Highlights

- Latest knowledge regarding the impact of virgin olive oil constituents on membranes.
- Oleic acid has therapeutic effects by modulating membrane structure and function.
- Minor constituents localize at different membrane levels.
- Minor constituents have therapeutic effects complementary to oleic acid.

Membrane composition and dynamics: a target of bioactive virgin olive oil constituents

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Abbreviations

ABCA1, ATP-binding cassette sub-family A member 1; AD, Alzheimer's disease; apoB48, apolipoprotein B-48; APP, amyloid precursor protein; BCMO1, β-carotene 15,15'-monooxygenase; BLAOA, bovine α-lactalbumin complexed with oleic acid; CD36, cluster of differentiation 36; ER, endoplasmic reticulum; FABPpm, plasma membrane fatty acid-binding protein; FAs, fatty acids; FAT, fatty acid translocase; FATP, fatty acid transport protein; GAP43, growth

associated protein-43; GH, growth hormone; GHSR, GH secretagogue receptor; GLUT, glucose transporter; GPCRs, G-protein-coupled receptors; HAMLET, human α-lactalbumin made lethal to tumor cells; HDLs, high-density lipoproteins; HER2, human epidermal growth factor receptor 2; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; 5-HT_{7A}: 5-hydroxytryptamine 7A; INSIG1, insulin-induced gene 1; InsP3, inositol 1,4,5-triphosphate; LDLs, low-density lipoproteins; MAP2, microtubule-associated protein-2; MUFAs, monounsaturated fatty acids; NF-κB, nuclear factor-κB; NeuroD2, neuronal differentiation protein-2; NPC1L1, Niemann-Pick C1-Like1; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PLCβ1α/β, phospholipase Cβ1; ROS, reactive oxygen species; SCAP, SREBP cleavage-activating protein; SFAs, saturated fatty acids; SLC2, solute carrier member 2; SREBPs, sterol regulatory element-binding proteins; StARD3, StAR-related lipid transfer domain protein 3; PUFAs, polyunsaturated fatty acids; SCD1, stearoyl-CoA desaturase 1; TRPA1, transient receptor potential A1; UBX, ubiquitin-like; UBXD8, UBX-domain-containing protein 8; VLDLs, very-low-density lipoproteins.

ABSTRACT

Contents

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The endogenous synthesis of lipids, which requires suitable dietary raw materials, is critical for the formation of membrane bilayers. In eukaryotic cells, phospholipids are the predominant membrane lipids and consist of hydrophobic acyl chains attached to a hydrophilic head group. The relative balance between saturated, monounsaturated, and polyunsaturated acyl chains is required for the organization and normal function of membranes. Virgin olive oil is the richest natural dietary source of the monounsaturated lipid oleic acid and is one of the key components of the healthy Mediterranean diet. Virgin olive oil also contains a unique constellation of many other lipophilic and amphipathic constituents whose health benefits are still being discovered. The focus of this review is the latest evidence regarding the impact of oleic acid and the minor constituents of virgin olive oil on the arrangement and behavior of lipid bilayers. We highlight the relevance of these interactions to the potential use of virgin olive oil in preserving the functional properties of membranes to maintain health and in modulating membrane functions that can be altered in several pathologies.

Keywords

Virgin olive oil; Oleic acid; Minor constituents; Phospholipids; Membrane; Health/Disease.

1. Introduction

Virgin olive oil plays a pivotal role as the main source of fat in the Mediterranean diet, a diet that has traditionally been linked to longevity in Mediterranean populations and is associated with a significant improvement in health status, as measured by reduced mortality from several chronic diseases [1-8].

More than 90% of olive oil is formed in the mesocarp of the drupe from the fruit of the olive tree (*Olea europaea* L.). Virgin olive oil (for olive oil classification and definitions see ref. [9]) is obtained exclusively from fresh and healthy olives by physical procedures under low thermal conditions (<27 °C) and is the only edible fat that can be consumed as a natural fruit product with no additives or preservatives. Spain is by far the largest producer of virgin olive oil in the world, accounting for approximately 50% (~1,600,000 tons) of total global production in 2012 [10]. More recently, due to the increasing recognition of the unique health properties of virgin olive oil, interest in virgin olive oil production has extended to countries outside the Mediterranean region, such as western Africa, Argentina, Australia, Azerbaijan, Brazil, China, India, Japan, Mexico, New Zealand, South Africa, and the USA.

The main bioactive constituents of virgin olive oil include monounsaturated oleic acid and a variety of compounds present in lower quantities ("minor constituents") [3, 4, 9, 11-15; and references within the manuscript]. Almost all fatty acids (FAs) exist as a complex in the form of triacylglycerol, an ester derived from glycerol and 3 FAs (saponifiable fraction, >98%). The triacylglycerol content of virgin olive oil is responsible for its hydrophobicity.

The minor constituents (unsaponifiable fraction, up to 1.5%) contribute to the specific properties of virgin olive oil, including its oxidative stability and unique flavor, as well as its color (the pigments in virgin olive oil include carotenoids and chlorophylls).

Cells can simultaneously acquire or import a spectrum of precursors through endocytosis of circulating lipoproteins of endogenous or exogenous (dietary) origin, which affects the biogenesis and remodeling of membranes. In this review, we will address the impact of the bioactive constituents of virgin olive oil on the modulation of membrane composition and function in the context of health and disease, as well as the potential therapeutic applications of these bioactive constituents.

2. Virgin olive oil: major and minor bioactive constituents

2.1. Oleic acid

FAs are carboxylic acids and often contain a long, unbranched aliphatic chain. FAs are categorized as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), based on their structural and chemical properties. SFAs do not contain any double bonds or other functional groups along the chain, which is fully saturated with hydrogen atoms. The principal dietary SFAs are palmitic (16:0) and stearic (18:0) acids, which are composed of 16 and 18 carbon atoms, respectively. MUFAs contain one pair of carbon atoms linked by a *cis* double bond. Oleic acid (18:1n-9), which contains 18 carbon atoms with a double bond at the 9^{th} carbon from the methyl end of the FA molecule, is the major

dietary MUFA and represents 55 to 83% of the total FAs in virgin olive oil (Table 1). Carbon chains containing 2 or more *cis* double bonds, with the first double bond located between either the 3rd and 4th or the 6th and 7th carbon atom from the methyl end of the FA molecule, belong to the n-3 or n-6, respectively, PUFA families. These families cannot be synthesized by the human body (double bonds can be introduced into all positions of the FA chain with the exception of the n-3 and n-6 positions) and therefore must be obtained from the diet as α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6) or their long-chain PUFA derivatives. Of these FAs, eicosapentaenoic acid (20:5n-3), docosahexaenoic acid (22:6n-3), dihomo-γ-linolenic acid (20:3n-6), and arachidonic acid (20:4n-6) are the most metabolically significant. The concentrations of SFAs (palmitic + stearic acids) and PUFAs (α-linolenic + linoleic acids) in virgin olive oil range from 8 to 25% and from 3 to 21% of the total FAs, respectively. Some parameters, such as the area of production, altitude, climate, fruit variety, and stage of maturity of the fruit can greatly affect the FA composition of virgin olive oil [16].

Oleic acid is the primary component of virgin olive oil (~83% oleic acid in position *sn*-2 of the triacylglycerols) and is also found in peanut oil (~59% oleic acid in position *sn*-2 of the triacylglycerols) and canola oil (~37% oleic acid in position *sn*-2 of the triacylglycerols). However, stearoyl-CoA desaturase 1 (SCD1, similar to the mouse orthologue *mScd1*), which is anchored in the endoplasmic reticulum (ER) of hepatocytes and adipose tissue cells [17], plays a central role in partitioning endogenous and exogenous (dietary) FAs into metabolically active or inactive pools and is the rate-limiting enzyme for the biosynthesis of MUFAs (mainly oleic acid) from SFAs. SCD1 catalyzes the insertion of a *cis* double

bond into the 9th carbon atom of palmitic and stearic acids. Oleic acid is a key component of triacylglycerols and membrane lipids [18]. Importantly, oleic acid is the most common FA in nature, as well as in our diet (generally, oleic acid supplies an amount of calories equivalent or greater than the amount provided by SFAs and PUFAs combined). Tight restrictions on SFA consumption (<10% of total daily calories; less than 7% for high-risk individuals) and PUFA consumption (<10%) have been recommended. By contrast, oleic acid may provide up to 20-25% of total daily calories. In fact, oleic acid is one of the most abundant FA in the human body (Table 2) [19-25].

2.2. Minor constituents

Like other fruits and vegetables, olives synthesize a variety of compounds (>230 "minor constituents") in response to stress conditions including infection, wounds, UV radiation, and the relatively high temperatures of the Mediterranean climate. In contrast to other vegetable oils, which must be extracted from seeds using solvents to be made edible, virgin olive oil retains almost all of the properties of olives, including the chemical composition, biochemical status, and organoleptic properties. In addition to complex environmental and agronomical factors, the average concentration of the minor constituents in virgin olive oil also depends on technological factors [26].

Among the several minor constituents of virgin olive oil (Figs. 1-3), the most abundant fraction is hydrocarbons (squalene and, in smaller amounts, the carotenoids β-carotene and lutein). Other minor constituents of virgin olive oil include phytosterols, such as β-sitosterol, Δ5-avenasterol, and campesterol,

triterpenic compounds in the form of dialcohols (erythrodiol and uvaol) or acids (oleanolic and maslinic acids), and phenolic compounds, representing the polar fraction. The main classes of phenolic compounds in virgin olive oil are secoiridoids, as ester derivatives of elenolic acid in either its aglyconic form or glycosylated with hydroxytyrosol (oleuropein) or tyrosol (ligustroside); simple phenols, in the form of alcohols (tyrosol and hydroxytyrosol) or acids (*p*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, gallic, gentisic, syringic, vanillic, protocathechuic, and *p*-hydroxybenzoic acids); hydroxyisochromans formed by the reaction between hydroxytyrosol and benzaldehyde or vanillin; flavonoids (luteolin, apigenin, and quercetin); and the lignans (+)-pinoresinol, 1-(+)-acetoxypinoresinol, and 1-(+)-hydroxypinoresinol. The lipophilic phenols include tocopherols (α-, β-, γ-, and δ-tocopherols) and tocotrienols (α-, β-, γ-, and δ-tocotrienols), with α-tocopherol as the predominant constituent in virgin olive oil.

3. Oleic acid: membrane interactions and functions

After ingestion, lingual and pancreatic lipases hydrolyze the ester bonds of virgin olive oil triacylglycerols, yielding 2 major digestion products: *sn*-2-monoacylglycerols (mainly *sn-2*-monooleoylglycerol) and FAs (mainly oleic acid). These products are then taken up by enterocytes, re-esterified into triacylglycerols, and incorporated with other lipids (e.g., small amounts of cholesteryl esters, mainly cholesteryl oleoyl ester), lipid-soluble vitamins, sugars, and apolipoproteins (mainly apoB48) into chylomicrons for subsequent secretion into the bloodstream. Similarly, the liver secretes very-low-density lipoproteins (VLDLs), which are rich in endogenous triacylglycerols from FAs

synthesized *de novo* or taken up from the circulating pool of FAs complexed with albumin. The triacylglycerols of chylomicrons and VLDLs undergo hydrolysis by lipoprotein lipase, which is located at the surface of cells. The uptake of FAs from the cell surroundings has long been thought to occur via simple diffusion, a passive process dependent on the activity of intracellular metabolism. This process involves the creation of a trans-membrane, downhill concentration gradient and includes the flip-flop molecular mechanism of FA transport [27] and membrane transporters (e.g., FABPpm, FAT/CD36, FATP family proteins, and caveolin-1), which facilitate FA endocytosis. In addition, chylomicrons and VLDLs, which may or may not be affected by partial hydrolysis, can be recognized by membrane receptors (e.g., apoB48 and VLDL receptors) [28-31], internalized, and directed to the endosomal-lysosomal system. Here, the *sn*-1(3)-ester bonds in triacylglycerols and the C-3 ester bond in cholesteryl esters are hydrolyzed by lysosomal acid lipase, which produces significant amounts of intracellular oleic acid and *sn-2*-monooleoylglycerol for membrane biogenesis, either by acylation of glycerophosphate to phosphatidic acid (via the Kennedy pathway) or by remodeling of *de novo* synthesized glycerophospholipids via deacylation-reacylation or via the monoacylglycerol pathway. The regular consumption of virgin olive oil increases the concentration (up to 15%) of oleic acid among the plasma membrane lipids [32-34].

3.1. Oleic acid in membrane lipid structure

Cell membranes contain hundreds of different lipid species, which can be categorized into 3 main classes: glycerophospholipids, sphingolipids, and sterols (cholesterol in animals) (Fig. 4). Cholesterol contains a 4-membered

cyclopentanophenanthrene ring structure, a hydroxyl group, and a short hydrophobic tail and imparts stiffness to membranes, accentuating the potential hydrophobic incompatibility between the hydrophobic transmembrane domains of proteins and the lipid bilayer core [35]. Additional complexity of glycerophospholipids is achieved through differences in the FA chains and their linkages by ester bonds in the *sn*-1 and *sn*-2 positions and by alkyl ether or alkenyl ether bonds in the *sn*-1 position of the glycerol backbone. The lipid backbone of sphingolipids is one molecule of ceramide (sphingosine amide linked to a FA), which can be further modified and extended with a phosphocholine head group or glycans to form sphingomyelin or glycosphingolipids, respectively [36]. A considerable part of our genome is required to synthesize, metabolize, and regulate the pool of FAs for *de novo* biogenesis of membranes, and the role of exogenous (dietary) FAs in these processes remains largely unknown.

The incorporation of oleic acid into the phospholipids of the cell membrane can regulate the membrane structure and, in turn, alter the biophysical properties of the membrane. The lamellar phase is recognized as the most common lipid arrangement and is oriented perpendicular to the membrane surface in cells. The fluid lamellar phase ($L\alpha$ or liquid crystalline) is found in most membrane regions and domains, such as coated pits, caveolae, synaptosomes, or lipid rafts [liquid-ordered (l_0) phase]; however, membrane lipids can also organize into non-lamellar phases ($L\varepsilon$ frustrated membranes), including hexagonal (H_I and H_{II}), cubic (micellar and bicontinuous), and rhombohedral phases [37]. Unlike stearic acid and elaidic acid (*trans*-18:1n-9), oleic acid favors the organization of

membranes into H_{II} non-lamellar phases, which drastically changes the temperature range of the stability of the single Lα phase, decreasing the lamellar-to-hexagonal (Lα-to-H_{II}) phase transition temperature in lipid membranes [38-40]. This propensity of oleic acid to stabilize the H_{II} phase and to induce a negative curvature strain on lipid bilayers is related to the molecular shape of oleic acid. The *cis* double bond of oleic acid produces a bend at a ~120° angle in the middle of the carbon chain that confers a boomerang-shaped structure. In elaidic acid (the *trans* isomer of oleic acid), the 2 parts of the carbon chain are almost linear, similar to stearic acid, resulting in a rod-shape structure. Linear molecules pack together closely in a given space and cause little disorganization of the lipid bilayer, whereas bent molecules cannot pack together to form such ordered and rigid arrays. The kinked lipid chain of oleic acid reduces the order within the lipid bilayer, helps to maintain the hydration level, and increases membrane fluidity [41, 42]. Oleic acid and 2-hydroxyoleic acid (a synthetic, hydroxylated analog of oleic acid) were recently reported to induce lateral lipid heterogeneity in a model mimicking cell membrane structure [43]. Both FAs have a similar ability to modulate the liquid-ordered/liquid-disordered structure ratio and the microdomain lipid composition, conceivably affecting lipid rafts [44, 45]. The effects of different naturally occurring FAs (stearic, α-linolenic, linoleic, eicosapentaenoic, docosahexaenoic, and arachidonic acids) and the corresponding synthetic 2-hydroxylated FA derivatives were also compared, but the greatest disordering effect was caused by oleic acid and 2-hydroxyoleic acid, suggesting that the presence of an 18-carbon acyl chain in combination with a single *cis* double bond produced the most potent reorganizing effect on the model membranes.

3.2. Oleic acid in membrane lipid function

Oleic acid may have important consequences as an environmental factor by altering some targeted molecular functions in the cell through the modulation of membrane properties, while either embedded in the membrane or in its free form upon release from the *sn*-2 position of phospholipids via phospholipases. In general, different proteins interact with specific regions of the membrane, which are clearly aligned into the l_0 and Lε organizations [37]. For example, sterol regulatory element-binding proteins (SREBPs) belong to a family of transcription factors that orchestrates the expression of a broad array of genes required for the synthesis of cholesterol [by transcription of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase] and FAs (by transcription of SCD1)[46]. SREBPs are anchored in the membrane of the ER via 2 trans-membrane domains and are subject to negative feedback regulation by cholesterol and FAs [47]. This feedback mechanism is based on the ability of unsaturated FAs to reduce degradation of insulin-induced gene 1 (INSIG1), an ER membrane protein that plays a crucial role in the SREBP cleavage-activating protein (SCAP)/SREBP transport system for cholesterol synthesis and uptake [48, 49]. After ubiquitylation of INSIG1, oleic acid but not stearic acid blocks the binding of ubiquitylated INSIG1 with the ubiquitin-like (UBX)-domain-containing protein 8 (UBXD8), thereby circumventing ER-associated degradation of INSIG1 by stabilizing INSIG1. INSIG1 then binds the polytopic membrane protein SCAP, blocking the proteolytic activation of SREBP. This decrease in cellular levels of cholesterol has been shown to induce a rearrangement of lipid rafts, thereby

affecting the localization and activity of integral proteins, including platforms (e.g., FAT/CD36) that facilitate the binding and uptake of MUFAs and PUFAs [50].

As an example, one role for oleic acid in this complex dynamic motion of cross-sectional distribution in membrane lipids, which stabilizes the bilayer structure and regulates the embedded proteins therein, has been proposed for both the catalytic and proton-translocase activities of urothelial V-ATPase [51]. Comparison of the effects of the supply of oleic or linoleic acid to superficial umbrella cell membranes of the urinary bladder mucosa revealed that the ATP hydrolytic activity of V-ATPase increases while the V-ATPase proton translocation rate decreases in oleic acid-rich membranes of urothelial endocytic vesicles. Similar findings were observed when the activity of the Na/K-ATPase was explored in reconstituted systems containing membrane lipids bearing FA chains of different lengths and degrees of unsaturation, supporting an uncoupling or slippage phenomenon analogous to that observed in P-ATPase-type ion pumps [52]. Accordingly, oleic acid is proposed to mediate an increase in the minimal hexagonally packed particle size and create an appropriate topological setting for optimal ATPase function in the membrane urothelium.

Due to its ability to organize membranes into Lε frustrated lamellar phases, oleic acid facilitates membrane docking and activity of other amphitropic proteins. Thus, Lε domains delineate regions for the recruitment and interaction of heterotrimeric Gαβγ proteins, G-protein-coupled receptors (GPCRs), and related signaling molecules; by contrast, l_0 regions form domains that enable the interaction of dissociated Gα subunits from the Gβγ dimers with effector proteins

[37, 53]. GPCRs, which are the largest family of membrane-spanning proteins (~800 members in the human genome), can detect an expansive array of extracellular signals or ligands, including photons, ions, odors, pheromones, hormones, and neurotransmitters [54, 55]. In addition to oleic acid-induced rearrangement of lipid bilayer structure into H_{II} nonlamellar phases, the activation of heterotrimeric $G\alpha\beta\gamma$ proteins induces conformational changes in the immediate lipid environment. The $G\alpha$ subunit exchanges bound GDP for GTP and dissociates from the $G\beta\gamma$ dimer, which retains its affinity for hexagonal-phase membrane regions close to GPCRs [56]. Post-translational modification of heterotrimeric $G\alpha\beta\gamma$ proteins, including myristoylation, palmitoylation, and isoprenylation, also favors the anchoring of proteins to the membrane and the formation of non-lamellar-prone regions [56]. Furthermore, oleic acid regulates G-proteins concentrated in lipid raft/caveolae domains. The G-protein-coupled growth hormone (GH) secretagogue receptor (GHSR), which is activated by ghrelin, has been shown to assemble into lipid rafts [57]. Ghrelin binds to GHSR and provokes increased levels of intracellular diacylglycerol, inositol 1,4,5-triphosphate (InsP3), and Ca^{2+} by the action of phospholipase $C\beta1$ ($PLC\beta1\alpha/\beta$) on membrane phosphatidylinositol via $G_{q/11}\alpha$ proteins [58]. Oleic acid markedly improves the response of GHSR to ghrelin via a mechanism that involves the suppression of ligand-induced receptor internalization by blocking GHSR membrane-trafficking and uncoupling GHSR from the endocytic pathway [59]. Notably, the responses of other GPCRs, such as the 5-hydroxytryptamine 7A (5-HT_{7A}) receptor, are selectively stimulated by oleic acid, which confers more efficient ligand coupling of the 5-HT_{7A} receptor with $G_s\alpha$ subtypes of G proteins

in the environment of lipid rafts [60]. Therefore, oleic acid provides multiple adaptive membrane sites for the plasticity of GPCRs, whereas G-proteins promote and perpetuate the concurrence of dynamic multi-domain systems of non-lamellar membranes with fluid lamellar phases.

3.3. Oleic acid in membrane lipid therapy

Accumulating data from multiple lines of evidence suggest that dietary FAs are linked not only to the promotion of health but also to the pathogenesis of disease. In particular, oleic acid from virgin olive oil has been shown to have beneficial effects in reducing the risk of major chronic diseases [3, 9, 11, 12, 15], many of which are associated with abnormalities in the type and/or abundance of the lipids in cell membranes [37].

In contrast to the consumption of soybean oil (which is rich in linoleic acid and low in oleic acid), virgin olive oil intake has been shown to induce a marked reduction in blood pressure in hypertensive animals [61]. This effect was not only acute (with a post-prandial peak at 2-4 h) and stable for 3-4 days but was also sustained after the administration of virgin olive oil for 2 weeks. Importantly, the administration of oleic acid, but not stearic or elaidic acid, mimicked the hypotensive effects of virgin olive oil. These findings suggest a scenario in which the signaling pathways of adrenergic receptors (α- and β-adrenoreceptors), key elements in the central and peripheral control of blood pressure, are affected by the increase in H_{II}-prone membrane regions in response to the administration of virgin olive oil or oleic acid. Several lines of evidence indicate adaptive changes in the bilayer structure to support the

regulation of the α2-adrenoreceptor/G protein/adenylyl cyclase-cAMP/protein kinase A (PKA) vasodilatory pathway [62-64]. First, membranes from the aorta of animals feature reduced expression of G-proteins, including $G\alpha i_2$, $G\alpha i_3$, $G\alpha_{q/11}$, and PLCβ1α/β. Second, the reduction in blood pressure correlated with a decrease in the Lα-to-H_{II} phase transition in aortic membranes and an increase in the dose of oleic acid administered to the animals. The effect of oleic acid on blood pressure reduction is mediated, at least in part, by an enhanced production of vasodilatory stimuli (e.g., cAMP and PKA) and a decrease in vasoconstriction pathways (InsP3, Ca^{2+}, diacylglycerols, and Rho kinase). This conclusion is supported by earlier studies demonstrating that the ingestion of virgin olive oil reduces blood pressure [32, 65] as well as the need for antihypertensive medication [66] in humans.

Glucose uptake from the bloodstream is the rate-limiting step in whole-body glucose utilization and is regulated by specific transporter GLUT membrane proteins. Normal glucose transport and utilization is critical for preventing glucose intolerance that leads to insulin resistance and the development of type 2 diabetes [67]. Members of the GLUT family (which includes 14 GLUT proteins in humans) are encoded by the solute carrier member 2 (SLC2) genes and are composed of ~500 amino acid residues. GLUT family members are categorized into 3 classes based on sequence similarity: Class 1 (GLUTs 1-4, 14), Class 2 (GLUTs 5, 7, 9, and 11), and Class 3 (GLUTs 6, 8, 10, 12, and HMIT). GLUTs are integral membrane proteins that contain 12 membrane-spanning helices, a single N-linked glycosylation site, and a relatively large, central cytoplasmic linker domain and feature topologies in which both their N and C termini are

exposed on the cytoplasmic side of the membrane. GLUT4 is the predominant isoform expressed in insulin-sensitive tissues, fat, and muscle. The insertion of GLUT4 into the plasma membrane and fusion with insulin-dependent docked GLUT4-containing vesicles is governed by the ratio of unsaturated FAs and SFAs in the phospholipids of model membranes [42], suggesting that membranes rich in oleic acid and poor in SFAs have increased flexibility and promote the capacity for GLUT4-dependent glucose transport into cells. In a mouse skeletal cell line, oleic acid has been shown to reverse SFA-induced impairment of insulin-responsive GLUT4 recycling by preventing aberrant PKCθ activation [68]. These findings are in agreement with human studies demonstrating that dietary fats rich in oleic acid and poor in SFAs improve insulin sensitivity in the post-prandial state [69, 70]. Type 2 diabetes is commonly linked with nonalcoholic fatty liver disease; however, the impact of FAs on hepatic membranes and the intolerance of hepatic cells to insulin remain poorly understood and controversial [71]. More evident is the protective effects of oleic acid, when incorporated into hepatic membranes, against models of drug-induced liver toxicity [72] or aging-related liver apoptosis [73].

There is increasing evidence that lipid rafts may be targets for neuronal development and regeneration, highlighting the potential for lipid-based strategies against progressive neurodegenerative disorders such as Alzheimer's disease (AD) [74, 75]. Neuronal lipid rafts are involved in cellular polarity as well as pro-survival and pro-growth receptor signaling pathways for neuronal sprouting [76] and are found in the membrane sites surrounding pre- and post-synaptic proteins [77]. An additional event in neuronal membrane repair is the

coalescence of smaller-scale lipid rafts into larger lipid rafts through lateral membrane distribution, which occurs via lipid-lipid and lipid-protein interactions. In neurons, oleic acid activates peroxisome-proliferator-activated receptors, promoting axonal growth, neuronal clustering, and the transcription of key genes for neuronal differentiation, including neurogenic basic helix-loop-helix/neuronal differentiation protein-2 (NeuroD2), axonal growth-associated protein-43 (GAP43), and microtubule-associated protein-2 (MAP2) [78-80] Detailed postmortem analysis of FAs in lipid rafts in the frontal cortex grey matter of patients who suffered from severe Alzheimer's-type dementia compared to samples from neurologically normal individuals revealed a significant decrease in oleic and docosahexaenoic acids [74]. The most striking observation was the negative relationship between oleic and stearic acids in lipid rafts. This observation suggests that oleic acid is important for preserving the homeoviscous state of membranes in neuronal cells. In support of this concept, oleic acid decreases the levels of the toxic amyloid-β peptide, which reciprocally interacts with cholesterol in lipid rafts [81], and amyloid plaques in a mouse model of AD [82]. Furthermore, the incorporation of oleic acid into membranes of neuroblastoma cells reduces the toxic effects of palmitic acid and rescues cells from apoptosis [83]. The incorporation of oleic acid into the brain mitochondrial membranes after the administration of virgin olive oil could also reduce oxidative stress associated with age [84].

The deprotonated form of oleic acid also plays an active role as a lipid cofactor of partially unfolded α-lactalbumin in the tumoricidal action of the oligomeric complex HAMLET (human α-lactalbumin made lethal to tumor cells), which

induces membrane disruption in tumor cells but not healthy cells [85-89]. In contrast to SFAs, PUFAs, and *trans*-MUFAs, oleic acid shares specific structural features required for both optimal HAMLET formation and engagement of targets involved in tumor cell death. Membrane binding studies with a HAMLET-like substance (BLAOA, bovine α-lactalbumin complexed with oleic acid) suggest that acyl-chain mismatch or immiscibility of membrane lipid components may provide local sites for HAMLET interaction and accumulation. The negative intrinsic curvature of oleic acid and the bilayer-penetrating helix in the partially unfolded α-lactalbumin more easily disrupt the membrane by blebbing, with subsequent shedding of small lipid aggregates, causing the membrane to collapse [90]. In addition to interaction with tumor cell membranes, HAMLET becomes rapidly internalized and translocates into the nucleus [91]. The conserved death response of tumor cells to HAMLET is dependent on oncogenic transformation, proteasome inhibition, nucleosome-histone binding, mitochondrial damage, and ion channel activation. The interactions of HAMLET with membranes as well as subsequent biological perturbations in tumor cells all require both the protein and oleic acid.

4. Minor constituents: membrane interactions and functions

4.1. Squalene

Squalene is a polyunsaturated aliphatic hydrocarbon that contains 6 isoprene units. It is structurally related to β-carotene, ubiquinol-10 (reduced form of coenzyme Q_{10}), and vitamins K_1, D, and E. In virgin olive oil, squalene is protected from oxidation, and squalene confers stability to virgin olive oil [92, 93]. The content of squalene in virgin olive oil ranges from 800 to 12000 mg/kg,

accounting for more than 50% of the unsaponifiable fraction of virgin olive oil. In humans, approximately 60 to 85% of dietary squalene (up to 200 mg/day) is absorbed and distributed ubiquitously by triacylglycerol-rich lipoproteins in tissues, with the greatest accumulation in the skin [94]. However, squalene can be endogenously synthesized by sequential reactions in which HMG-CoA is first converted to farnesyl pyrophosphate, followed by head-to-head condensation of 2 molecules of farnesyl pyrophosphate catalyzed by squalene synthase, and reduction by NADPH to yield squalene. Squalene is an important precursor in the formation of eukaryotic sterols and bacterial hopanoids. For the biosynthesis of cholesterol, the enzymes squalene monooxygenase and oxidocyclase convert squalene into the cyclic derivative lanosterol, which is converted to cholesterol via a series of 19 reactions. SREBPs mainly regulate the transcription of the rate-limiting enzyme HMG-CoA reductase [95]. However, cholesterol can also feed back to stimulate the proteasomal degradation of squalene monooxygenase as an additional post-translational control point in the regulation of sterol biosynthesis pathways and to preserve the demand for the isoprenoids farnesol and geranylgeraniol [96]. Farnesylation and geranylgeranylation of members of the Ras superfamily of GTPases and other membrane proteins have been shown to be critical in the correct targeting of these proteins to the inner surface of the plasma membrane [53].

In spite of its nonpolar nature, squalene can be partitioned within the hydrophobic core of the lipid bilayer, with the extended length —or the coiled conformation [97]— oriented parallel to the membrane phospholipid chains and in the center plane of the bilayer. In this conformation, squalene plays a major

role in maintaining the electrochemical gradient that moves ions across membranes [98]. Studies of model membranes of giant vesicles, which mimic the native environment of mammalian cells for the study of membrane lipid-protein interactions, have revealed that squalene increases the polarity and hydrophobic interactions of reconstituted trans-membrane-spanning regions of pore-forming proteins such as aquaporins, facilitating the functional regulation of these proteins as well as the trans-membrane route for water movement [99]. The stabilizing effects of squalene on membranes are related to the increased rigidity and size of regions adopting the inverse hexagonal H_{II} phase [100, 101]. This dynamically folded conformation of squalene is retained when linked to gemcitabine, a synthetic tumoricidal pyrimidine nucleoside, leading to self-organized, stable, and mono-dispersed nanoparticles composed of inverse hexagonal phases that increase gemcitabine amphipathy and improve gemcitabine diffusion through lipid membranes [102, 103]. Grafting of the squalenoyl moiety onto the gemcitabine molecule accelerates gemcitabine diffusion within cholesterol-rich domains, indicating squalenylation as a new nanotechnology platform for targeted delivery of drugs with anticancer and antiviral properties [104, 105].

The natural enrichment of squalene in skin surface lipids suggests that this terpene molecule protects against external chemical, physical, or microbial signals and stressors on the skin surface [94]. The most intensely researched field is solar UV irradiation (UVB, 290-320 nm; UVA, 320-400 nm), with efforts attempting to target the increased risk of tumor development associated with UV overexposure. Molecules such as DNA, *trans*-urocanic acid, melanin precursors,

tryptophan, and endogenous or bacterial porphyrins can selectively absorb in the UV wavelength range (mainly UVA) and hence catalyze oxidative cytostatic, cytotoxic, or immunomodulating photoreactions. When the α-tocopherol and ubiquinol-10 line of defense is overwhelmed, a wide range of relatively hydrosoluble by-products of varying polarity and reactivity are rapidly generated by oxidative degradation of squalene, which is the most efficient quencher of singlet oxygen in skin surface lipids [94, 106]. Oral administration of squalene decreases UV-induced DNA damage and prevents photo-aging in human skin [107]. In addition to its radical scavenging capacity (either alone or in virgin olive oil) in membranes and skin disorders [108, 109], squalene is effective against several degenerative diseases [4, 110-112] and has been used as a vaccine adjuvant [113].

4.2. β-Carotene and lutein

Carotenoids are polyenoic terpenoids with 8 condensed isoprene units containing conjugated *trans* double bonds. They include the family of carotenes (e.g., β-carotene) and xanthophylls (e.g., lutein). Carotenes are pure hydrocarbons and contain no oxygen atoms, whereas xanthophylls contain oxygen atoms within hydroxyl groups and/or epoxides. Carotenoids are ubiquitous constituents in the membranes of all photosynthetic organisms and are essential for the assembly and stability of protein complexes and for membrane dynamics via carotenoid-lipid-protein interactions in thylakoid membranes [114]. They also have photoprotective properties and participate in protecting elongation factors of the translational machinery from stress damage in the membranes of green plants and other types of photosynthetic organisms.

Carotenoids cannot be synthesized by vertebrates *de novo* and thus are sequestered from the diet. In virgin olive oil, the content of major carotenoids ranges from 4 mg/kg for β-carotene to 10 mg/kg for lutein, although virgin olive oil also contains β-cryptoxanthin and 5,6-epoxy xanthophylls, including neoxanthin, violaxanthin, antheraxanthin, and their furanoxides [115, 116]. Carotenoids are susceptible to reactions including isomerization (*trans* to *cis*) and oxidation, which increase the average shelf life of virgin olive oil [117].

Carotenoids levels in human blood and tissues are critical for maintaining human health. In the intestinal mucosa, approximately half of the dietary β-carotene is irreversibly converted to 11-*cis*-retinal via β-carotene 15,15'-monooxygenase (BCMO1) activity. Retinal is then reduced to retinol (vitamin A) by retinol dehydrogenases (stoichiometric conversion of 2 mol of retinol formed per 1 mol of β-carotene cleaved) [118, 119]; the β-ionone ring also converts pro-vitamin A (1 mol of retinol) to β-cryptoxanthin [120]. Retinal can also be oxidized to retinoic acid. The capacity of the liver to metabolize β-carotene is 4 times greater than that of the intestine [121]. The absorption of retinol and intact carotenoids is affected by several dietary factors, including the food matrix and interactions between carotenoids and other food components such as fiber, lipids, phytosterols, and other carotenoids [120, 122]. Lutein is the most abundant carotenoid in human triacylglycerol-rich lipoproteins after the ingestion of virgin olive oil [123]. The retinol metabolite retinoic acid, via retinoic acid and X receptors, may modulate the expression of the homeobox transcription factor ISX, which represses the expression of BCMO1 and membrane protein transporters involved in retinol and carotenoid intestinal absorption [120, 124].

In biological membranes, carotenoids are present as carotenoid-protein complexes and as direct components of the lipid phase, demonstrating an ability to form self-assemblies [125]. The conformation of carotenoids in membranes is of great importance because the energy associated with spectroscopic transitions is directly related to the antioxidant properties exhibited by carotenoids in lipid environments [126]. According to the most recent study of atomistic molecular dynamics simulations of β-carotene and zeaxanthin (a structural isomer of lutein) immersed in a model lipid membrane [127], β-carotene can adopt a relatively flat profile along tilt angles of the polyenic chain —with respect to the membrane normal axis— from 90˚ (perpendicular to lipid tails) to 35˚. Three minimal energy profiles for the simulations of β-carotene were observed at 90˚, 60˚, and 40˚, indicating substantial variability of the orientation of β-carotene within membranes. The orientation axis of β-carotene, which is nearly parallel with the membrane surface within hydrophobic tails, displays a broad distribution of the β-ionone ring centered at one of the leaflets. The average tilt angle for zeaxanthin was lower (approximately 30˚), likely due to electrostatic interactions between the hydroxyl groups of zeaxanthin with the polar lipid heads of the membrane. By contrast, the entire ε ring of lutein can rotate around the C6'-C7' bond, which facilitates the interaction of the hydroxyl groups located at the opposite ends of the molecule with the same polar membrane surface [128] and the unambiguous orientation following the pattern of zeaxanthin and parallel with respect to the plane of the membrane [129]. This result is in agreement with a specific position of lutein involving one membrane-spanning segment and one horizontally oriented segment and with the tendency

of lutein to accumulate in the liquid-disordered phase of membranes [130]. These studies are also consistent with the β-carotene-mediated induction of membrane lateral asymmetry by lateral diffusion and a decrease in the lipid membrane surface area, as opposed to the rigidity of membranes containing lutein [114, 131]. The lower effective conjugated chain length for β-carotene predicts a lesser antioxidant character than lutein within membranes through enhanced charge donation and reduced charge acceptance [126, 132]. Differences in the membrane spatial distribution of β-carotene and lutein, as well as subsequent differences in their antioxidant properties, may constitute an advantage to synergistically trap in-tandem (lutein → β-carotene) triplet-excited molecules of sensitizers, singlet oxygen, or free radicals and to prevent the propagation cycle of lipid peroxidation from the interface of the membrane to the inner region of the membrane. In support of this notion, there is evidence that unpaired electrons flow from the oxidative-derived radical cations of astaxanthin, which is anchored in the lipid/water interface, to β-carotene in the inner portion of the membrane. The resulting β-carotene radical cation tends to rotate and move toward the polar interface in the membrane, facilitating the regeneration of β-carotene by interaction of the β-carotene radical cation with ascorbate or α-tocopherol [133].

In the rod outer segment disc membranes of photoreceptor cells, the visual pigment rhodopsin is a retinal photoreceptor protein (a prototypical GPCR) of bipartite structure consisting of the transmembrane protein opsin and the light-sensitive chromophore 11-*cis*-retinal. The reactions that eventually result in the conversion of light energy (photons) into a photoreceptor electrical response

require a constant supply of 11-*cis*-retinal from β-carotene and pro-vitamin A carotenoids. The retina is very sensitive to oxidative stress and lipid peroxidation due to abundant illumination, high respiratory demands for oxygen, and a large presence of docosahexaenoic acid, which contains 6 double bonds and thus is easily oxidized. To prevent oxidative damage to the retina, lutein (zeaxanthin and *meso*-zeaxanthin —a metabolite of lutein) accumulates at very high concentrations in the *macula lutea* (up to 1 mM in the central fovea) via the involvement of specific carotenoid-binding proteins [StAR-related lipid transfer domain protein 3 (StARD3), for lutein] [134]. Lutein exhibits high efficiency in protecting against lipid peroxidation in membranes composed of raft-forming mixtures as well as in models of photoreceptor outer segments [135] and modulates the properties of lipid raft functions around membrane proteins such as the transient receptor potential A1 (TRPA1) ion channel in primary sensory neurons [136]. There is clinical evidence that lutein, β-carotene, and vitamins C and E reduce the risk of age-related macular degeneration [137, 138]. These carotenoids have also been proposed to favor optical density of the macular pigment [139] and protect against age-related cataracts [140], cognitive decline [141], atrial fibrillation [142], osteoporotic fractures [143], and vascular aging [144].

4.3. β-Sitosterol, Δ5-avenasterol, and campesterol

While cholesterol is the major sterol in animals, β-sitosterol, Δ5-avenasterol, and campesterol, in addition to brassicasterol and stigmasterol, are the most commonly occurring sterols (phytosterols) in the plant kingdom. These sterols are structurally similar and contain 4 strongly hydrophobic hydrocarbon rings

(planar steroid nucleus), a hydroxyl group at the 3-carbon atom of the A-ring, and an alkyl tail (side chain). Therefore, these molecules display amphipathic properties. Phytosterols differ from cholesterol only by an additional ethyl group (β-sitosterol) or methyl group (campesterol) at the 24-carbon atom or by an additional *trans* double bond at the 22-carbon atom (stigmasterol, as compared to brassicasterol) in the side chain. Carbon atom 24 in β-sitosterol and campesterol has R chirality, whereas the equivalent carbon atom in brassicasterol and stigmasterol has S chirality and lower flexibility due to the *trans* configuration of the side chain double bond [145]. Mammals do not synthesize phytosterols endogenously. The sterol β-sitosterol is the most abundant in virgin olive oil, comprising 80% to 92% of the total sterols (at least 1000 mg/kg, with a maximum of 3000 mg/kg). Following β-sitosterol, the most important sterols in virgin olive oil are Δ5-avenasterol (4-14%) and campesterol (0.5-4%). These constituents help maintain virgin olive oil stability by inhibiting polymerization reactions during heating at frying temperature. The sterol profile is affected by the olive fruit variety and the degree of ripening [146, 147] and is considered a fingerprint for authentic virgin olive oil [148, 149].

When β-sitosterol is added to mixed micelles (model intestinal solutions) containing bile salts, phosphatidylcholine, FAs, and cholesterol, there is a specific, sharp decrease in the maximum solubilization of cholesterol that is dependent on the preferential solubilization of β-sitosterol at similar micellar solubilization sites according to the strength of affinity [150]. This restriction due to dynamic competition of cholesterol and β-sitosterol in processes of self-assembly of nutrients in the intestinal lumen may explain the ability of β-

sitosterol to decrease the rate of cholesterol transport to the intestinal wall, which is one of the main molecular mechanisms of phytosterol action on cholesterol plasma levels. The polytopic Niemann-Pick C1-Like1 (NPC1L1) trans-membrane protein is required for phytosterol (and cholesterol) entry in enterocytes [151] . Phytosterols and non-esterified cholesterol are pumped back into the intestinal lumen via the ATP-binding cassette sub-family G-member proteins ABCG5 and ABCG8 or may be transported to the basolateral membrane of the enterocyte for ABCA1 (ATP-binding cassette sub-family A member 1)-mediated biogenesis of HDLs (high-density low lipoproteins), whereas cholesterol is esterified via the enzyme acetyl-coA acetyltransferase 2, packed into chylomicrons, and drained into the lymph system [152]. These complex mechanisms are responsible for the assimilation of approximately 50% of dietary cholesterol and less than 5% of phytosterols. Notably, virgin olive oil intake induces an increase in the plasma levels of β-sitosterol, which is inversely correlated with the intestinal absorption of dietary cholesterol and the plasma levels of total cholesterol and LDLs (low-density lipoproteins) in asymptomatic elderly people at high cardiovascular risk [153]. Both β-sitosterol and FAs (oleic acid and PUFAs) can act as signaling molecules for the transcriptional repression of NPC1L1 in the intestine and liver [154]. These pathways lead to the insertion of β-sitosterol and, eventually, other phytosterols into membranes and the partial replacement of cholesterol.

β-Sitosterol has lower condensing and ordering potency than cholesterol in binary and multicomponent model membranes composed of phosphatidylcholine, sphingomyelin, ganglioside, and/or ceramide [155]. In

conjunction with the similar in-plane organization of β-sitosterol and β-sitostanol (a saturated form of β-sitosterol) in Langmuir monolayers of glycerophospholipids [155, 156], these data suggest that the alkyl tail, rather than the ring structure, in β-sitosterol accounts for the difference in membrane organization compared with cholesterol. Interestingly, disturbances in the molecular packing and morphology of model membranes due to the replacement of cholesterol by β-sitosterol are more pronounced when glycerophospholipids have MUFA chains compared with SFA chains [156]. Accordingly, atomistic molecular dynamics simulation studies on lipid matrices reminiscent of rafts (rich in saturated lipids) or less ordered membranes (rich in unsaturated lipids) have shown that the lower cholesterol tilt and the best ordering as a function of tilt in saturated bilayers promotes higher ordering of the neighboring acyl chains [157, 158]. As expected, phytosterols are inferior to cholesterol in promoting the formation of membrane domains and can inhibit the incorporation of exogenous cholesterol into lipid rafts at a molar ratio of 1:4 (cholesterol:phytosterol) [159]. Therefore, the incorporation of increasing amounts of phytosterols into bilayers could gradually diminish the l_0 phase. The magnitude of these effects depends on the geometry of the phytosterol because the transition temperature from the lamellar to Lα phase in bilayer vesicles is reduced according to the following order of strength (compared to cholesterol): β-sitosterol > stigmasterol > campesterol > cholesterol [160]. This indicates that β-sitosterol is the least favorable phytosterol for stabilizing the interactions of lipid molecules constituting membranes. In binary phosphatidylcholine/sphingomyelin model membranes, β-sitosterol also induces a stronger contraction of area per lipid than stigmasterol [161].

The cholesterol-lowering effects of phytosterols (mainly β-sitosterol) in plasma and cell membranes have been shown to maintain cardiovascular health [162-164]. Circulating β-sitosterol, Δ5-avenasterol, and campesterol are biomarkers for predicting glucose intolerance, and Δ5-avenasterol and campesterol confer lower risk for type 2 diabetes [165]. Furthermore, several studies have reported that phytosterol and, in particular, β-sitosterol supplementation replaces cholesterol and reduces growth and survival signals routed through caveolar rafts in membranes of several cancer cell lines [155, 156]. In some types of cancer, the feedback regulation of cholesterol absorption and the lost of endogenous cholesterol synthesis result in the formation of regions prone to lipid raft development in the membranes of cancer cells [166-168]. Among the many mechanisms by which phytosterols exhibit anti-cancer activity *in vitro* are the modulation of ceramide metabolism, liver X receptor activation, cell cycle progression, and apoptosis [169].

In mice, increased circulating levels of phytosterols result in elevated levels of phytosterols in the brain, most likely via delivery to the endothelial cells of the blood-brain barrier followed by subsequent re-secretion from the basolateral plasma membrane [170]. This accumulation of β-sitosterol is relatively lower than campesterol, and the process is virtually irreversible [171]. Within the brain, in a cell-type specific manner, β-sitosterol can modulate the expression of genes involved in lipid uptake and metabolism. The substitution of approximately 10% of membrane cholesterol by β-sitosterol reduces processing of α-secretase-mediated amyloid precursor protein (APP) in lipid rafts and

promotes the specific re-distribution of APP, which impedes its cleavage by α-secretase, in non-raft regions of hippocampal cells [172]. Studies of platelets, which contain more than 95% of circulating APP, have revealed that β-sitosterol displaces cholesterol from lipid rafts, inhibits high-cholesterol-induced platelet amyloid-β peptide efflux, and increases pathways (β- and γ-secretase activities) for APP processing [173]. β-Sitosterol also enhances mitochondrial function by promoting inner mitochondrial membrane fluidity in hippocampal cells [174]. These anti-amyloidogenic and brain ATP-preserving/boosting effects of β-sitosterol suggest its potential for use in the prevention and management of neurodegenerative diseases such as AD.

4.4. Erythrodiol, uvaol, oleanolic, and maslinic acids

Naturally occurring triterpenic compounds in the form of dialcohols (erythrodiol and uvaol) or acids (oleanolic and maslinic acids) are composed of 30-carbon atom skeletons structured as 5 6-membered rings. Erythrodiol and uvaol are 2 triterpenic compounds that are widely distributed in the plant kingdom, either in free form or esterified with FAs, whereas oleanolic acid and maslinic acid are found as free acids or as part of triterpenoid saponins. In the olive fruit, the methyl group at the 28-carbon atom of α- and β-amyrins can be substituted by a hydroxymethyl group to give uvaol and erythrodiol or by a carboxylic group to give ursolic and oleanolic acids. An additional hydroxyl group at the 2-carbon atom of oleanolic acid yields maslinic acid [175]. Therefore, uvaol (ursan-12-en-3β,28-diol) and ursolic acid (ursan-12-en-3β-ol-28-oic acid) have the carbon skeleton of ursane (derived from α-amyrin), whereas erythrodiol (olean-12-en-3β,28-diol), oleanolic acid (olean-12-en-3β-ol-28-oic acid), and maslinic acid

(olean-12-en-2α,3β-diol-28-oic acid) have the carbon skeleton of oleanane (derived from β-amyrin). Erythrodiol and uvaol account for approximately 60% and 40% of triterpenic dialcohols, respectively. Oleanolic and maslinic acids account for 98-99% of mono- and di-hydroxy pentacyclic triterpenic acids. The complete sequence of these reactions occurs in the green epicarp of the olive fruit as well as in the leaf and are significantly influenced by olive variety and the progression of olive fruit or leaf ontogeny [146, 147, 176, 177]. Together with sterols, erythrodiol and uvaol are relevant markers for authenticating the genuine virgin olive oil and detecting adulteration. The sum of erythrodiol and uvaol in virgin olive oil must be less than 4.5% of the total sterol content [149]. The concentrations of these non-steroidal triterpenic compounds in virgin olive oil are approximately 75 mg/kg erythrodiol, 20 mg/kg uvaol, 100 mg/kg oleanolic acid, 50 mg/kg maslinic acid, and 4 mg/kg ursolic acid [147, 178].

Pentacyclic triterpenic acids alter the membrane phase properties induced by cholesterol; the anisotropy and the nonuniformity of the bilayer environment also influence the relative position of pentacyclic triterpenic acids in synthetic model membranes [179]. Ursolic and maslinic acids partition into fluid lipid domains close to the membrane-water interface and compete with cholesterol to form hydrogen bonds with carbonyl oxygen atoms in neighboring lipid acyl chains. This partitioning induces the segregation of cholesterol within the bilayer and inverted micellar (H_{II} phase) structures. By contrast, oleanolic acid penetrates more deeply into membranes, may adopt a parallel stacking conformation [180], and acts to alter the presence of l_0 cholesterol-rich domains. In the membranes of human keratinocytes, oleanolic and ursolic acids are

integrated to a similar extent into lipid rafts, but only oleanolic acid has the ability to inhibit the release of ceramides in response to UVA radiation [181]. Structure-activity analysis indicates that chemical functions at key positions on ring E (a carboxylic group at the 17-carbon atom, which is near 2 methyl groups on the 20-carbon atom) are essential for enabling lipid raft stabilization by tight packing of oleanolic acid with sphingolipids in a manner resembling that of cholesterol. In agreement, the geminal methyl groups in the oleanane scaffold contribute differently to the efficiency of biological activities when compared to the vicinal methyl groups in the ursane scaffold [182]. Ceramides from plasma membrane-localized sphingomyelin immediately self-associate and form ceramide-enriched membrane domains [183] . This raft fusion not only facilitates cholesterol efflux and the replacement of cholesterol by ceramides in rafts [184] but also facilitates cell death pathways [185]. Substantial amounts of ceramide, either by *de novo* synthesis or via the action of acid or neutral sphingomyelinase, are present in isolated rafts/caveolae, even in stimulated cells [186]. Therefore, membrane ordering by oleanolic acid could be a mechanism for limiting the transverse motion of ceramide molecules as well as signaling that stems from these domains.

Pentacyclic triterpene dialcohols and acids have been shown to decrease *in vitro* LDL susceptibility to oxidation [187], *in vitro* membrane lipid peroxidation [188, 189], plasma lipid levels and blood pressure [190, 191] (by promoting nitric-oxide-dependent vasorelaxation [192, 193]) in experimental hypertension, and *ex vivo* secretion of pro-inflammatory cytokines in human peripheral blood mononuclear cells [194]. These molecules also have vasodepressor, cardiotonic,

and antidysrhythmic activity in animal models [195] and modulate prostaglandin I_2 synthesis in human coronary smooth muscle cells [196]. The potency in protecting LDLs against oxidation is as follows: maslinic acid > uvaol > erythrodiol [187]. Maslinic acid and uvaol block LDL binding sites prone to oxidative processes, but maslinic acid also scavenges free radicals. Uvaol and erythrodiol are the most effective in preventing LDL-supported thrombin generation. Oleanolic acid has protective effects against hepatic insulin resistance [197], inflammation [198, 199], and thrombosis [198] and is a potent endogenous agonist of the G-protein-coupled bile acid receptor TGR5 —a novel pharmacological target for the treatment of metabolic syndrome [200]— via activation of subunit $G\alpha_s$ in lipid rafts [201]. Recent evidence suggests a relevant role for oleanolic acid in the prevention of type 2 diabetes because the regular consumption of virgin olive oil enriched in oleanolic acid improved outcomes in prediabetic patients with impaired fasting glucose and impaired glucose tolerance [202]. In addition, erythrodiol, uvaol, oleanolic, and maslinic acids inhibit the growth of tumor cell lines from various human cancers, including colon, brain, and breast cancer [203-207]. Oleanolic acid can suppress migration and invasion [208] and potentiate the radiosensitivity [207] of tumor cells and enhance the activity of antitumor drugs [209]. Due to the high tumor uptake and tumor-specific targeting of oleanolic acid, new hybrid radiolabeled nanoparticles containing oleanolic acid have been experimentally used in an all-in-one multimodal imaging system for the diagnosis and therapeutic monitoring of tumors [210]. Erythrodiol and oleanolic acid are also effective against experimental autoimmune encephalomyelitis by restricting the infiltration of inflammatory cells into the central nervous system and preventing disruption of

the blood-brain barrier, suggesting their potential for the prevention and management of multiple sclerosis and other autoimmune and/or neuro-inflammatory diseases [211].

4.5. Secoiridoids, phenyl-alcohols, phenyl-acids, hydroxyisochromans, flavonoids, and lignans

Among secondary metabolites, phenolic compounds are the most widely distributed in plants, with more than 8000 phenolic structures known. Phenolic compounds are ubiquitous in all plant organs and are involved in the defense against UV radiation, extreme thermal conditions, and aggression by pathogens or predators, tolerance to water shortage, allelopathy, soil microbial activity, organic matter decomposition, and mineralization. Phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups.

The phenolic fraction in virgin olive oil (and olive leaf) contains at least 36 structurally different phenolic compounds, which can be divided into 6 major groups based on similarities in structure: secoiridoids, phenyl-alcohols, phenyl-acids, hydroxyisochromans, flavonoids, and lignans [212-215]. Different factors have an influence on the variability and concentration of phenolic compounds in virgin olive oil, which may exceed 1500 mg/kg, including the cultivar (variety and age) and region of olive tree growth, olive fruit maturation, and virgin olive oil processing [216, 217]. The secoiridoids are typical in the botanical family of *Oleaceae*, which includes *O. europaea* L (olive tree) and may account for more than 80-90% of the total phenolic compounds in virgin olive oil. The most abundant secoiridoids in virgin olive oil are the dialdehyde form of decarboxymethyl elenolic acid linked to hydroxytyrosol, the dialdehyde form of

decarboxymethyl elenolic acid linked to tyrosol, oleuropein aglycone (elenolic acid linked to hydroxytyrosol), ligustroside aglycone (elenolic acid linked to tyrosol), and hydroxytyrosol acetate (up to 600, 400, 300, 50, and 15 mg/kg, respectively). The predominant phenyl-alcohols in virgin olive oil are hydroxytyrosol (3,4-dihydroxyphenylethanol) and tyrosol (4-hydroxyphenylethanol) (up to 200 and 180 mg/kg, respectively). Hydroxycinnamic structures (which contain 9 carbon atoms, C6-C3) and hydrobenzoic structures (which contain 7 carbon atoms, C6-C1) comprise the phenyl-acids found in virgin olive oil, which include *p*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, gallic, gentisic, syringic, vanillic, protocathechuic, and *p*-hydroxybenzoic acids (up to 4 mg/kg per analyte). Two hydroxyisochromans formed by the reaction of hydroxytyrosol with benzaldehyde (1-phenyl-6,7-dihydroxyisochroman) or vanillin [1-(3-methoxy-4-hydroxy-phenyl)-6,7-dihydroxyisochroman] have also been identified in virgin olive oil (up to 0.4 and 1.5 mg/kg, respectively). The structure of flavonoids is based on a flavan nucleus consisting of 3 phenolic rings referred to as the A, B, and C rings. Variations in the heterocyclic C ring give rise to the main groups of flavonoids: flavones, flavanols, flavonols, flavanones, isoflavones, and anthocyanins. The major flavonoids in virgin olive oil are the flavones luteolin and apigenin (up to 10 and 3 mg/kg, respectively) and the flavonol quercetin (up to 1 mg/kg). (+)-Pinoresinol, 1-(+)-acetoxypinoresinol, and 1-(+)-hydroxypinoresinol are the most common lignans in virgin olive oil (up to 180, 80, and 20 mg/kg, respectively). The phenolic fraction plays a significant role in the sensory properties (bitterness, pungency, and astringency) of virgin olive oil and in the protection and stability of virgin olive oil against oxidation. Secoiridoids may

undergo hydrolysis under gastric conditions, resulting in significant increases in the amounts of hydroxytyrosol and tyrosol [13, 218] and, to a lesser extent, oleuropein and oleuropein aglycone [219, 220] entering the small intestine. Secoiridoids exhibit an absorption efficiency of approximately 55 to 66% and an excretion rate of only 5 to 16% of the total amount ingested [221]. A wide range of different phenolic compounds has been detected in multiple animal tissues, including brain tissue, after a single ingestion of the phenolic compounds found in virgin olive oil [222].

Studies of unilamellar membranes have revealed that phenolic compounds (the dialdehyde form of decarboxymethyl elenolic acid linked to hydroxytyrosol, oleuropein aglycone, oleuropein, hydroxytyrosol, and hydroxytyrosol acetate) remain at the lipid membrane surface area, almost parallel to the bilayer, which they do not penetrate [223]. This is most likely due to the hydrophilic properties and the conformational mobility of phenolic non-planar structures, in contrast to hydroxyisochromans with planar and lipophilic phenyl substituents, which favors the deeper membrane localization of the phenolic molecule [224]. In phospholipid/water systems, oleuropein exhibits a high affinity for phospholipid acyl chains, promoting no major changes in the gel-to-Lα phase transition and a tendency to form inverted H_{II} phase structures in zwitterionic phospholipid-containing membranes but inducing dramatic perturbations at the surface of membranes formed with negatively charged phospholipids [225]. Similar membrane localization and interaction, with most of the molecule exposed to the aqueous phase, have been reported for flavonoids, including luteolin [226] and quercetin [227]. Incorporation of phenolic compounds into the membrane may

induce a deformation of the membrane surface toward a negative membrane curvature. Therefore, the accommodation of lipid membrane dynamics by phenolic compounds dictates the ability of phenolic compounds to delay lipid peroxidation by trapping peroxyl radicals from the aqueous phase and to prevent the propagation cycle of lipid peroxidation from the interface to the inner membrane. This response may be heightened by cross-talk with hydrophilic, amphipathic, and/or hydrophobic natural antioxidants, including other phenolic compounds. The combination of phenolic compounds with ascorbate or α-tocopherol has an additive or synergistic effect against oxidative stress on membranes [223]. Phenolic compounds also contribute to the regeneration and conservation of α-tocopherol molecules [228, 229]. Oleuropein aglycone, oleuropein, and/or hydroxytyrosol confer antioxidant protection against oxidative injury in membranous systems, including human red blood cells [230], liver cells [231], and retinal pigment epithelial cells [232]. The antioxidant mechanisms of phenolic compounds include reduction of oxygen concentration, termination of free radicals, decomposition of primary products of oxidation to non-radical species, and prevention of continuous hydrogen abstraction from substrates and chelators of metal ions [233]. Independent of redox-sensitive processes, phenolic compounds may exert additional biological activities at the membrane level. For example, oleuropein may entropically interfere with the *in vitro* aggregation of islet amyloid polypeptide (amylin) in the membrane of human pancreatic β-cells, thereby avoiding the toxic effects of amylin aggregates, which often involve nonspecific membrane permeabilization and destruction of pancreatic β-cells [234, 235]. The pore-forming effect of α-synuclein oligomers on synthetic membrane vesicles can be similarly prevented

by apigenin, which contains a critical dihydroxyphenyl ring structure, alone or as part of the flavone scaffold [236]. *In vivo*, oleuropein also plays a role in blocking the membrane stressor activity of amyloid-β peptide aggregates in an invertebrate model of AD and musculoskeletal degenerative diseases such as inclusion body myositis [237]. Studies of the interaction of phenolic compounds with integral membrane proteins indicate that hydroxytyrosol may accelerate the oscillation of the norepinephrine transporter between the outward-facing mode (accessible to the extracellular medium) and the inward-facing mode (open to the intracellular milieu) in an *in vitro* model of differentiated sympathetic neurons [238].

Prospective epidemiological studies, some randomized prevention trials, and many short-term studies of intermediate endpoints suggest that the intake of virgin olive oil phenolic compounds —in combination with oleic acid— may protect against cardiovascular diseases [3, 14, 239-243] and different types of cancer [13, 216, 240, 241, 244, 245] via synergistic and overlapping protective antioxidant and anti-inflammatory effects as well as other effects yet to be identified. The plasma incorporation of phenolic compounds following virgin olive oil intake has been shown to improve antioxidant status in elderly people [246] and endothelial dysfunction in patients with early atherosclerosis [247]. *In vitro*, phenolic compounds can regulate genes involved in nitric oxide metabolism, proteolysis of connective tissues, differentiation coupled to membrane-bound oxidases, and nuclear factor-κB (NF-κB) signaling [248, 249]. The cooperation of hydroxytyrosol + tyrosol or *p*-coumaric + caffeic acid diminishes homocysteine-induced endothelial dysfunction by inhibiting

adhesion molecules in a redox-independent manner [250], whereas the cooperation of hydroxytyrosol + caffeic acid protects DNA from oxidative damage while exerting an antiproliferative effect on tumor cells [251]. Similar cellular effects, including reduced reactive oxygen species (ROS) and reduced proliferation, have been reported for caffeic, syringic, and protocathechuic acids [252]. Recent evidence also suggests that luteolin inhibits human prostate tumor growth by suppressing vascular endothelial growth factor 2-mediated angiogenesis [253] and apoptosis [254]. Other *in vitro* anticancer effects of luteolin include irreversible changes in mitochondrial membrane structure/function [255] and the reversal of the epithelial-mesenchymal transition [134], which may act in concert with the ability of luteolin to enhance the activity of antitumor drugs [256]. In addition, ferulic acid potentiates the radiosensitivity of human cervical carcinoma cells [257]. Secoiridoids, flavonoids, and lignans strongly inhibit the overexpression of fatty acid synthase [258] and the proto-oncogene HER2 (human epidermal growth factor receptor 2) [259] in tumor cells. Hydroxytyrosol, tyrosol, caffeic acid, and (+)-pinoresinol exert anticancer effects by inhibiting invasion [260] and selectively activating the ataxia telangiectasia mutated-p53 cascade [261]. Additional novel health effects and mechanisms of phenolic compounds found in virgin olive oil are rapidly being identified. *In vitro*, hydroxytyrosol, tyrosol, 1-(+)-acetoxypinoresinol, oleuropein aglycones, and notably, the dialdehyde form of decarboxymethyl elenolic acid linked to tyrosol have strong activity against *Helicobacter pylori* [262]. These results are in agreement with the observation that the administration of virgin olive oil rich in phenolic compounds is moderately effective in eradicating *H. pylori* in patients with *H. pylori*-positive status [263].

This suggests a potential role for these phenolic compounds as chemo-preventative agents for peptic or gastric ulcers. Oleuropein operates at a dose one order of magnitude lower than ranitidine in experimental injury of the gastric mucosa, improving the total antioxidant capacity and cell membrane integrity [264]. Hydroxytyrosol has antifungal activity [265], and luteolin exerts antibacterial and synergistic activity by altering outer and inner membrane permeability in amoxicillin-resistant *Escherichia coli* [266]. Oleuropein has lipid-lowering effects and confers protection against SFA (palmitic acid)-induced hepatocellular steatosis [267]. The supplementation of diets with hydroxytyrosol improves the inflammatory response in experimental models of chronic colonic inflammation with clinical and histological similarity to human ulcerative colitis [268]. Tyrosol has a protective effect against mitochondrial dysfunction-induced dopaminergic neuronal death via activation of the phosphoinositide 3-kinase (PI3K)/PKB signaling pathway, along with up-regulation of antioxidant enzymes in an *in vitro* model of AD [269]. In mitochondria isolated from the hippocampus, cortex, and striatum of transgenic mice doubly expressing amyloid-β precursor protein and presenilin 1, luteolin nearly completely restores mitochondrial function by a mechanism associated with ROS scavenging and/or stabilization of the electron transport chain [270]. Luteolin also confers protection against ER stress-induced neuronal cell death in the hippocampus in an experimental corticosterone-treated depression model [271]. Phenolic acids and flavonoids are active in the prevention or delay of cataract development [272]. Luteolin inhibits NF-κB binding activity and the activator-protein-1 pathway as well as their downstream target genes in gestational tissues, reducing the secretion of pro-inflammatory cytokines, prostaglandins, and metalloproteinases in fetal

membranes, amnion, and myometrial cells exposed to pro-inflammatory stress [273], indicating a potential therapeutic use of luteolin in pre-term birth. The plethora of beneficial effects associated with the phenolic compounds described above supports the potential of virgin olive oil in treating metabolic syndrome, as well as infectious, neurodegenerative, and autoimmune diseases, all of which are reaching pandemic levels and increasingly affecting human life. In 2011, following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority concluded that a cause and effect relationship had been established between the consumption of virgin olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives) and the protection of LDL particles from oxidative damage [274]. There is the hope that new health claims related to virgin olive oil consumption will be recognized in the near future.

4.6. Tocopherols and tocotrienols

Essential in the human diet, vitamin E is a general term that is used to describe a family of 8 different, naturally occurring forms: 4 tocopherols (α-, β-, γ-, and δ-tocopherols) and 4 tocotrienols (α-, β-, γ-, and δ-tocotrienols). A chromanol ring bearing a phenolic group and a lipophilic side chain are the functional domains of vitamin E [275, 276]. The number of methyl groups attached at the 5-carbon atom and/or 7-carbon atom on the chromanol ring, in addition to a common methyl group at the 8-carbon atom, designates each form. The α form is the most highly methylated, with a maximum of 3 methyl groups on the chromanol ring, compared with the β and γ forms, which have 2 methyl groups (the common methyl group and one at the 5-carbon atom or 7-carbon atom, respectively), and

the δ form, which contains only the common methyl group on the chromanol ring. Structurally, tocopherols and tocotrienols differ in the branched side chain at the 2-carbon atom, which is a saturated isoprenoid C_{16} side chain in tocopherols and a farnesyl isoprenoid side chain with *trans* double bonds at the 3'-, 7'-, and 11'-carbon atoms in tocotrienols. α-Tocopherol is the most biologically active form of vitamin E and accounts for nearby 95% of the total vitamin E found in virgin olive oil (up to 300 mg/kg). The concentrations of the different forms of vitamin E in virgin olive oil are approximately 300 mg/kg α-tocopherol, 3 mg/kg α-tocotrienol, 4 mg/kg β-tocopherol, 1 mg/kg β-tocotrienol, 2 mg/kg δ-tocopherol, 12 mg/kg γ-tocopherol, and 5 mg/kg γ-tocotrienol. Different factors influence the variability and concentration of tocopherols in virgin olive oil, including the cultivar (variety and age) and region of olive tree growth, olive fruit maturation, harvesting and processing conditions, and virgin olive oil storage conditions [277-284]. These natural antioxidants not only provide nutritional value to virgin olive oil but also contribute to its stability with respect to oxidation, even during heating at frying temperature, thereby preserving its quality.

Among the members of the vitamin E family of compounds, α-tocopherol is selectively enriched in the plasma and tissues via independent activities of a hepatic α-tocopherol transfer protein that retains α-tocopherol, as well as the catabolizing cytochrome P-450 system, which preferentially degrades all other forms of vitamin E [285]. The lipophilic side chain enables the anchoring and penetration of tocopherols and tocotrienols into biological membranes, where

they function as chain-terminating hydrogen atom donors to lipid peroxy radicals [286-289]. Tocopherols and tocotrienols are inserted into one leaflet of the lipid bilayer, parallel to membrane phospholipid chains with the active hydroxyl group at the lipid-water interface, and exert mainly the same mobility [290]. They show preferential affinity for and co-localize with PUFAs and partition into non-raft domains rich in PUFAs [288]. This orientation and localization influence membrane conformational dynamics; in some cases, tocopherols modulate the structure of unsaturated phospholipid bilayers in a similar manner as cholesterol in lipid rafts [291]. At membrane tocopherol concentrations above 10 mol%, the main transition temperature decreases continuously in a concentration-dependent manner, except for α- and δ-tocopherols, which stabilize a modulated Pβ phase (in between the gel-to-Lα phase transition). γ-Tocopherol fluidizes the bilayer more efficiently than α- and δ-tocopherols but not as efficiently as cholesterol. At a narrow composition range between 7.5 and 10 mol% and coupled to the bilayer curvature, tocopherols favor the asymmetric ripple Pβ' phase, which is characterized by corrugations of the membrane surface with well-defined periodicity and a bilayer thickness that is different in the 2 arms of the ripple and increases with increasing tocopherol concentration in the membrane. By contrast, tocotrienols are more readily transferred to membranes and cause more minor ordering disturbances than tocopherols. This intricate arrangement of tocopherols and tocotrienols in membranes optimizes protection against peroxy-radicals created within PUFA chains as well as the regeneration of tocopheroxyl/tocotrienoxyl radicals by water-soluble reducing agents such as ascorbate and phenolic

compounds; such arrangement has membrane-stabilizing and reparative effects [292].

Evidence suggests that corresponding tocopherols and tocotrienols display similar potency for radical scavenging in homogenous solution systems and liposomal membranes [293, 294]. The hydrophobic prenyl chain in the different vitamin E forms appears not to significantly affect hydrogen donor propensities, which increase with increasing methylation degree of the chromanol ring ($\alpha > \beta = \gamma > \delta$). Thus, a high plasma concentration of vitamin E, implying a high total plasma antioxidant capacity, has been associated with lower risks of cardiovascular disease and some types of cancer [275, 295, 296]. Vitamin E has also been shown to play a critical role in central nervous system health. The therapeutic benefit of vitamin E is related to the prevention and management of neurodegenerative disorders such as AD and Parkinson's disease [297, 298]. Moreover, increasing evidence suggests novel mechanisms of action for vitamin E independent of antioxidant activity and a different spectrum of biological activities for tocopherols and tocotrienols [299, 300].

5. Conclusions and future directions

The role of evolution in creating lipid bilayers to allow for the interaction of cells with the environment is interesting. This review provides compelling evidence regarding how oleic acid and the minor constituents of virgin olive oil interact with and regulate the functions of membranes. Oleic acid can be efficiently incorporated into phospholipids, forming the core of the lipid bilayer and regulating the activity of integral or peripheral membrane proteins. Minor

constituents exhibit a wide range of lipophilicity, which causes preferential localization of these constituents within the membrane or on the bilayer/water interface (Fig. 5). Despite the low concentrations of some minor constituents of virgin olive oil, their spatial confinement in the membrane may greatly enhance their local concentration, synergy, and co-localization in relevant regions or microdomains. However, further work needs to be done to confidently establish that oleic acid and the minor constituents of virgin olive oil are absorbed, metabolized, and directed to target membranes while retaining their therapeutic benefits, as well as to gain mechanistic insights into the multilateral and cooperative actions of these bioactive entities in membrane lipid and protein dynamics in health and disease.

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7. References

[1] G. Buckland, N. Travier, A. Agudo, A. Fonseca-Nunes, C. Navarro, P. Lagiou, C. Demetriou, P. Amiano, M. Dorronsoro, M.D. Chirlaque, J.M. Huerta, E. Molina, M.J. Perez, E. Ardanaz, C. Moreno-Iribas, J.R. Quiros, A. Naska, D. Trichopoulos, M.C. Giurdanella, R. Tumino, C. Agnoli, S. Grioni, S. Panico, A. Mattiello, G. Masala, C.

Sacerdote, S. Polidoro, D. Palli, A. Trichopoulou, C.A. Gonzalez, Olive oil intake and breast cancer risk in the Mediterranean countries of the European Prospective Investigation into Cancer and Nutrition study, Int. J. Cancer 131 (2012) 2465-2469.

[2] G. Buckland, N. Travier, A. Barricarte, E. Ardanaz, C. Moreno-Iribas, M.J. Sanchez, E. Molina-Montes, M.D. Chirlaque, J.M. Huerta, C. Navarro, M.L. Redondo, P. Amiano, M. Dorronsoro, N. Larranaga, C.A. Gonzalez, Olive oil intake and CHD in the European Prospective Investigation into Cancer and Nutrition Spanish cohort, Br. J. Nutr. 108 (2012) 2075-2082.

[3] S. Lopez, B. Bermudez, L.M. Varela, A. Ortega, S. Jaramillo, R. Abia, F.J. Muriana, Olives and olive oil: Diet and health impacts, CAB Rev. 7 (2012) 1-10.

[4] J.M. Lou-Bonafonte, C. Arnal, M.A. Navarro, J. Osada, Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development, Mol. Nutr. Food Res. 56 (2012) 1043-1057.

[5] E. Ros, Olive oil and CVD: accruing evidence of a protective effect, Br. J. Nutr. 108 (2012) 1931-1933.

[6] R. Estruch, E. Ros, J. Salas-Salvado, M.I. Covas, D. Corella, F. Aros, E. Gomez-Gracia, V. Ruiz-Gutierrez, M. Fiol, J. Lapetra, R.M. Lamuela-Raventos, L. Serra-Majem, X. Pinto, J. Basora, M.A. Munoz, J.V. Sorli, J.A. Martinez, M.A. Martinez-Gonzalez, P.S. Investigators, Primary prevention of cardiovascular disease with a Mediterranean diet, N. Engl. J. Med. 368 (2013) 1279-1290.

[7] C. Feart, C. Samieri, B. Alles, P. Barberger-Gateau, Potential benefits of adherence to the mediterranean diet on cognitive health, Proc. Nutr. Soc. 72 (2013) 140-152.

[8] A. Giacosa, R. Barale, L. Bavaresco, P. Gatenby, V. Gerbi, J. Janssens, B. Johnston, K. Kas, C. La Vecchia, P. Mainguet, P. Morazzoni, E. Negri, C. Pelucchi, M. Pezzotti, M. Rondanelli, Cancer prevention in Europe: the Mediterranean diet as a protective choice, Eur. J. Cancer Prev. 22 (2013) 90-95.

[9] B. Bermudez, S. Lopez, A. Ortega, L.M. Varela, Y.M. Pacheco, R. Abia, F.J. Muriana, Oleic acid in olive oil: from a metabolic framework toward a clinical perspective, Curr. Pharm. Des. 17 (2011) 831-843.

[10] International Olive Oil Council, http://www.internationaloliveoil.org/estaticos/view/131-world-olive-oil-figures

[11] C.A. de la Lastra, An up-date of olive oil and bioactive constituents in health: molecular mechanisms and clinical implications, Curr. Pharm. Des. 17 (2011) 752-753.

[12] A. Ortega, L.M. Varela, B. Bermudez, S. Lopez, R. Abia, F.J. Muriana, Dietary fatty acids linking postprandial metabolic response and chronic diseases, Food Funct. 3 (2012) 22-27.

[13] A. Cardeno, M. Sanchez-Hidalgo, C.A. de la Lastra, An up-date of olive oil phenols in inflammation and cancer: molecular mechanisms and clinical implications, Curr. Med. Chem. 20 (2013) 4758-4776.

[14] S. Martin-Pelaez, M.I. Covas, M. Fito, A. Kusar, I. Pravst, Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims, Mol. Nutr. Food Res. 57 (2013) 760-771.

[15] H. Sales-Campos, P.R. Souza, B.C. Peghini, J.S. da Silva, C.R. Cardoso, An overview of the modulatory effects of oleic acid in health and disease, Mini Rev. Med. Chem. 13 (2013) 201-210.

[16] P. Inglese, F. Famiani, F. Galvano, M. Servili, S. Esposto, S. Urbani, Factors affecting extra-virgin olive oil composition, John Wiley & Sons Inc, 2010.

[17] T.A. Lagace, N.D. Ridgway, The role of phospholipids in the biological activity and structure of the endoplasmic reticulum, Biochim. Biophys. Acta 1833 (2013) 2499-2510.

[18] L. Hodson, B.A. Fielding, Stearoyl-CoA desaturase: rogue or innocent bystander?, Prog. Lipid Res. 52 (2013) 15-42.

[19] A. Kotronen, T. Seppanen-Laakso, J. Westerbacka, T. Kiviluoto, J. Arola, A.L. Ruskeepaa, H. Yki-Jarvinen, M. Oresic, Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum, Obesity 18 (2010) 937-944.

[20] A. Andersson, C. Nalsen, S. Tengblad, B. Vessby, Fatty acid composition of skeletal muscle reflects dietary fat composition in humans, Am. J. Clin. Nutr. 76 (2002) 1222-1229.

[21] G. Rocquelin, L. Guenot, P.O. Astorg, M. David, Phospholipid content and fatty acid composition of human heart, Lipids 24 (1989) 775-780.

[22] G. Bahrami, M. Masoumi, Z. Rahimi, Co-existence of fatty acids changes in aorta artery and adipose tissue; comparison between CAD and non CAD patients, J. Thromb. Thrombolysis 27 (2009) 185-190.

[23] T. Fraser, H. Tayler, S. Love, Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease, Neurochem. Res. 35 (2010) 503-513.

[24] J. Guest, M. Garg, A. Bilgin, R. Grant, Relationship between central and peripheral fatty acids in humans, Lipids Health Dis. 12 (2013) 79.

[25] Y. Min, C. Lowy, S. Islam, F.S. Khan, R. Swaminathan, Relationship between red cell membrane fatty acids and adipokines in individuals with varying insulin sensitivity, Eur. J. Clin. Nutr. 65 (2011) 690-695.

[26] E. Frankel, A. Bakhouche, J. Lozano-Sanchez, A. Segura-Carretero, A. Fernandez-Gutierrez, Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of byproducts as alternative sources of polyphenols, J. Agric. Food Chem. 61 (2013) 5179-5188.

[27] V.A. Ngo, R.K. Kalia, A. Nakano, P. Vashishta, Molecular mechanism of flip-flop in triple-layer oleic-acid membrane: correlation between oleic acid and water, J. Phys. Chem. B 116 (2012) 13416-13423.

[28] Y.M. Pacheco, R. Abia, J.S. Perona, M. Reina, V. Ruiz-Gutierrez, E. Montero, F.J. Muriana, Triacylglycerol-rich lipoproteins interact with human vascular cells in a lipid-dependent fashion, J. Agric. Food Chem. 49 (2001) 5653-5661.

[29] L.M. Varela, A. Ortega, B. Bermudez, S. Lopez, Y.M. Pacheco, J. Villar, R. Abia, F.J. Muriana, A high-fat meal promotes lipid-load and apolipoprotein B-48 receptor transcriptional activity in circulating monocytes, Am. J. Clin. Nutr. 93 (2011) 918-925.

[30] B. Bermudez, S. Lopez, L.M. Varela, A. Ortega, Y.M. Pacheco, W. Moreda, R. Moreno-Luna, R. Abia, F.J. Muriana, Triglyceride-rich lipoprotein regulates APOB48 receptor gene expression in human THP-1 monocytes and macrophages, J. Nutr. 142 (2012) 227-232.

[31] L.M. Varela, A. Ortega-Gomez, S. Lopez, R. Abia, F.J. Muriana, B. Bermudez, The effects of dietary fatty acids on the postprandial triglyceride-rich lipoprotein/apoB48 receptor axis in human monocyte/macrophage cells, J. Nutr. Biochem. 24 (2013) 2031-2039.

[32] V. Ruiz-Gutierrez, F.J. Muriana, A. Guerrero, A.M. Cert, J. Villar, Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources, J. Hypertens. 14 (1996) 1483-1490.

[33] R. Alemany, J.S. Perona, J.M. Sanchez-Dominguez, E. Montero, J. Canizares, R. Bressani, P.V. Escriba, V. Ruiz-Gutierrez, G protein-coupled receptor systems and their lipid environment in health disorders during aging, Biochim. Biophys. Acta 1768 (2007) 964-975.

[34] E. Escrich, M. Solanas, R. Moral, R. Escrich, Modulatory effects and molecular mechanisms of olive oil and other dietary lipids in breast cancer, Curr. Pharm. Des. 17 (2011) 813-830.

[35] P.V. Escriba, J.M. Gonzalez-Ros, F.M. Goni, P.K. Kinnunen, L. Vigh, L. Sanchez-Magraner, A.M. Fernandez, X. Busquets, I. Horvath, G. Barcelo-Coblijn, Membranes: a meeting point for lipids, proteins and therapies, J. Cell. Mol. Med. 12 (2008) 829-875.

[36] A. Nohturfft, S.C. Zhang, Coordination of lipid metabolism in membrane biogenesis, Annu. Rev. Cell. Dev. Biol. 25 (2009) 539-566.

[37] P.V. Escriba, Membrane-lipid therapy: a new approach in molecular medicine, Trends Mol. Med. 12 (2006) 34-43.

[38] S.S. Funari, F. Barcelo, P.V. Escriba, Effects of oleic acid and its congeners, elaidic and stearic acids, on the structural properties of phosphatidylethanolamine membranes, J. Lipid Res. 44 (2003) 567-575.

[39] J. Prades, S.S. Funari, P.V. Escriba, F. Barcelo, Effects of unsaturated fatty acids and triacylglycerols on phosphatidylethanolamine membrane structure, J. Lipid Res. 44 (2003) 1720-1727.

[40] T.N. Engelbrecht, A. Schroeter, T. Hauss, R.H. Neubert, Lipophilic penetration enhancers and their impact to the bilayer structure of stratum corneum lipid model membranes: neutron diffraction studies based on the example oleic acid, Biochim. Biophys. Acta 1808 (2011) 2798-2806.
[41] S. Leekumjorn, H.J. Cho, Y. Wu, N.T. Wright, A.K. Sum, C. Chan, The role of fatty acid unsaturation in minimizing biophysical changes on the structure and local effects of bilayer membranes, Biochim. Biophys. Acta 1788 (2009) 1508-1516.
[42] R.N. Weijers, Lipid composition of cell membranes and its relevance in type 2 diabetes mellitus, Curr. Diabetes Rev. 8 (2012) 390-400.
[43] M. Ibarguren, D.J. Lopez, J.A. Encinar, J.M. Gonzalez-Ros, X. Busquets, P.V. Escriba, Partitioning of liquid-ordered/liquid-disordered membrane microdomains induced by the fluidifying effect of 2-hydroxylated fatty acid derivatives, Biochim. Biophys. Acta 1828 (2013) 2553-2563.
[44] J. Prades, S.S. Funari, M. Gomez-Florit, O. Vogler, F. Barcelo, Effect of a 2-hydroxylated fatty acid on cholesterol-rich membrane domains, Mol. Membr. Biol. 29 (2012) 333-343.
[45] S.R. Shaikh, A.C. Dumaual, A. Castillo, D. LoCascio, R.A. Siddiqui, W. Stillwell, S.R. Wassall, Oleic and docosahexaenoic acid differentially phase separate from lipid raft molecules: a comparative NMR, DSC, AFM, and detergent extraction study, Biophys. J. 87 (2004) 1752-1766.
[46] J. Ye, R.A. DeBose-Boyd, Regulation of cholesterol and fatty acid synthesis, Cold Spring Harb. Perspect. Biol. 3 (2011) a004754.

[47] J.N. Lee, X. Zhang, J.D. Feramisco, Y. Gong, J. Ye, Unsaturated fatty acids inhibit proteasomal degradation of Insig-1 at a postubiquitination step, J. Biol. Chem. 283 (2008) 33772-33783.

[48] Y. Gong, J.N. Lee, P.C. Lee, J.L. Goldstein, M.S. Brown, J. Ye, Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake, Cell Metab. 3 (2006) 15-24.

[49] X.Y. Dong, S.Q. Tang, Insulin-induced gene: a new regulator in lipid metabolism, Peptides 31 (2010) 2145-2150.

[50] R. Ehehalt, R. Sparla, H. Kulaksiz, T. Herrmann, J. Fullekrug, W. Stremmel, Uptake of long chain fatty acids is regulated by dynamic interaction of FAT/CD36 with cholesterol/sphingolipid enriched microdomains (lipid rafts), BMC Cell Biol. 9 (2008) 45.

[51] E.J. Grasso, M.B. Scalambro, R.O. Calderon, Differential response of the urothelial V-ATPase activity to the lipid environment, Cell Biochem. Biophys. 61 (2011) 157-168.

[52] M.C. Berman, Slippage and uncoupling in P-type cation pumps; implications for energy transduction mechanisms and regulation of metabolism, Biochim. Biophys. Acta 1513 (2001) 95-121.

[53] O. Vogler, J.M. Barcelo, C. Ribas, P.V. Escriba, Membrane interactions of G proteins and other related proteins, Biochim. Biophys. Acta 1778 (2008) 1640-1652.

[54] A. Krishnan, M.S. Almen, R. Fredriksson, H.B. Schioth, The origin of GPCRs: identification of mammalian like rhodopsin, adhesion, glutamate and frizzled GPCRs in fungi, PLoS One 7 (2012) e29817.

[55] A.J. Venkatakrishnan, X. Deupi, G. Lebon, C.G. Tate, G.F. Schertler, M.M. Babu, Molecular signatures of G-protein-coupled receptors, Nature 494 (2013) 185-194.

[56] O. Vogler, J. Casas, D. Capo, T. Nagy, G. Borchert, G. Martorell, P.V. Escriba, The Gbetagamma dimer drives the interaction of heterotrimeric Gi proteins with nonlamellar membrane structures, J. Biol. Chem. 279 (2004) 36540-36545.

[57] V.D. Dixit, E.M. Schaffer, R.S. Pyle, G.D. Collins, S.K. Sakthivel, R. Palaniappan, J.W. Lillard, Jr., D.D. Taub, Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells, J. Clin. Invest. 114 (2004) 57-66.

[58] A.D. Howard, S.D. Feighner, D.F. Cully, J.P. Arena, P.A. Liberator, C.I. Rosenblum, M. Hamelin, D.L. Hreniuk, O.C. Palyha, J. Anderson, P.S. Paress, C. Diaz, M. Chou, K.K. Liu, K.K. McKee, S.S. Pong, L.Y. Chaung, A. Elbrecht, M. Dashkevicz, R. Heavens, M. Rigby, D.J. Sirinathsinghji, D.C. Dean, D.G. Melillo, A.A. Patchett, R. Nargund, P.R. Griffin, J.A. DeMartino, S.K. Gupta, J.M. Schaeffer, R.G. Smith, L.H. Van der Ploeg, A receptor in pituitary and hypothalamus that functions in growth hormone release, Science 273 (1996) 974-977.

[59] P.J. Delhanty, A. van Kerkwijk, M. Huisman, B. van de Zande, M. Verhoef-Post, C. Gauna, L. Hofland, A.P. Themmen, A.J. van der Lely, Unsaturated fatty acids prevent desensitization of the human growth hormone secretagogue receptor by blocking its internalization, Am. J. Physiol. Endocrinol. Metab. 299 (2010) E497-505.

[60] G.L. Alberts, C.L. Chio, W.B. Im, Allosteric modulation of the human 5-HT(7A) receptor by lipidic amphipathic compounds, Mol. Pharmacol. 60 (2001) 1349-1355.

[61] S. Teres, G. Barcelo-Coblijn, M. Benet, R. Alvarez, R. Bressani, J.E. Halver, P.V. Escriba, Oleic acid content is responsible for the reduction in blood pressure induced by olive oil, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 13811-13816.

[62] R. Alemany, S. Teres, C. Baamonde, M. Benet, O. Vogler, P.V. Escriba, 2-hydroxyoleic acid: a new hypotensive molecule, Hypertension 43 (2004) 249-254.

[63] Q. Yang, R. Alemany, J. Casas, K. Kitajka, S.M. Lanier, P.V. Escriba, Influence of the membrane lipid structure on signal processing via G protein-coupled receptors, Mol. Pharmacol. 68 (2005) 210-217.

[64] R. Alemany, O. Vogler, S. Teres, C. Egea, C. Baamonde, F. Barcelo, C. Delgado, K.H. Jakobs, P.V. Escriba, Antihypertensive action of 2-hydroxyoleic acid in SHRs via modulation of the protein kinase A pathway and Rho kinase, J. Lipid Res. 47 (2006) 1762-1770.

[65] P.V. Escriba, J.M. Sanchez-Dominguez, R. Alemany, J.S. Perona, V. Ruiz-Gutierrez, Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives, Hypertension 41 (2003) 176-182.

[66] L.A. Ferrara, A.S. Raimondi, L. d'Episcopo, L. Guida, A. Dello Russo, T. Marotta, Olive oil and reduced need for antihypertensive medications, Arch. Intern. Med. 160 (2000) 837-842.

[67] M. Mueckler, B. Thorens, The SLC2 (GLUT) family of membrane transporters, Mol. Aspects Med. 34 (2013) 121-138.

[68] Y. Tsuchiya, H. Hatakeyama, N. Emoto, F. Wagatsuma, S. Matsushita, M. Kanzaki, Palmitate-induced down-regulation of sortilin and impaired GLUT4 trafficking in C2C12 myotubes, J. Biol. Chem. 285 (2010) 34371-34381.

[69] S. Lopez, B. Bermudez, Y.M. Pacheco, J. Villar, R. Abia, F.J. Muriana, Distinctive postprandial modulation of beta cell function and insulin sensitivity by dietary fats: monounsaturated compared with saturated fatty acids, Am. J. Clin. Nutr. 88 (2008) 638-644.

[70] S. Lopez, B. Bermudez, A. Ortega, L.M. Varela, Y.M. Pacheco, J. Villar, R. Abia, F.J. Muriana, Effects of meals rich in either monounsaturated or saturated fat on lipid concentrations and on insulin secretion and action in subjects with high fasting triglyceride concentrations, Am. J. Clin. Nutr. 93 (2011) 494-499.

[71] A. Chabowski, M. Zendzian-Piotrowska, A. Nawrocki, J. Gorski, Not only accumulation, but also saturation status of intramuscular lipids is significantly affected by PPARgamma activation, Acta Physiol. 205 (2012) 145-158.

[72] J. Hwang, Y.H. Chang, J.H. Park, S.Y. Kim, H. Chung, E. Shim, H.J. Hwang, Dietary saturated and monounsaturated fats protect against acute acetaminophen hepatotoxicity by altering fatty acid composition of liver microsomal membrane in rats, Lipids Health Dis. 10 (2011) 184.

[73] R.I. Bello, C. Gomez-Diaz, M.I. Buron, P. Navas, J.M. Villalba, Differential regulation of hepatic apoptotic pathways by dietary olive and sunflower oils in the aging rat, Exp. Gerontol. 41 (2006) 1174-1184.

[74] V. Martin, N. Fabelo, G. Santpere, B. Puig, R. Marin, I. Ferrer, M. Diaz, Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex, J. Alzheimer's Dis. 19 (2010) 489-502.

[75] E. Evangelisti, D. Wright, M. Zampagni, R. Cascella, C. Fiorillo, S. Bagnoli, A. Relini, D. Nichino, T. Scartabelli, B. Nacmias, S. Sorbi, C. Cecchi, Lipid rafts mediate amyloid-induced calcium dyshomeostasis and oxidative stress in Alzheimer's disease, Curr. Alzheimer Res. 10 (2013) 143-153.

[76] B.P. Head, H.H. Patel, P.A. Insel, Interaction of membrane/lipid rafts with the cytoskeleton: Impact on signaling and function: Membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling, Biochim. Biophys. Acta (2013) doi: 10.1016/j.bbamem.2013.07.018 [Epub ahead of print].

[77] A.M. Sebastiao, M. Colino-Oliveira, N. Assaife-Lopes, R.B. Dias, J.A. Ribeiro, Lipid rafts, synaptic transmission and plasticity: impact in age-related neurodegenerative diseases, Neuropharmacology 64 (2013) 97-107.

[78] A. Tabernero, E.M. Lavado, B. Granda, A. Velasco, J.M. Medina, Neuronal differentiation is triggered by oleic acid synthesized and released by astrocytes, J. Neurochem. 79 (2001) 606-616.

[79] R.A. Rodriguez-Rodriguez, A. Tabernero, A. Velasco, E.M. Lavado, J.M. Medina, The neurotrophic effect of oleic acid includes dendritic differentiation and the expression of the neuronal basic helix-loop-helix transcription factor NeuroD2, J. Neurochem. 88 (2004) 1041-1051.

[80] A. Bento-Abreu, A. Tabernero, J.M. Medina, Peroxisome proliferator-activated receptor-alpha is required for the neurotrophic effect of oleic acid in neurons, J. Neurochem. 103 (2007) 871-881.

[81] X. Yang, S. Askarova, J.C. Lee, Membrane biophysics and mechanics in Alzheimer's disease, Mol. Neurobiol. 41 (2010) 138-148.

[82] Z. Amtul, D. Westaway, D.F. Cechetto, R.F. Rozmahel, Oleic acid ameliorates amyloidosis in cellular and mouse models of Alzheimer's disease, Brain Pathol. 21 (2011) 321-329.

[83] A. Bolognesi, A. Chatgilialoglu, L. Polito, C. Ferreri, Membrane lipidome reorganization correlates with the fate of neuroblastoma cells supplemented with fatty acids, PLoS One 8 (2013) e55537.

[84] J.J. Ochoa, R. Pamplona, M.C. Ramirez-Tortosa, S. Granados-Principal, P. Perez-Lopez, A. Naudi, M. Portero-Otin, M. Lopez-Frias, M. Battino, J.L. Quiles, Age-related changes in brain mitochondrial DNA deletion and oxidative stress are differentially modulated by dietary fat type and coenzyme Q(10), Free Radic. Biol. Med. 50 (2011) 1053-1064.

[85] M. Svensson, A. Hakansson, A.K. Mossberg, S. Linse, C. Svanborg, Conversion of alpha-lactalbumin to a protein inducing apoptosis, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 4221-4226.

[86] A. Fontana, B. Spolaore, P. Polverino de Laureto, The biological activities of protein/oleic acid complexes reside in the fatty acid, Biochim. Biophys. Acta 1834 (2013) 1125-1143.

[87] J.C. Ho, P. Storm, A. Rydstrom, B. Bowen, F. Alsin, L. Sullivan, I. Ambite, K.H. Mok, T. Northen, C. Svanborg, Lipids as tumoricidal components of human alpha-lactalbumin made lethal to tumor cells (HAMLET): unique and shared effects on signaling and death, J. Biol. Chem. 288 (2013) 17460-17471.

[88] T. Nakamura, T. Aizawa, R. Kariya, S. Okada, M. Demura, K. Kawano, K. Makabe, K. Kuwajima, Molecular mechanisms of the cytotoxicity of human alpha-lactalbumin made lethal to tumor cells (HAMLET) and other protein-oleic acid complexes, J. Biol. Chem. 288 (2013) 14408-14416.

[89] Z. Xiao, A. Mak, K. Koch, R.B. Moore, A molecular complex of bovine milk protein and oleic acid selectively kills cancer cells in vitro and inhibits tumour growth in an orthotopic rat bladder tumour model, BJU Int. 112 (2013) E201-210.

[90] H. Wen, W.R. Glomm, O. Halskau, Cytotoxicity of bovine alpha-lactalbumin: Oleic acid complexes correlates with the disruption of lipid membranes, Biochim. Biophys. Acta 1828 (2013) 2691-2699.

[91] C. Duringer, A. Hamiche, L. Gustafsson, H. Kimura, C. Svanborg, HAMLET interacts with histones and chromatin in tumor cell nuclei, J. Biol. Chem. 278 (2003) 42131-42135.

[92] E. Psomiadou, M. Tsimidou, Stability of virgin olive oil. 1. Autoxidation studies, J. Agric. Food Chem. 50 (2002) 716-721.

[93] E. Psomiadou, M. Tsimidou, Stability of virgin olive oil. 2. Photo-oxidation studies, J. Agric. Food Chem. 50 (2002) 722-727.

[94] C. De Luca, G. Valacchi, Surface lipids as multifunctional mediators of skin responses to environmental stimuli, Mediators Inflamm. 2010 (2010) 321494.

[95] X.Y. Dong, S.Q. Tang, J.D. Chen, Dual functions of Insig proteins in cholesterol homeostasis, Lipids Health Dis. 11 (2012) 173.

[96] S. Gill, J. Stevenson, I. Kristiana, A.J. Brown, Cholesterol-dependent degradation of squalene monooxygenase, a control point in cholesterol synthesis beyond HMG-CoA reductase, Cell Metab. 13 (2011) 260-273.

[97] M. Spanova, D. Günther, Squalene – biochemistry, molecular biology, process biotechnology, and applications, Eur. J. Lipid Sci. Tech. 113 (2011) 1299-1320.

[98] T. Hauss, S. Dante, N.A. Dencher, T.H. Haines, Squalane is in the midplane of the lipid bilayer: implications for its function as a proton permeability barrier, Biochim. Biophys. Acta 1556 (2002) 149-154.

[99] J.S. Hansen, A. Vararattanavech, T. Vissing, J. Torres, J. Emneus, C. Helix-Nielsen, Formation of giant protein vesicles by a lipid cosolvent method, Chembiochem. 12 (2011) 2856-2862.

[100] M. Spanova, D. Zweytick, K. Lohner, L. Klug, E. Leitner, A. Hermetter, G. Daum, Influence of squalene on lipid particle/droplet and membrane organization in the yeast Saccharomyces cerevisiae, Biochim. Biophys. Acta 1821 (2012) 647-653.

[101] S.F. Gilmore, A.I. Yao, Z. Tietel, T. Kind, M.T. Facciotti, A.N. Parikh, Role of squalene in the organization of monolayers derived from lipid extracts of Halobacterium salinarum, Langmuir 29 (2013) 7922-7930.

[102] P. Couvreur, L.H. Reddy, S. Mangenot, J.H. Poupaert, D. Desmaele, S. Lepetre-Mouelhi, B. Pili, C. Bourgaux, H. Amenitsch, M. Ollivon, Discovery of new hexagonal supramolecular nanostructures formed by squalenoylation of an anticancer nucleoside analogue, Small 4 (2008) 247-253.

[103] A. Ambike, V. Rosilio, B. Stella, S. Lepetre-Mouelhi, P. Couvreur, Interaction of self-assembled squalenoyl gemcitabine nanoparticles with phospholipid-cholesterol monolayers mimicking a biomembrane, Langmuir 27 (2011) 4891-4899.

[104] D. Desmaele, R. Gref, P. Couvreur, Squalenoylation: a generic platform for nanoparticular drug delivery, J. Control Release 161 (2012) 609-618.

[105] H.M. Ali, A. Maksimenko, G. Urbinati, H. Chapuis, M. Raouane, D. Desmaele, H. Yasuhiro, H. Harashima, P. Couvreur, L. Massaad-Massade, Effects of silencing RET/PTC1 oncogene in papillary thyroid carcinoma by siRNA-squalene nanoparticles with and without fusogenic companion GALA-Cholesterol, Thyroid (2013) [Epub ahead of print].

[106] A. Wei, T. Shibamoto, Antioxidant activities of essential oil mixtures toward skin lipid squalene oxidized by UV irradiation, Cutan. Ocul. Toxicol. 26 (2007) 227-233.

[107] S. Cho, C.W. Choi, D.H. Lee, C.H. Won, S.M. Kim, S. Lee, M.J. Lee, J.H. Chung, High-dose squalene ingestion increases type I procollagen and decreases ultraviolet-induced DNA damage in human skin in vivo but is associated with transient adverse effects, Clin. Exp. Dermatol. 34 (2009) 500-508.

[108] P. Viola, M. Viola, Virgin olive oil as a fundamental nutritional component and skin protector, Clin. Dermatol. 27 (2009) 159-165.

[109] K. Wolosik, M. Knas, A. Zalewska, M. Niczyporuk, A.W. Przystupa, The importance and perspective of plant-based squalene in cosmetology, J. Cosmet. Sci. 64 (2013) 59-66.

[110] L.H. Reddy, P. Couvreur, Squalene: A natural triterpene for use in disease management and therapy, Adv. Drug. Deliv. Rev. 61 (2009) 1412-1426.

[111] H.N. Bhilwade, N. Tatewaki, H. Nishida, T. Konishi, Squalene as novel food factor, Curr. Pharm. Biotechnol. 11 (2010) 875-880.

[112] S.K. Kim, F. Karadeniz, Biological importance and applications of squalene and squalane, Adv. Food Nutr. Res. 65 (2012) 223-233.

[113] M.A. Tegenge, R.J. Mitkus, A physiologically-based pharmacokinetic (PBPK) model of squalene-containing adjuvant in human vaccines, J. Pharmacokinet. Pharmacodyn. 40 (2013) 545-556.

[114] I. Domonkos, M. Kis, Z. Gombos, B. Ughy, Carotenoids, versatile components of oxygenic photosynthesis, Prog. Lipid Res. 52 (2013) 539-561.

[115] B. Gandul-Rojas, M.I. Minguez-Mosquera, Chlorophyll and carotenoid composition in virgin olive oils from various spanish olive varieties, J. Sci. Food Agric. 72 (1996) 31-39.

[116] L. Gallardo-Guerrero, M. Roca, I. Minguez-Mosquera, Distribution of chlorophylls and carotenoids in ripening olives and between oil and alperujo when processed using a two-phase extraction system, J. Am. Chem. Soc. 79 (2002) 105-109.

[117] R. Aparicio-Ruiz, B. Gandul-Rojas, Thermal degradation kinetics of neoxanthin, violaxanthin, and antheraxanthin in virgin olive oils, J. Agric. Food Chem. 60 (2012) 5180-5191.

[118] J. Paik, A. During, E.H. Harrison, C.L. Mendelsohn, K. Lai, W.S. Blaner, Expression and characterization of a murine enzyme able to cleave beta-carotene. The formation of retinoids, J. Biol. Chem. 276 (2001) 32160-32168.

[119] T. Kowatz, D. Babino, P. Kiser, K. Palczewski, J. von Lintig, Characterization of human beta,beta-carotene-15,15'-monooxygenase (BCMO1) as a soluble monomeric enzyme, Arch. Biochem. Biophys. 539 (2013) 214-222.

[120] E.H. Harrison, Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids, Biochim. Biophys. Acta 1821 (2012) 70-77.

[121] J.A. Novotny, S.R. Dueker, L.A. Zech, A.J. Clifford, Compartmental analysis of the dynamics of beta-carotene metabolism in an adult volunteer, J. Lipid Res. 36 (1995) 1825-1838.

[122] B. Gleize, M. Steib, M. Andre, E. Reboul, Simple and fast HPLC method for simultaneous determination of retinol, tocopherols, coenzyme Q(10) and carotenoids in complex samples, Food Chem. 134 (2012) 2560-2564.

[123] A. Perez-Galvez, Y.M. Pacheco, B. Bermudez, S. Lopez, R. Abia, F.J. Muriana, J. Villar, J. Garrido-Fernández, Postprandial evolution of the carotenoid content in the triacylglycerol-rich lipoprotein fraction after a single ingestion of virgin olive oil in humans, Food Res. Int. 38 (2005) 1097-1102.

[124] G.P. Lobo, S. Hessel, A. Eichinger, N. Noy, A.R. Moise, A. Wyss, K. Palczewski, J. von Lintig, ISX is a retinoic acid-sensitive gatekeeper that controls intestinal beta,beta-carotene absorption and vitamin A production, FASEB J. 24 (2010) 1656-1666.

[125] A. Sujak, W. Okulski, W.I. Gruszecki, Organisation of xanthophyll pigments lutein and zeaxanthin in lipid membranes formed with dipalmitoylphosphatidylcholine, Biochim. Biophys. Acta 1509 (2000) 255-263.

[126] J. Cerezo, J. Zuniga, A. Bastida, A. Requena, J.P. Ceron-Carrasco, L.A. Eriksson, Antioxidant properties of beta-carotene isomers and their role in photosystems: insights from Ab initio simulations, J. Phys. Chem. A 116 (2012) 3498-3506.

[127] J. Cerezo, J. Zuniga, A. Bastida, A. Requena, J.P. Ceron-Carrasco, Conformational changes of beta-carotene and zeaxanthin immersed in a model membrane through atomistic molecular dynamics simulations, Phys. Chem. Chem. Phys. 15 (2013) 6527-6538.

[128] W.I. Gruszecki, A. Sujak, K. Strzalka, A. Radunz, G.H. Schmid, Organisation of xanthophyll-lipid membranes studied by means of specific pigment antisera, spectrophotometry and monomolecular layer technique lutein versus zeaxanthin, Z. Naturforsch. C 54 (1999) 517-525.

[129] A. Sujak, J. Gabrielska, W. Grudzinski, R. Borc, P. Mazurek, W.I. Gruszecki, Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects, Arch. Biochem. Biophys. 371 (1999) 301-307.
[130] A. Wisniewska, J. Widomska, W.K. Subczynski, Carotenoid-membrane interactions in liposomes: effect of dipolar, monopolar, and nonpolar carotenoids, Acta Biochim. Pol. 53 (2006) 475-484.
[131] W.I. Gruszecki, K. Strzalka, Carotenoids as modulators of lipid membrane physical properties, Biochim. Biophys. Acta 1740 (2005) 108-115.
[132] A. Martinez, M.A. Rodriguez-Girones, A. Barbosa, M. Costas, Donator acceptor map for carotenoids, melatonin and vitamins, J. Phys. Chem. A 112 (2008) 9037-9042.
[133] R.M. Han, J.P. Zhang, L.H. Skibsted, Reaction dynamics of flavonoids and carotenoids as antioxidants, Molecules 17 (2012) 2140-2160.
[134] B. Li, P. Vachali, J.M. Frederick, P.S. Bernstein, Identification of StARD3 as a lutein-binding protein in the macula of the primate retina, Biochemistry 50 (2011) 2541-2549.
[135] A. Wisniewska-Becker, G. Nawrocki, M. Duda, W.K. Subczynski, Structural aspects of the antioxidant activity of lutein in a model of photoreceptor membranes, Acta Biochim. Pol. 59 (2012) 119-124.
[136] G. Horvath, E. Szoke, A. Kemeny, T. Bagoly, J. Deli, L. Szente, S. Pal, K. Sandor, J. Szolcsanyi, Z. Helyes, Lutein inhibits the function of the transient receptor potential A1 ion channel in different in vitro and in vivo models, J. Mol. Neurosci. 46 (2012) 1-9.

[137] T. Aslam, C. Delcourt, R. Silva, F.G. Holz, A. Leys, A. Garcia-Layana, E. Souied, Micronutrients in age-related macular degeneration, Ophthalmologica 229 (2013) 75-79.

[138] E. Yonova-Doing, P.G. Hysi, C. Venturini, K.M. Williams, A. Nag, S. Beatty, S.H. Liew, C.E. Gilbert, C.J. Hammond, candidate gene study of macular response to supplemental lutein and zeaxanthin, Exp. Eye Res. 115C (2013) 172-177.

[139] J. Feeney, C. Finucane, G.M. Savva, H. Cronin, S. Beatty, J.M. Nolan, R.A. Kenny, Low macular pigment optical density is associated with lower cognitive performance in a large, population-based sample of older adults, Neurobiol. Aging 34 (2013) 2449-2456.

[140] Y.H. Cui, C.X. Jing, H.W. Pan, Association of blood antioxidants and vitamins with risk of age-related cataract: a meta-analysis of observational studies, Am. J. Clin. Nutr. 98 (2013) 778-786.

[141] E.J. Johnson, R. Vishwanathan, M.A. Johnson, D.B. Hausman, A. Davey, T.M. Scott, R.C. Green, L.S. Miller, M. Gearing, J. Woodard, P.T. Nelson, H.Y. Chung, W. Schalch, J. Wittwer, L.W. Poon, Relationship between serum and brain carotenoids, alpha-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the Georgia Centenarian Study, J. Aging Res. 2013 (2013) 951786.

[142] J. Karppi, S. Kurl, T.H. Makikallio, K. Ronkainen, J.A. Laukkanen, Low levels of plasma carotenoids are associated with an increased risk of atrial fibrillation, Eur. J. Epidemiol. 28 (2013) 45-53.

[143] Z. Dai, R. Wang, L.W. Ang, Y.L. Low, J.M. Yuan, W.P. Koh, Protective effects of dietary carotenoids on risk of hip fracture in men: The Singapore chinese health study, J. Bone Miner. Res. (2013) doi: 10.1002/jbmr.2041. [Epub ahead of print].

[144] T. Wolak, E. Paran, Can carotenoids attenuate vascular aging?, Vascul. Pharmacol. 59 (2013) 63-66.

[145] R.A. Moreau, B.D. Whitaker, K.B. Hicks, Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses, Prog. Lipid Res. 41 (2002) 457-500.

[146] M. Fuentes de Mendoza, C. De Miguel Gordillo, J. Marin Expoxito, J. Sanchez Casas, M. Martinez Cano, D. Martin Vertedor, M.N. Franco Baltasar, Chemical composition of virgin olive oils according to the ripening in olives, Food Chem. 141 (2013) 2575-2581.

[147] M. Lukic, I. Lukic, M. Krapac, B. Sladonja, V. Pilizota, Sterols and triterpene diols in olive oil as indicators of variety and degree of ripening, Food Chem. 136 (2013) 251-258.

[148] D. Garcia-Gonzalez, M. Viera, N. Tena, R. Aparicio, Evaluation of the methods based on triglycerides and sterols for the detection of hazelnut oil in olive oil, Grasas Aceites 58 (2007) 344-350.

[149] B. Mathison, D. Holstege, A rapid method to determine sterol, erythrodiol, and uvaol concentrations in olive oil, J. Agric. Food Chem. 61 (2013) 4506-4513.

[150] K. Matsuoka, E. Rie, S. Yui, C. Honda, K. Endo, Competitive solubilization of cholesterol and beta-sitosterol with changing biliary lipid compositions in model intestinal solution, Chem. Phys. Lipids 165 (2012) 7-14.

[151] S.W. Park, Intestinal and hepatic niemann-pick c1-like 1, Diabetes Metab. J. 37 (2013) 240-248.

[152] L. Jia, J.L. Betters, L. Yu, Niemann-pick C1-like 1 (NPC1L1) protein in intestinal and hepatic cholesterol transport, Annu. Rev. Physiol. 73 (2011) 239-259.

[153] V. Escurriol, M. Cofan, M. Serra, M. Bullo, J. Basora, J. Salas-Salvado, D. Corella, I. Zazpe, M.A. Martinez-Gonzalez, V. Ruiz-Gutierrez, R. Estruch, E. Ros, Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet, Eur. J. Nutr. 48 (2009) 373-382.

[154] Y. Park, T.P. Carr, Unsaturated fatty acids and phytosterols regulate cholesterol transporter genes in Caco-2 and HepG2 cell lines, Nutr. Res. 33 (2013) 154-161.

[155] K. Hac-Wydro, Studies on beta-sitosterol and ceramide-induced alterations in the properties of cholesterol/sphingomyelin/ganglioside monolayers, Biochim. Biophys. Acta 1828 (2013) 2460-2469.

[156] K. Hac-Wydro, The effect of beta-sitosterol on the properties of cholesterol/phosphatidylcholine/ganglioside monolayers--the impact of monolayer fluidity, Colloids Surf. B Biointerfaces 110 (2013) 113-119.

[157] J. Aittoniemi, T. Rog, P. Niemela, M. Pasenkiewicz-Gierula, M. Karttunen, I. Vattulainen, Tilt: major factor in sterols' ordering capability in membranes, J. Phys. Chem. B 110 (2006) 25562-25564.

[158] T. Rog, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, Ordering effects of cholesterol and its analogues, Biochim. Biophys. Acta 1788 (2009) 97-121.

[159] S. Rozner, L. Verkhovski, Y. Nissimov, A. Aserin, R. Vilensky, D. Danino, C.C. Zouboulis, Y. Milner, N. Garti, Inhibition of cholesterol transport into skin cells in cultures by phytosterol-loaded microemulsion, Chem. Phys. Lipids 153 (2008) 109-118.

[160] K.K. Halling, J.P. Slotte, Membrane properties of plant sterols in phospholipid bilayers as determined by differential scanning calorimetry,

resonance energy transfer and detergent-induced solubilization, Biochim. Biophys. Acta 1664 (2004) 161-171.

[161] K. Hac-Wydro, P. Wydro, P. Dynarowicz-Latka, M. Paluch, Cholesterol and phytosterols effect on sphingomyelin/phosphatidylcholine model membranes--thermodynamic analysis of the interactions in ternary monolayers, J. Colloid Interface Sci. 329 (2009) 265-272.

[162] F. Marangoni, A. Poli, Phytosterols and cardiovascular health, Pharmacol. Res. 61 (2010) 193-199.

[163] S.P. Choudhary, L.S. Tran, Phytosterols: perspectives in human nutrition and clinical therapy, Curr. Med. Chem. 18 (2011) 4557-4567.

[164] J. Plat, D. Mackay, S. Baumgartner, P.M. Clifton, H. Gylling, P.J. Jones, Progress and prospective of plant sterol and plant stanol research: report of the Maastricht meeting, Atherosclerosis 225 (2012) 521-533.

[165] H. Cederberg, H. Gylling, T.A. Miettinen, J. Paananen, J. Vangipurapu, J. Pihlajamaki, T. Kuulasmaa, A. Stancakova, U. Smith, J. Kuusisto, M. Laakso, Non-cholesterol sterol levels predict hyperglycemia and conversion to type 2 diabetes in finnish men, PLoS One 8 (2013) e67406.

[166] S. Staubach, F.G. Hanisch, Lipid rafts: signaling and sorting platforms of cells and their roles in cancer, Expert Rev. Proteomics 8 (2011) 263-277.

[167] N. Defamie, M. Mesnil, The modulation of gap-junctional intercellular communication by lipid rafts, Biochim. Biophys. Acta 1818 (2012) 1866-1869.

[168] K.S. George, S. Wu, Lipid raft: A floating island of death or survival, Toxicol. Appl. Pharmacol. 259 (2012) 311-319.

[169] P.G. Bradford, A.B. Awad, Modulation of signal transduction in cancer cells by phytosterols, Biofactors 36 (2010) 241-247.

[170] P.J. Jansen, D. Lutjohann, K. Abildayeva, T. Vanmierlo, T. Plosch, J. Plat, K. von Bergmann, A.K. Groen, F.C. Ramaekers, F. Kuipers, M. Mulder, Dietary plant sterols accumulate in the brain, Biochim. Biophys. Acta 1761 (2006) 445-453.

[171] T. Vanmierlo, O. Weingartner, S. van der Pol, C. Husche, A. Kerksiek, S. Friedrichs, E. Sijbrands, H. Steinbusch, M. Grimm, T. Hartmann, U. Laufs, M. Bohm, H.E. de Vries, M. Mulder, D. Lutjohann, Dietary intake of plant sterols stably increases plant sterol levels in the murine brain, J. Lipid Res. 53 (2012) 726-735.

[172] J. Wang, F. Wu, C. Shi, Substitution of membrane cholesterol with beta-sitosterol promotes nonamyloidogenic cleavage of endogenous amyloid precursor protein, Neuroscience 247 (2013) 227-233.

[173] C. Shi, J. Liu, F. Wu, X. Zhu, D.T. Yew, J. Xu, β-sitosterol inhibits high cholesterol-induced platelet β-amyloid release, J. Bioenerg. Biomembr. 43 (2011) 691-697.

[174] C. Shi, F. Wu, J. Xu, Incorporation of β-sitosterol into mitochondrial membrane enhances mitochondrial function by promoting inner mitochondrial membrane fluidity, J. Bioenerg. Biomembr. 45 (2013) 301-305.

[175] N. Stiti, S. Triki, M.-A. Hartmann, Formation of triterpenoids throughout Olea europaea fruit ontogeny, Lipids 42 (2007) 55-67.

[176] A. Guinda, M. Rada, T. Delgado, P. Gutierrez-Adanez, J.M. Castellano, Pentacyclic triterpenoids from olive fruit and leaf, J. Agric. Food Chem. 58 (2010) 9685-9691.

[177] J. Peragon, Time course of pentacyclic triterpenoids from fruits and leaves of olive tree (Olea europaea L.) cv. picual and cv. cornezuelo during ripening, J. Agric. Food Chem. 61 (2013) 6671-6678.

[178] Y. Allouche, A. Jimenez, M. Uceda, M.P. Aguilera, J.J. Gaforio, G. Beltran, Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars, J. Agric. Food Chem. 57 (2009) 3604-3610.

[179] J. Prades, O. Vogler, R. Alemany, M. Gomez-Florit, S.S. Funari, V. Ruiz-Gutierrez, F. Barcelo, Plant pentacyclic triterpenic acids as modulators of lipid membrane physical properties, Biochim. Biophys. Acta 1808 (2011) 752-760.

[180] A.C. Teixeira, A.R. Garcia, L.M. Ilharco, A.M. Goncalves da Silva, A.C. Fernandes, Phase behaviour of oleanolic acid, pure and mixed with stearic acid: Interactions and crystallinity, Chem. Phys. Lipids 163 (2010) 655-666.

[181] M. Bayer, P. Proksch, I. Felsner, H. Brenden, Z. Kohne, R. Walli, T.N. Duong, C. Gotz, J. Krutmann, S. Grether-Beck, Photoprotection against UVAR: effective triterpenoids require a lipid raft stabilizing chemical structure, Exp. Dermatol. 20 (2011) 955-958.

[182] M.B. Sporn, K.T. Liby, M.M. Yore, L. Fu, J.M. Lopchuk, G.W. Gribble, New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress, J. Nat. Prod. 74 (2011) 537-545.

[183] F.M. Goni, A. Alonso, Effects of ceramide and other simple sphingolipids on membrane lateral structure, Biochim. Biophys. Acta. 1788 (2009) 169-177.

[184] S. Grether-Beck, M. Salahshour-Fard, A. Timmer, H. Brenden, I. Felsner, R. Walli, J. Fullekrug, J. Krutmann, Ceramide and raft signaling are linked with each other in UVA radiation-induced gene expression, Oncogene 27 (2008) 4768-4778.

[185] Y. Uchida, Ceramide signaling in mammalian epidermis, Biochim. Biophys. Acta (2013) doi: 10.1016/j.bbalip.2013.09.003. [Epub ahead of print].

[186] W.J. van Blitterswijk, A.H. van der Luit, R.J. Veldman, M. Verheij, J. Borst, Ceramide: second messenger or modulator of membrane structure and dynamics?, Biochem. J. 369 (2003) 199-211.
[187] Y. Allouche, G. Beltran, J.J. Gaforio, M. Uceda, M.D. Mesa, Antioxidant and antiatherogenic activities of pentacyclic triterpenic diols and acids, Food Chem. Toxicol. 48 (2010) 2885-2890.
[188] M.P. Montilla, A. Agil, M.C. Navarro, M.I. Jimenez, A. Garcia-Granados, A. Parra, M.M. Cabo, Antioxidant activity of maslinic acid, a triterpene derivative obtained from Olea europaea, Planta Med. 69 (2003) 472-474.
[189] J.S. Perona, R. Cabello-Moruno, V. Ruiz-Gutierrez, The role of virgin olive oil components in the modulation of endothelial function, J. Nutr. Biochem. 17 (2006) 429-445.
[190] L.I. Somova, F.O. Shode, P. Ramnanan, A. Nadar, Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies africana leaves, J. Ethnopharmacol. 84 (2003) 299-305.
[191] L.O. Somova, A. Nadar, P. Rammanan, F.O. Shode, Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension, Phytomedicine 10 (2003) 115-121.
[192] R. Rodriguez-Rodriguez, J.S. Perona, M.D. Herrera, V. Ruiz-Gutierrez, Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats, J. Agric. Food Chem. 54 (2006) 2096-2102.
[193] R. Rodriguez-Rodriguez, M.D. Herrera, M.A. de Sotomayor, V. Ruiz-Gutierrez, Pomace olive oil improves endothelial function in spontaneously hypertensive rats by increasing endothelial nitric oxide synthase expression, Am. J. Hypertens. 20 (2007) 728-734.

[194] A. Marquez-Martin, R. De La Puerta, A. Fernandez-Arche, V. Ruiz-Gutierrez, P. Yaqoob, Modulation of cytokine secretion by pentacyclic triterpenes from olive pomace oil in human mononuclear cells, Cytokine 36 (2006) 211-217.

[195] L.I. Somova, F.O. Shode, M. Mipando, Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol, Phytomedicine 11 (2004) 121-129.

[196] J. Martinez-Gonzalez, R. Rodriguez-Rodriguez, M. Gonzalez-Diez, C. Rodriguez, M.D. Herrera, V. Ruiz-Gutierrez, L. Badimon, Oleanolic acid induces prostacyclin release in human vascular smooth muscle cells through a cyclooxygenase-2-dependent mechanism, J. Nutr. 138 (2008) 443-448.

[197] X. Wang, R. Liu, W. Zhang, X. Zhang, N. Liao, Z. Wang, W. Li, X. Qin, C. Hai, Oleanolic acid improves hepatic insulin resistance via antioxidant, hypolipidemic and anti-inflammatory effects, Mol. Cell. Endocrinol. 376 (2013) 70-80.

[198] W. Lee, E.J. Yang, S.K. Ku, K.S. Song, J.S. Bae, Anti-inflammatory effects of oleanolic acid on LPS-induced inflammation in vitro and in vivo, Inflammation 36 (2013) 94-102.

[199] R.S. Santos, P.L. Silva, G.P. de Oliveira, C.L. Santos, F.F. Cruz, E.F. de Assis, H.C. de Castro-Faria-Neto, V.L. Capelozzi, M.M. Morales, P. Pelosi, C.R. Gattass, P.R. Rocco, Oleanolic acid improves pulmonary morphofunctional parameters in experimental sepsis by modulating oxidative and apoptotic processes, Respir. Physiol. Neurobiol. 189 (2013) 484-490.

[200] V. Stepanov, K. Stankov, M. Mikov, The bile acid membrane receptor TGR5: a novel pharmacological target in metabolic, inflammatory and neoplastic disorders, J. Recept. Signal Transduct. Res. 33 (2013) 213-223.

[201] D.D. Jensen, C.B. Godfrey, C. Niklas, M. Canals, M. Kocan, D.P. Poole, J.E. Murphy, F. Alemi, G.S. Cottrell, C. Korbmacher, N.A. Lambert, N.W. Bunnett, C.U. Corvera, The bile acid receptor TGR5 does not interact with beta-arrestins or traffic to endosomes but transmits sustained signals from plasma membrane rafts, J. Biol. Chem. 288 (2013) 22942-22960.

[202] J.M. Castellano, A. Guinda, T. Delgado, M. Rada, J.A. Cayuela, Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes, Diabetes 62 (2013) 1791-1799.

[203] Y. Allouche, F. Warleta, M. Campos, C. Sanchez-Quesada, M. Uceda, G. Beltran, J.J. Gaforio, Antioxidant, antiproliferative, and pro-apoptotic capacities of pentacyclic triterpenes found in the skin of olives on MCF-7 human breast cancer cells and their effects on DNA damage, J. Agric. Food Chem. 59 (2011) 121-130.

[204] S.Y. Park, C.W. Nho, D.Y. Kwon, Y.H. Kang, K.W. Lee, J.H. Park, Maslinic acid inhibits the metastatic capacity of DU145 human prostate cancer cells: possible mediation via hypoxia-inducible factor-1alpha signalling, Br. J. Nutr. 109 (2013) 210-222.

[205] F.J. Reyes-Zurita, E.E. Rufino-Palomares, P.P. Medina, L. Garcia-Salguero, J. Peragon, M. Cascante, J.A. Lupianez, Antitumour activity on extrinsic apoptotic targets of the triterpenoid maslinic acid in p53-deficient Caco-2 adenocarcinoma cells, Biochimie. 95 (2013) 2157-2167.

[206] E.E. Rufino-Palomares, F.J. Reyes-Zurita, L. Garcia-Salguero, K. Mokhtari, P.P. Medina, J.A. Lupianez, J. Peragon, Maslinic acid, a triterpenic anti-tumoural agent, interferes with cytoskeleton protein expression in HT29 human colon-cancer cells, J. Proteomics 83 (2013) 15-25.

[207] J. Wang, M. Yu, L. Xiao, S. Xu, Q. Yi, W. Jin, Radiosensitizing effect of oleanolic acid on tumor cells through the inhibition of GSH synthesis in vitro, Oncol. Rep. 30 (2013) 917-924.

[208] G. Guo, W. Yao, Q. Zhang, Y. Bo, Oleanolic acid suppresses migration and invasion of malignant glioma cells by inactivating MAPK/ERK signaling pathway, PLoS One 8 (2013) e72079.

[209] J. Wei, H. Liu, M. Liu, N. Wu, J. Zhao, L. Xiao, L. Han, E. Chu, X. Lin, Oleanolic acid potentiates the antitumor activity of 5-fluorouracil in pancreatic cancer cells, Oncol. Rep. 28 (2012) 1339-1345.

[210] S.M. Kim, M.K. Chae, M.S. Yim, I.H. Jeong, J. Cho, C. Lee, E.K. Ryu, Hybrid PET/MR imaging of tumors using an oleanolic acid-conjugated nanoparticle, Biomaterials 34 (2013) 8114-8121.

[211] R. Martin, M. Hernandez, C. Cordova, M.L. Nieto, Natural triterpenes modulate immune-inflammatory markers of experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis, Br. J. Pharmacol. 166 (2012) 1708-1723.

[212] A. Bendini, L. Cerretani, A. Carrasco-Pancorbo, A.M. Gomez-Caravaca, A. Segura-Carretero, A. Fernandez-Gutierrez, G. Lercker, Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade, Molecules 12 (2007) 1679-1719.

[213] S. Christophoridou, P. Dais, Detection and quantification of phenolic compounds in olive oil by high resolution 1H nuclear magnetic resonance spectroscopy, Anal. Chim. Acta 633 (2009) 283-292.

[214] C. Romero, M. Brenes, Analysis of total contents of hydroxytyrosol and tyrosol in olive oils, J. Agric. Food Chem. 60 (2012) 9017-9022.

[215] T.A. Enache, A. Amine, C.M. Brett, A.M. Oliveira-Brett, Virgin olive oil ortho-phenols-electroanalytical quantification, Talanta 105 (2013) 179-186.

[216] S. Cicerale, L.J. Lucas, R.S. Keast, Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil, Curr. Opin. Biotechnol. 23 (2012) 129-135.

[217] A. Dagdelen, G. Tumen, M. Ozcan, E. Dundar, Phenolics profiles of olive fruits (Olea europaea L.) and oils from Ayvalık, Domat and Gemlik varieties at different ripening stages, Food Chem. 136 (2013) 41-45.

[218] L. Rubio, M.J. Motilva, A. Macia, T. Ramo, M.P. Romero, Development of a phenol-enriched olive oil with both its own phenolic compounds and complementary phenols from thyme, J. Agric. Food Chem. 60 (2012) 3105-3112.

[219] M. Suarez, R.M. Valls, M.P. Romero, A. Macia, S. Fernandez, M. Giralt, R. Sola, M.J. Motilva, Bioavailability of phenols from a phenol-enriched olive oil, Br. J. Nutr. 106 (2011) 1691-1701.

[220] M. de Bock, E.B. Thorstensen, J.G. Derraik, H.V. Henderson, P.L. Hofman, W.S. Cutfield, Human absorption and metabolism of oleuropein and hydroxytyrosol ingested as olive (Olea europaea L.) leaf extract, Mol. Nutr. Food Res. 57 (2013) 2079-2085.

[221] M.N. Vissers, P.L. Zock, A.J. Roodenburg, R. Leenen, M.B. Katan, Olive oil phenols are absorbed in humans, J. Nutr. 132 (2002) 409-417.

[222] A. Serra, L. Rubio, X. Borras, A. Macia, M.P. Romero, M.J. Motilva, Distribution of olive oil phenolic compounds in rat tissues after administration of a phenolic extract from olive cake, Mol. Nutr. Food Res. 56 (2012) 486-496.

[223] F. Paiva-Martins, M.H. Gordon, P. Gameiro, Activity and location of olive oil phenolic antioxidants in liposomes, Chem. Phys. Lipids 124 (2003) 23-36.
[224] G.I. Togna, A.R. Togna, M. Franconi, C. Marra, M. Guiso, Olive oil isochromans inhibit human platelet reactivity, J. Nutr. 133 (2003) 2532-2536.
[225] N. Caturla, L. Perez-Fons, A. Estepa, V. Micol, Differential effects of oleuropein, a biophenol from Olea europaea, on anionic and zwiterionic phospholipid model membranes, Chem. Phys. Lipids 137 (2005) 2-17.
[226] H.A. Scheidt, D. Huster, The interaction of small molecules with phospholipid membranes studied by 1H NOESY NMR under magic-angle spinning, Acta Pharmacol. Sin. 29 (2008) 35-49.
[227] P. Kosinova, K. Berka, M. Wykes, M. Otyepka, P. Trouillas, Positioning of antioxidant quercetin and its metabolites in lipid bilayer membranes: implication for their lipid-peroxidation inhibition, J. Phys. Chem. B 116 (2012) 1309-1318.
[228] A. Bendini, L. Cerretani, S. Vecchi, A. Carrasco-Pancorbo, G. Lercker, Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions, J. Agric. Food Chem. 54 (2006) 4880-4887.
[229] M. Pazos, M.L. Andersen, I. Medina, L.H. Skibsted, Efficiency of natural phenolic compounds regenerating alpha-tocopherol from alpha-tocopheroxyl radical, J. Agric. Food Chem. 55 (2007) 3661-3666.
[230] F. Paiva-Martins, J. Fernandes, S. Rocha, H. Nascimento, R. Vitorino, F. Amado, F. Borges, L. Belo, A. Santos-Silva, Effects of olive oil polyphenols on erythrocyte oxidative damage, Mol. Nutr. Food Res. 53 (2009) 609-616.

[231] J. Li, G. Yang, S. Wang, L. Jiang, X. Liu, C. Geng, L. Zhong, M. Chen, The protective effects of hydroxytyrosol against ortho-phenylphenol-induced DNA damage in HepG2 cells, Toxicol. Mech. Methods 22 (2012) 432-437.

[232] X. Zou, Z. Feng, Y. Li, Y. Wang, K. Wertz, P. Weber, Y. Fu, J. Liu, Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: activation of Nrf2 and JNK-p62/SQSTM1 pathways, J. Nutr. Biochem. 23 (2012) 994-1006.

[233] F. Shahidi, M. Naczk, Phenolics in food and nutraceuticals: Sources, applications and health effects, CRC Press, 2004.

[234] S. Rigacci, V. Guidotti, M. Bucciantini, M. Parri, C. Nediani, E. Cerbai, M. Stefani, A. Berti, Oleuropein aglycon prevents cytotoxic amyloid aggregation of human amylin, J. Nutr. Biochem. 21 (2010) 726-735.

[235] K. Pillay, P. Govender, A direct fluorescence-based technique for cellular localization of amylin, Biotechnol. Appl. Biochem. 60 (2013) 384-392.

[236] M. Caruana, J. Neuner, T. Hogen, F. Schmidt, F. Kamp, C. Scerri, A. Giese, N. Vassallo, Polyphenolic compounds are novel protective agents against lipid membrane damage by alpha-synuclein aggregates in vitro, Biochim. Biophys. Acta 1818 (2012) 2502-2510.

[237] L. Diomede, S. Rigacci, M. Romeo, M. Stefani, M. Salmona, Oleuropein aglycone protects transgenic C. elegans strains expressing Abeta42 by reducing plaque load and motor deficit, PLoS One 8 (2013) e58893.

[238] B. Luzon-Toro, A. Geerlings, S. Hilfiker, Hydroxytyrosol increases norepinephrine transporter function in pheochromocytoma cells, Nucl. Med. Biol. 35 (2008) 801-804.

[239] M.I. Covas, Bioactive effects of olive oil phenolic compounds in humans: reduction of heart disease factors and oxidative damage, Inflammopharmacology 16 (2008) 216-218.

[240] J. Lopez-Miranda, F. Perez-Jimenez, E. Ros, R. De Caterina, L. Badimon, M.I. Covas, E. Escrich, J.M. Ordovas, F. Soriguer, R. Abia, C.A. de la Lastra, M. Battino, D. Corella, J. Chamorro-Quiros, J. Delgado-Lista, D. Giugliano, K. Esposito, R. Estruch, J.M. Fernandez-Real, J.J. Gaforio, C. La Vecchia, D. Lairon, F. Lopez-Segura, P. Mata, J.A. Menendez, F.J. Muriana, J. Osada, D.B. Panagiotakos, J.A. Paniagua, P. Perez-Martinez, J. Perona, M.A. Peinado, M. Pineda-Priego, H.E. Poulsen, J.L. Quiles, M.C. Ramirez-Tortosa, J. Ruano, L. Serra-Majem, R. Sola, M. Solanas, V. Solfrizzi, R. de la Torre-Fornell, A. Trichopoulou, M. Uceda, J.M. Villalba-Montoro, J.R. Villar-Ortiz, F. Visioli, N. Yiannakouris, Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008, Nutr. Metab. Cardiovasc. Dis. 20 (2010) 284-294.

[241] E.K. Pauwels, The protective effect of the Mediterranean diet: focus on cancer and cardiovascular risk, Med. Princ. Pract. 20 (2011) 103-111.

[242] F. Visioli, E. Bernardini, Extra virgin olive oil's polyphenols: biological activities, Curr. Pharm. Des. 17 (2011) 786-804.

[243] S.M. Ross, Effects of extra virgin olive oil phenolic compounds and the Mediterranean diet on cardiovascular health, Holist. Nurs. Pract. 27 (2013) 303-307.

[244] E. Escrich, R. Moral, M. Solanas, Olive oil, an essential component of the Mediterranean diet, and breast cancer, Public Health Nutr. 14 (2011) 2323-2332.

[245] I. Casaburi, F. Puoci, A. Chimento, R. Sirianni, C. Ruggiero, P. Avena, V. Pezzi, Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: a review of in vitro studies, Mol. Nutr. Food Res. 57 (2013) 71-83.

[246] M.J. Oliveras-Lopez, J.J. Molina, M.V. Mir, E.F. Rey, F. Martin, H.L. de la Serrana, Extra virgin olive oil (EVOO) consumption and antioxidant status in healthy institutionalized elderly humans, Arch. Gerontol. Geriatr. 57 (2013) 234-242.

[247] R.J. Widmer, M.A. Freund, A.J. Flammer, J. Sexton, R. Lennon, A. Romani, N. Mulinacci, F.F. Vinceri, L.O. Lerman, A. Lerman, Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis, Eur. J. Nutr. 52 (2013) 1223-1231.

[248] D. Palmieri, B. Aliakbarian, A.A. Casazza, N. Ferrari, G. Spinella, B. Pane, G. Cafueri, P. Perego, D. Palombo, Effects of polyphenol extract from olive pomace on anoxia-induced endothelial dysfunction, Microvasc. Res. 83 (2012) 281-289.

[249] J. Makino, R. Nakanishi, T. Kamiya, H. Hara, M. Ninomiya, M. Koketsu, T. Adachi, Luteolin suppresses the differentiation of THP-1 cells through the Inhibition of NOX2 mRNA expression and the membrane translocation of p47phox, J. Nat. Prod. 76 (2013) 1285-1290.

[250] C. Manna, D. Napoli, G. Cacciapuoti, M. Porcelli, V. Zappia, Olive oil phenolic compounds inhibit homocysteine-induced endothelial cell adhesion regardless of their different antioxidant activity, J. Agric. Food Chem. 57 (2009) 3478-3482.

[251] H.K. Obied, P.D. Prenzler, I. Konczak, A.U. Rehman, K. Robards, Chemistry and bioactivity of olive biophenols in some antioxidant and antiproliferative in vitro bioassays, Chem. Res. Toxicol. 22 (2009) 227-234.

[252] L. Zambonin, C. Caliceti, F. Vieceli Dalla Sega, D. Fiorentini, S. Hrelia, L. Landi, C. Prata, Dietary phenolic acids act as effective antioxidants in membrane models and in cultured cells, exhibiting proapoptotic effects in leukaemia cells, Oxid. Med. Cell. Longev. 2012 (2012) 839298.

[253] P. Pratheeshkumar, Y.O. Son, A. Budhraja, X. Wang, S. Ding, L. Wang, A. Hitron, J.C. Lee, D. Kim, S.P. Divya, G. Chen, Z. Zhang, J. Luo, X. Shi, Luteolin inhibits human prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis, PLoS One 7 (2012) e52279.

[254] X. Cai, W. Lu, T. Ye, M. Lu, J. Wang, J. Huo, S. Qian, X. Wang, P. Cao, The molecular mechanism of luteolin-induced apoptosis is potentially related to inhibition of angiogenesis in human pancreatic carcinoma cells, Oncol. Rep. 28 (2012) 1353-1361.

[255] Q. Chen, S. Liu, J. Chen, Q. Zhang, S. Lin, Z. Chen, J. Jiang, Luteolin induces mitochondria-dependent apoptosis in human lung adenocarcinoma cell, Nat. Prod. Commun. 7 (2012) 29-32.

[256] P.S. Rao, A. Satelli, M. Moridani, M. Jenkins, U.S. Rao, Luteolin induces apoptosis in multidrug resistant cancer cells without affecting the drug transporter function: involvement of cell line-specific apoptotic mechanisms, Int. J. Cancer 130 (2012) 2703-2714.

[257] S. Karthikeyan, G. Kanimozhi, N.R. Prasad, R. Mahalakshmi, Radiosensitizing effect of ferulic acid on human cervical carcinoma cells in vitro, Toxicol. In Vitro 25 (2011) 1366-1375.

[258] J.A. Menendez, A. Vazquez-Martin, C. Oliveras-Ferraros, R. Garcia-Villalba, A. Carrasco-Pancorbo, A. Fernandez-Gutierrez, A. Segura-Carretero, Analyzing effects of extra-virgin olive oil polyphenols on breast cancer-associated fatty acid

synthase protein expression using reverse-phase protein microarrays, Int. J. Mol. Med. 22 (2008) 433-439.

[259] J.A. Menendez, A. Vazquez-Martin, R. Garcia-Villalba, A. Carrasco-Pancorbo, C. Oliveras-Ferraros, A. Fernandez-Gutierrez, A. Segura-Carretero, tabAnti-HER2 (erbB-2) oncogene effects of phenolic compounds directly isolated from commercial extra-virgin olive oil (EVOO), BMC Cancer 8 (2008) 377.

[260] Y.Z. Hashim, I.R. Rowland, H. McGlynn, M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro, L. Kaisalo, K. Wahala, C.I. Gill, Inhibitory effects of olive oil phenolics on invasion in human colon adenocarcinoma cells in vitro, Int. J. Cancer 122 (2008) 495-500.

[261] L. Fini, E. Hotchkiss, V. Fogliano, G. Graziani, M. Romano, E.B. De Vol, H. Qin, M. Selgrad, C.R. Boland, L. Ricciardiello, Chemopreventive properties of pinoresinol-rich olive oil involve a selective activation of the ATM-p53 cascade in colon cancer cell lines, Carcinogenesis 29 (2008) 139-146.

[262] C. Romero, E. Medina, J. Vargas, M. Brenes, A. De Castro, In vitro activity of olive oil polyphenols against Helicobacter pylori, J. Agric. Food Chem. 55 (2007) 680-686.

[263] M. Castro, C. Romero, A. de Castro, J. Vargas, E. Medina, R. Millan, M. Brenes, Assessment of Helicobacter pylori eradication by virgin olive oil, Helicobacter 17 (2012) 305-311.

[264] M. Alirezaei, O. Dezfoulian, S. Neamati, M. Rashidipour, N. Tanideh, A. Kheradmand, Oleuropein prevents ethanol-induced gastric ulcers via elevation of antioxidant enzyme activities in rats, J. Physiol. Biochem. 68 (2012) 583-592.

[265] N. Zoric, I. Horvat, N. Kopjar, A. Vucemilovic, D. Kremer, S. Tomic, I. Kosalec, Hydroxytyrosol expresses antifungal activity in vitro, Curr. Drug Targets 14 (2013) 992-998.

[266] G. Eumkeb, S. Siriwong, K. Thumanu, Synergistic activity of luteolin and amoxicillin combination against amoxicillin-resistant Escherichia coli and mode of action, J. Photochem. Photobiol. B 117 (2012) 247-253.

[267] W. Hur, S.W. Kim, Y.K. Lee, J.E. Choi, S.W. Hong, M.J. Song, S.H. Bae, T. Park, S.J. Um, S.K. Yoon, Oleuropein reduces free fatty acid-induced lipogenesis via lowered extracellular signal-regulated kinase activation in hepatocytes, Nutr. Res. 32 (2012) 778-786.

[268] S. Sanchez-Fidalgo, L. Sanchez de Ibarguen, A. Cardeno, C.A. de la Lastra, Influence of extra virgin olive oil diet enriched with hydroxytyrosol in a chronic DSS colitis model, Eur. J. Nutr. 51 (2012) 497-506.

[269] P. Dewapriya, S.W. Himaya, Y.X. Li, S.K. Kim, Tyrosol exerts a protective effect against dopaminergic neuronal cell death in in vitro model of Parkinson's disease, Food Chem. 141 (2013) 1147-1157.

[270] N. Dragicevic, A. Smith, X. Lin, F. Yuan, N. Copes, V. Delic, J. Tan, C. Cao, R.D. Shytle, P.C. Bradshaw, Green tea epigallocatechin-3-gallate (EGCG) and other flavonoids reduce Alzheimer's amyloid-induced mitochondrial dysfunction, J. Alzheimer's Dis. 26 (2011) 507-521.

[271] M. Ishisaka, K. Kakefuda, M. Yamauchi, K. Tsuruma, M. Shimazawa, A. Tsuruta, H. Hara, Luteolin shows an antidepressant-like effect via suppressing endoplasmic reticulum stress, Biol. Pharm. Bull. 34 (2011) 1481-1486.

[272] P. Sunkireddy, S.N. Jha, J.R. Kanwar, S.C. Yadav, Natural antioxidant biomolecules promises future nanomedicine based therapy for cataract, Colloids Surf. B Biointerfaces 112 (2013) 554-562.

[273] C. Wall, R. Lim, M. Poljak, M. Lappas, Dietary flavonoids as therapeutics for preterm birth: luteolin and kaempferol suppress inflammation in human gestational tissues in vitro, Oxid. Med. Cell. Longev. 2013 (2013) 485201.

[274] C. Agostini, J. Bresson, S. Fairweather-Tati, A. Flynn, I. Golly, H. Korhonen, P. Lagiou, M. Lovik, R. Marchelli, A. Martin, B. Moseley, M. Neuhauser-Berthold, H. Przyrembel, S. Salminen, Y. Sanz, S. Strain, S. Strobel, I. Tetens, D. Tome, H. van Loveren, H. Verhagen, Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), “anti-inflammatory properties” (ID 1882), “contributes to the upper respiratory tract health” (ID 3468), “can help to maintain a normal function of gastrointestinal tract” (3779), and “contributes to body defences against external agents” (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006, EFSA J. 9 (2011) 2033-2058.

[275] M.L. Colombo, An update on vitamin E, tocopherol and tocotrienol-perspectives, Molecules 15 (2010) 2103-2113.

[276] E. Niki, M.G. Traber, A history of vitamin E, Ann. Nutr. Metab. 61 (2012) 207-212.

[277] S.C. Cunha, J.S. Amaral, J.O. Fernandes, M.B. Oliveira, Quantification of tocopherols and tocotrienols in portuguese olive oils using HPLC with three different detection systems, J. Agric. Food Chem. 54 (2006) 3351-3356.

[278] V. Papadimitriou, T.G. Sotiroudis, A. Xenakis, N. Sofikiti, V. Stavyiannoudaki, N.A. Chaniotakis, Oxidative stability and radical scavenging activity of extra virgin olive oils: an electron paramagnetic resonance spectroscopy study, Anal. Chim. Acta 573-574 (2006) 453-458.
[279] Y. Allouche, A. Jimenez, J.J. Gaforio, M. Uceda, G. Beltran, How heating affects extra virgin olive oil quality indexes and chemical composition, J. Agric. Food Chem. 55 (2007) 9646-9654.
[280] S. Casal, R. Malheiro, A. Sendas, B.P. Oliveira, J.A. Pereira, Olive oil stability under deep-frying conditions, Food Chem. Toxicol. 48 (2010) 2972-2979.
[281] A. Dagdelen, G. Tumen, M.M. Ozcan, E. Dundar, Determination of tocopherol contents of some olive varieties harvested at different ripening periods, Nat. Prod. Res. 26 (2012) 1454-1457.
[282] K. Yousfi, C.M. Weiland, J.M. Garcia, Effect of harvesting system and fruit cold storage on virgin olive oil chemical composition and quality of superintensive cultivated 'Arbequina' olives, J. Agric. Food Chem. 60 (2012) 4743-4750.
[283] M. Benito, J.M. Lasa, P. Gracia, R. Oria, M. Abenoza, L. Varona, A.C. Sanchez-Gimeno, Olive oil quality and ripening in super-high-density Arbequina orchard, J. Sci. Food Agric. 93 (2013) 2207-2220.
[284] G. Rigane, M. Boukhris, R.B. Salem, S. Sayadi, M. Bouaaziz, Analytical evaluation of two monovarietal virgin olive oils cultivated in the south of Tunisia: Jemri-Bouchouka and Chemlali-Tataouin cultivars, J. Sci. Food Agric. 93 (2013) 1242-1248.

[285] T.J. Sontag, R.S. Parker, Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status, J. Biol. Chem. 277 (2002) 25290-25296.

[286] E. Niki, N. Noguchi, Dynamics of antioxidant action of vitamin E, Acc. Chem. Res. 37 (2004) 45-51.

[287] J. Atkinson, R.F. Epand, R.M. Epand, Tocopherols and tocotrienols in membranes: a critical review, Free Radic. Biol. Med. 44 (2008) 739-764.

[288] J. Atkinson, T. Harroun, S.R. Wassall, W. Stillwell, J. Katsaras, The location and behavior of alpha-tocopherol in membranes, Mol. Nutr. Food Res. 54 (2010) 641-651.

[289] L. Ulatowski, D. Manor, Vitamin E trafficking in neurologic health and disease, Annu. Rev. Nutr. 33 (2013) 87-103.

[290] Y. Yoshida, Y. Saito, L.S. Jones, Y. Shigeri, Chemical reactivities and physical effects in comparison between tocopherols and tocotrienols: physiological significance and prospects as antioxidants, J. Biosci. Bioeng. 104 (2007) 439-445.

[291] M.A. Kamal, V.A. Raghunathan, Modulated phases of phospholipid bilayers induced by tocopherols, Biochim. Biophys. Acta 1818 (2012) 2486-2493.

[292] A.C. Howard, A.K. McNeil, P.L. McNeil, Promotion of plasma membrane repair by vitamin E, Nat. Commun. 2 (2011) 597.

[293] Y. Yoshida, E. Niki, N. Noguchi, Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects, Chem. Phys. Lipids 123 (2003) 63-75.

[294] A.F. Sonnen, H. Bakirci, T. Netscher, W.M. Nau, Effect of temperature, cholesterol content, and antioxidant structure on the mobility of vitamin E

constituents in biomembrane models studied by laterally diffusion-controlled fluorescence quenching, J. Am. Chem. Soc. 127 (2005) 15575-15584.
[295] M. Nagao, Y. Moriyama, K. Yamagishi, H. Iso, A. Tamakoshi, J.S. Group, Relation of serum alpha- and gamma-tocopherol levels to cardiovascular disease-related mortality among Japanese men and women, J. Epidemiol. 22 (2012) 402-410.
[296] S.J. Weinstein, U. Peters, J. Ahn, M.D. Friesen, E. Riboli, R.B. Hayes, D. Albanes, Serum alpha-tocopherol and gamma-tocopherol concentrations and prostate cancer risk in the PLCO Screening Trial: a nested case-control study, PLoS One 7 (2012) e40204.
[297] J.J. Sutachan, Z. Casas, S.L. Albarracin, B.R. Stab, 2nd, I. Samudio, J. Gonzalez, L. Morales, G.E. Barreto, Cellular and molecular mechanisms of antioxidants in Parkinson's disease, Nutr. Neurosci. 15 (2012) 120-126.
[298] F. Mangialasche, E. Westman, M. Kivipelto, J.S. Muehlboeck, R. Cecchetti, M. Baglioni, R. Tarducci, G. Gobbi, P. Floridi, H. Soininen, I. Kloszewska, M. Tsolaki, B. Vellas, C. Spenger, S. Lovestone, L.O. Wahlund, A. Simmons, P. Mecocci, AddNeuroMed consortium, Classification and prediction of clinical diagnosis of Alzheimer's disease based on MRI and plasma measures of alpha-/gamma-tocotrienols and gamma-tocopherol, J. Intern. Med. 273 (2013) 602-621.
[299] D.K. Das, Tocotrienols: potential drug targets for cardiovascular, cancer and neurological diseases, Curr. Pharm. Des. 17 (2011) 2145-2146.
[300] B. Aggarwal, K. Nesaretnam, Vitamin E tocotrienols: life beyond tocopherols, Genes Nutr. 7 (2012) 1.

Figure captions

Fig. 1. Chemical structures of squalene, carotenoids, sterols, triterpenic dialcohols, and triterpenic acids in virgin olive oil.

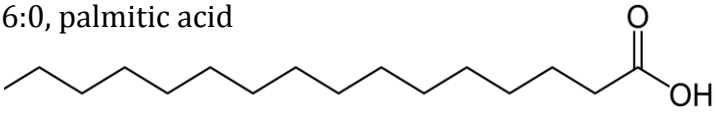
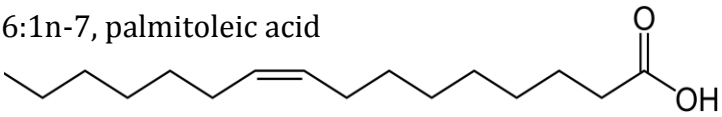
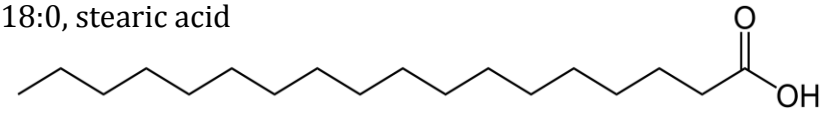
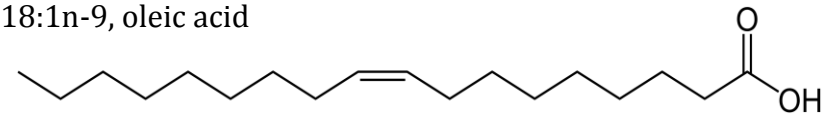
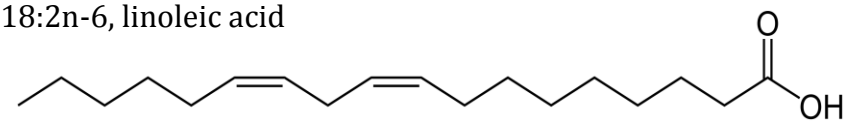
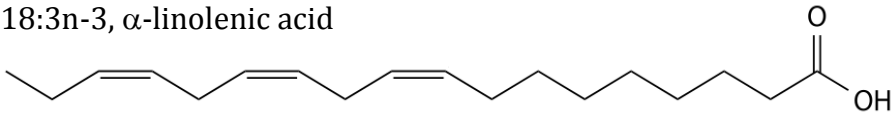
Fig. 2. Chemical structures of secoiridoids, phenyl-alcohols, and phenyl-acids in virgin olive oil.

Fig. 3. Chemical structures of hydroxyisochromans, flavonoids, lignans, tocopherols, and tocotrienols in virgin olive oil.

Fig. 4. Chemical structures of glycerophospholipids and sphingolipids containing oleic acid chains.

Fig. 5. Schematic representation illustrating the estimated positions of oleic acid and various minor constituents of virgin olive oil in a membrane. Lipid-water interfaces are shown in yellow. The chemical structures were obtained from the 3D Conformer utility in the PubChem Structure database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi#).

Table 1
Chemical structure and range of major fatty acids in virgin olive oil.

Fatty acid	Regulations[1] (%)
16:0, palmitic acid 	7.5-20.0
16:1n-7, palmitoleic acid 	0.3-3.5
18:0, stearic acid 	0.5-5.0
18:1n-9, oleic acid 	55.0-83.0
18:2n-6, linoleic acid 	3.5-21.0
18:3n-3, α-linolenic acid 	≤ 1.0
MUFAs, monounsaturated fatty acids	53-87
SFAs, saturated fatty acids	8-26
PUFAs, polyunsaturated fatty acids	3-22

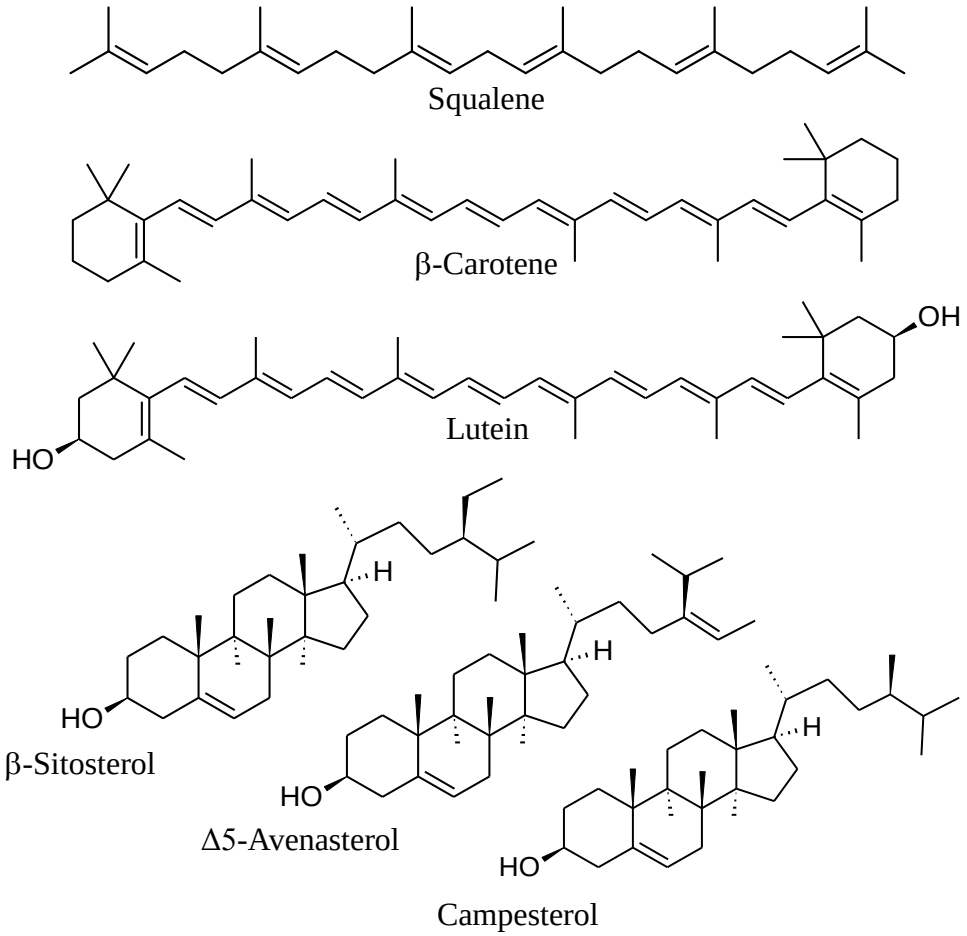
[1]International Olive Oil Council (www.internationaloliveoil.org).

Table 2
Mean values of major fatty acids in several human tissues.

Fatty acid	Subcutaneous adipose tissue [ref. 19]	Skeletal muscle[a] [ref. 20]	Skeletal muscle[b] [ref. 20]	Heart[c] [ref. 21]	Aorta [ref. 22]	Liver [ref. 19]	Brain[d] [ref. 23]	Cerebrospinal fluid [ref. 24]	Whole blood [ref. 24]	Erythrocyte[e] [ref. 25]
14:0, myristic acid	2	3	0.7	1	5	2	0.7	n.d.	n.d.	n.d.
16:0, palmitic acid	22	23	23	27	22	28	21	28	23	35
16:1n-7, palmitoleic acid	6	5	0.6	0.8	n.d.	4	2	3	3	n.d.
18:0, stearic acid	3	6	18	6	8	8	21	19	9	11
18:1n-9, oleic acid	50	51	15	12	31	35	17	25	21	18
18:2n-6, linoleic acid	11	10	30	16	9	12	0.9	7	20	19
18:3n-3, α-linolenic acid	1	0.8	0.3	0.2	8	0.9	n.d.	3	0.7	0.2
20:3n-6, dihomo-γ-linolenic acid	0.3	0.2	1	0.8	n.d.	0.8	1	1	2	n.d.
20:4n-6, arachidonic acid	0.6	0.3	10	16	3	4	9	3	8	5
20:5n-3, eicosapentaenoic acid	0.1	ND	1	0.4	3	0.5	n.d.	2	2	1
22:6n-3, docosahexaenoic acid	0.3	0.4	2	2	n.d.	3	15	1	4	2

[a]Skeletal muscle triglycerides.
[b]Skeletal muscle phospholipids.
[c]Heart phosphatidylcholine.
[d]Frontal neocortex (similar mean values are found in temporal and parietal neocortex).
[e]Erythrocyte phosphatidylcholine.
n.d., not determined.

Figure 1



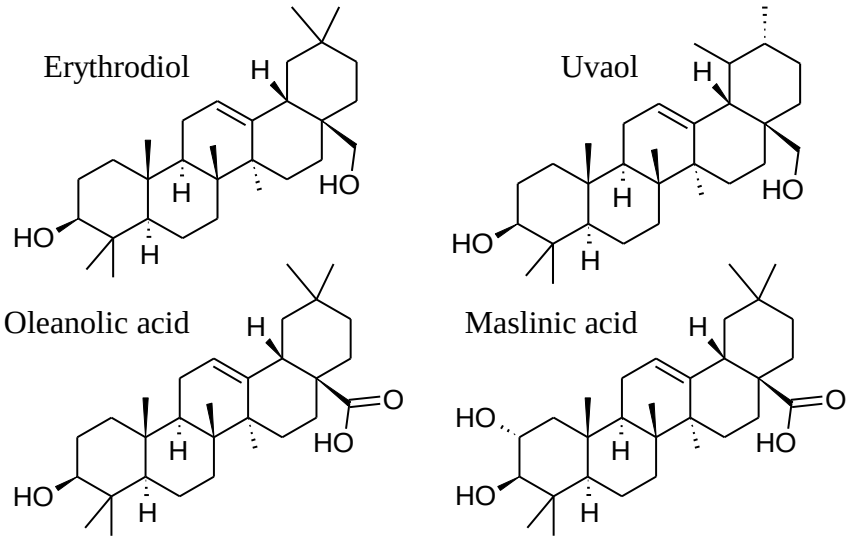


Fig. 1

Figure 2

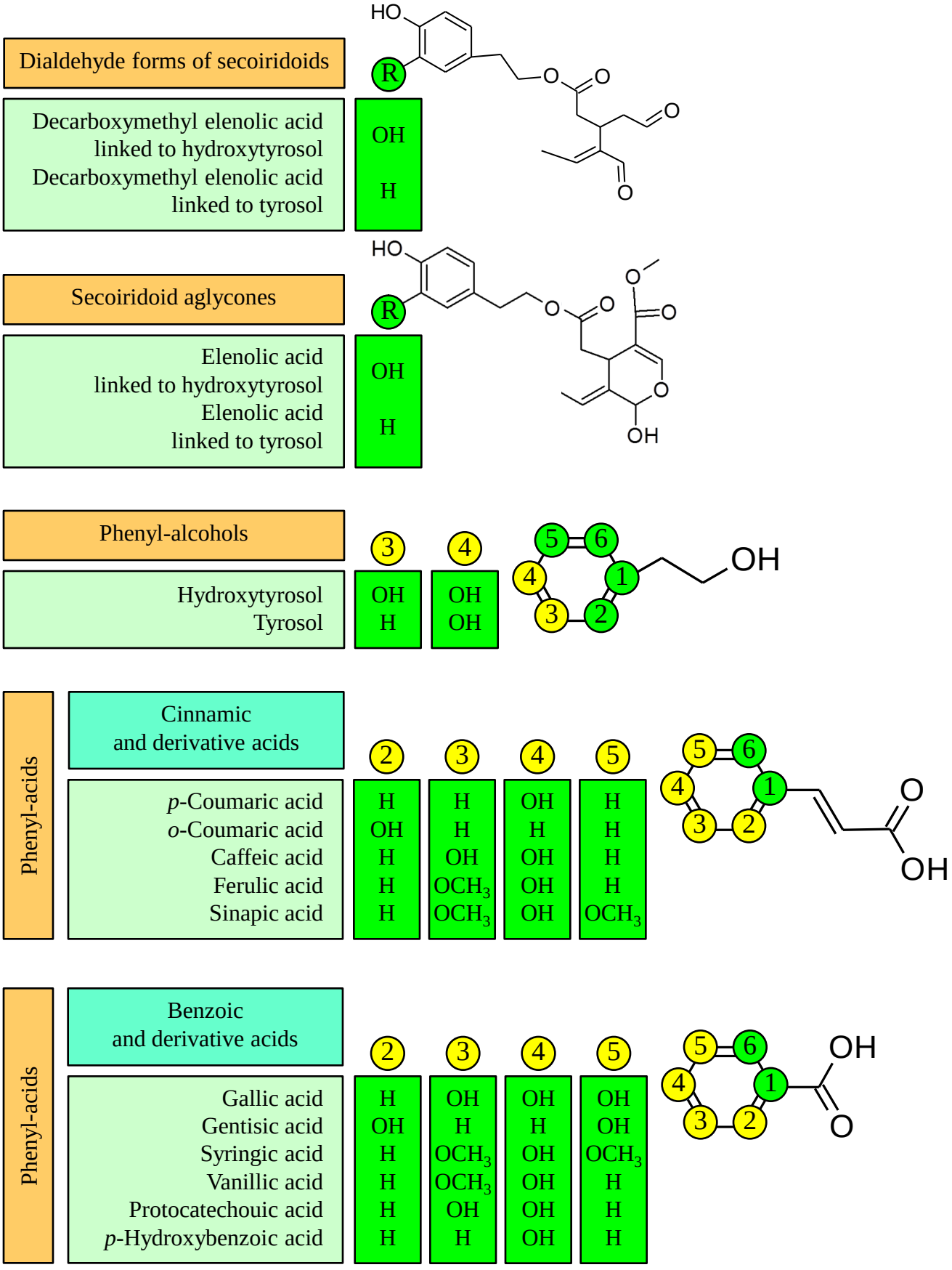


Fig. 2

Figure 3

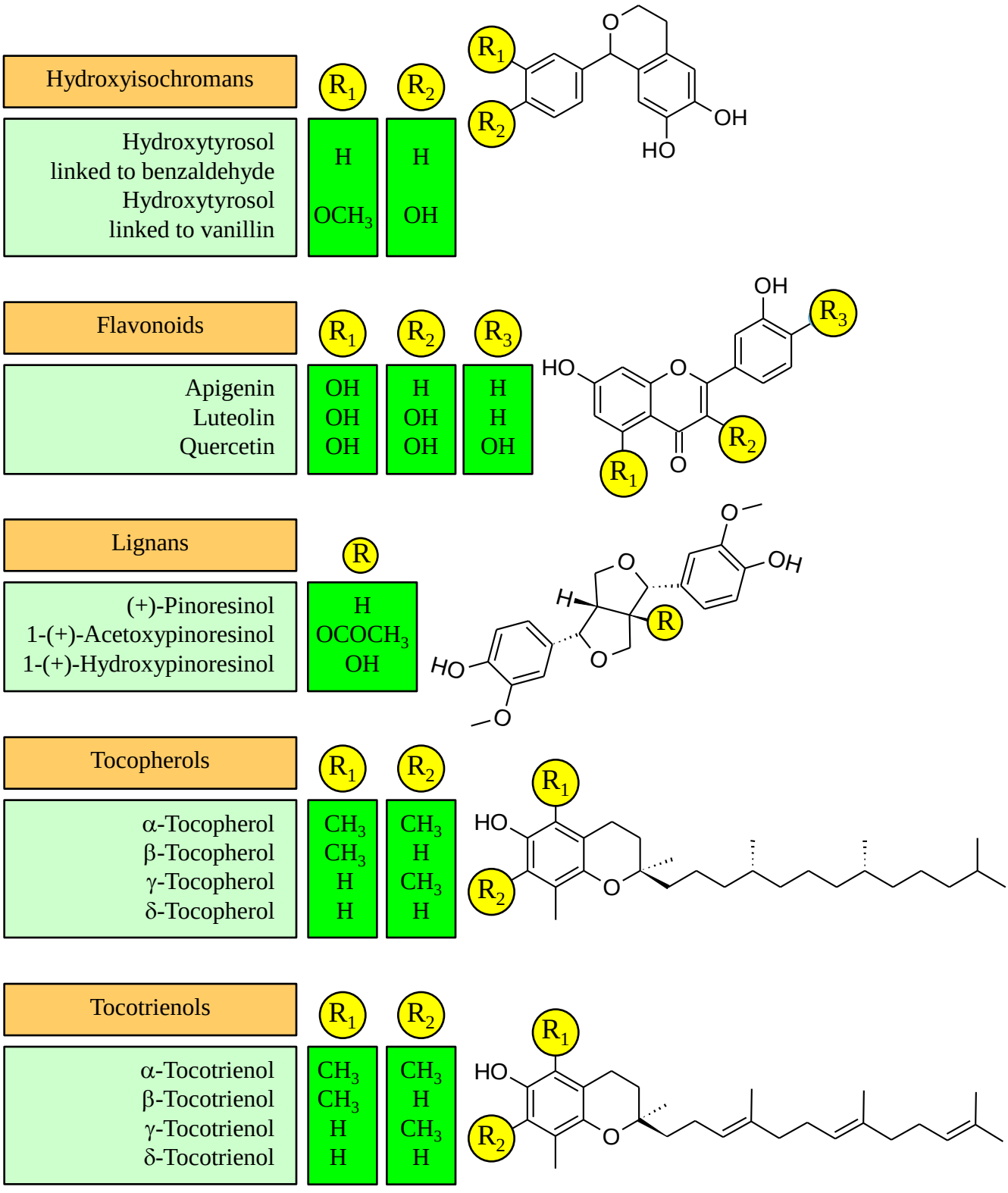


Fig. 3

Figure 4

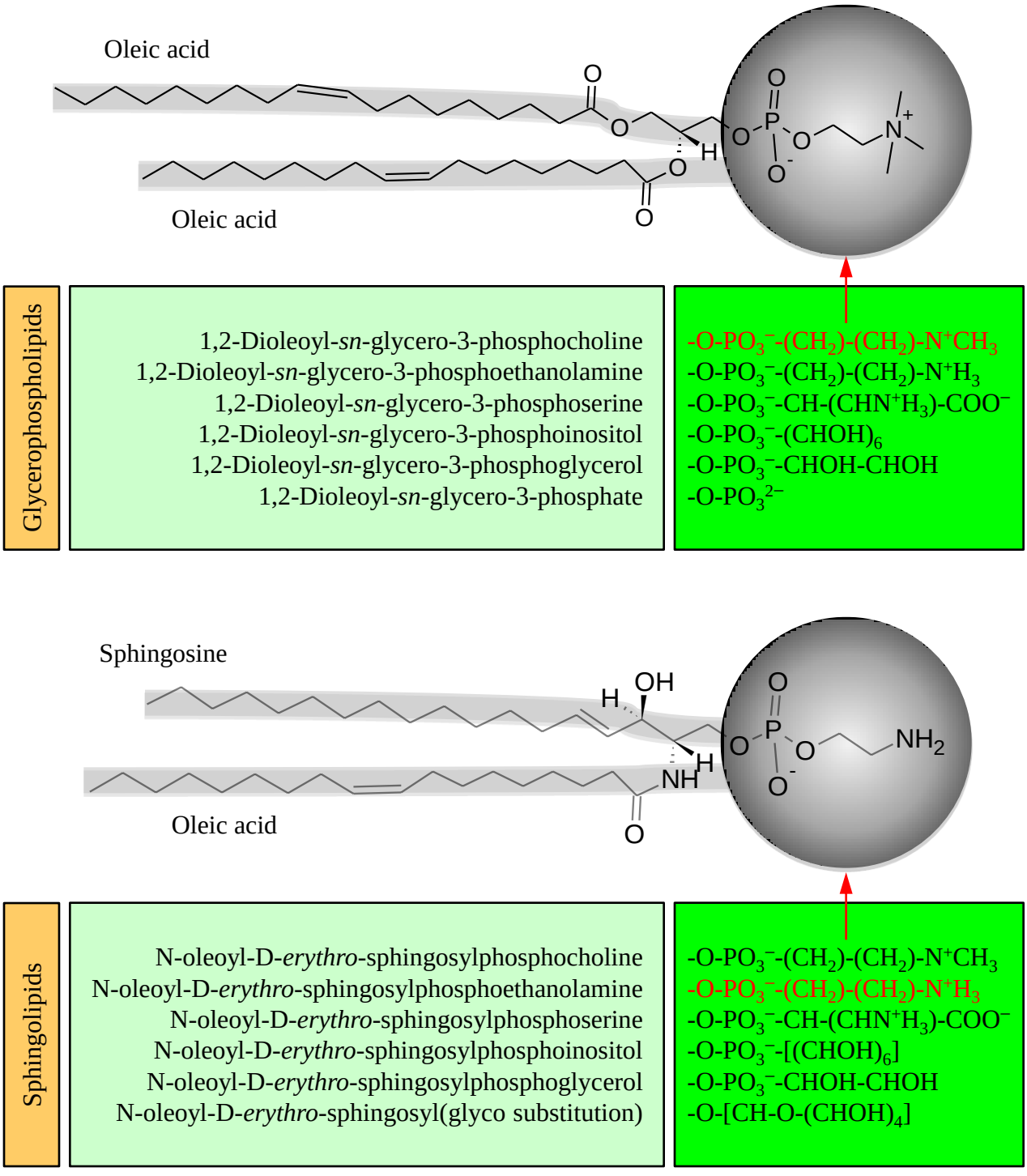
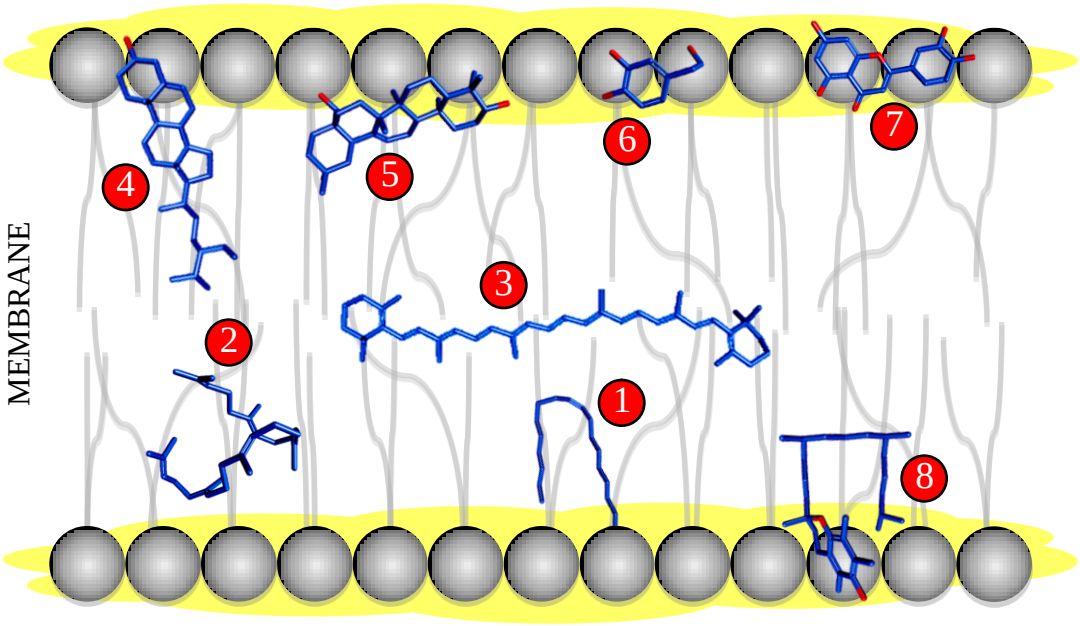


Fig. 4

Figure 5



1 Oleic acid
2 Squalene
3 β-Carotene
4 β-Sitosterol
5 Erythrodiol
6 Hydroxytyrosol
7 Luteolin
8 α-Tocopherol

Fig. 5