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Review

The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health ☆

Maitane Ibarguren [a,b,*,1], David J. López [a,b,*,1], Pablo V. Escribá [a]

[a] Laboratory of Molecular Cell Biomedicine, University of the Balearic Islands, Palma de Mallorca, Spain
[b] Lipopharma Therapeutics S.L., Palma de Mallorca, Spain

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ABSTRACT

This review deals with the effects of synthetic and natural fatty acids on the biophysical properties of membranes, and on their implication on cell function. Natural fatty acids are constituents of more complex lipids, like triacylglycerides or phospholipids, which are used by cells to store and obtain energy, as well as for structural purposes. Accordingly, natural and synthetic fatty acids may modify the structure of the lipid membrane, altering its microdomain organization and other physical properties, and provoking changes in cell signaling. Therefore, by modulating fatty acids it is possible to regulate the structure of the membrane, influencing the cell processes that are reliant on this structure and potentially reverting pathological cell dysfunctions that may provoke cancer, diabetes, hypertension, Alzheimer's and Parkinson's disease. The so-called Membrane Lipid Therapy offers a strategy to regulate the membrane composition through drug administration, potentially reverting pathological processes by re-adapting cell membrane structure. Certain fatty acids and their synthetic derivatives are described here that may potentially be used in such therapies, where the cell membrane itself can be considered as a target to combat disease. This article is part of a Special Issue entitled: Membrane Structure and Function: Relevance in the Cell's Physiology, Pathology and Therapy.

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Contents

1. Introduction

The plasma membrane represents the barrier of life, the structure that separates living cells from their surroundings. For many years, lipids were not considered to be involved in such important events as cell signaling or local hormonal regulation. Indeed, it was many years after linoleic acid (LA) was demonstrated to be an essential dietary constituent that the importance of this finding was recognized by the scientific community [1]. A major milestone was reached in 1979 with the discovery of the first biologically active phospholipid, the platelet-activating factor [2]. Since then, lipids have been found to fulfill some

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* Corresponding authors at: Laboratory of Molecular Cell Biomedicine, Department of Biology, University of the Balearic Islands, Crta. Valldemossa km. 7.5, 07122 Palma, Spain. Tel.: +34 971 17 33 31; fax: +34 971 17 31 84.
E-mail addresses: maitaneibarguren@hotmail.com (M. Ibarguren), davidlopez_95@hotmail.com (D.J. López).

[1] Both authors contributed equally to this work.

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unique biological roles, over and above their function as a source of energy or as simple building blocks of membranes. Indeed, it is now recognized that membrane lipids influence the trafficking of cellular constituents, as well as the activity of membrane proteins and signals.

Cell membranes are composed of thousands of different lipid molecules that interact dynamically to form the transient or stable structures that may be used by many proteins as platforms for their activity, and to enhance their interactions with other proteins. Lipids are a large and diverse group of naturally occurring organic compounds that share common physical properties, such as their solubility in non-polar organic solvents and general insolubility in water. In terms of membrane composition, lipids can be classified into different groups: glycerolipids, sphingolipids and terpene-derived lipids (e.g., sterols). Fatty acids may contribute to complex lipids, although they can be also found as free entities in the membrane. In addition, fatty acids are ubiquitously present in animal fats, vegetable oils or waxes.

Like lipids in general, fatty acids are now no longer considered as a mere energy source but rather, they have generated great interest due to their involvement in human health. In recent years, dietary recommendations have been made to decrease the intake of saturated and trans-fatty acids due to their negative cardiovascular effects, while mono- and polyunsaturated fatty acids are recommended for their cardio-protective benefits [3]. For instance, oleic acid (OA) has been associated with a reduction in blood pressure and a lower incidence of hypertension [4]. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have also been associated with the prevention of cardiovascular diseases and cancer [5], while the omega−6 polyunsaturated fatty acid (PUFA), gamma linolenic acid (γ-LNA), is known to possess anti-inflammatory properties [6]. In addition, altered levels of free fatty acids (FFAs) have been associated with pathological states, as in diseases like obesity, hypertension, diabetes mellitus, coronary heart disease, alcoholism, schizophrenia, Alzheimer's disease (AD), atherosclerosis and cancer [7]. A study carried out on autistic children between 5 and 8 years-of-age showed that they had increased plasma levels of most saturated fatty acids, except for propionic acid, while the concentration of PUFAs was decreased [8]. Other studies showed that both adult and elderly mice reproduced by assisted reproductive technologies, such as in vitro fertilization and intra-cytoplasmic sperm injection, contained lower monounsaturated fatty acid (MUFA) and higher PUFA levels. However, the levels of saturated fatty acids were altered in adult but not in old mice. All these changes might reflect potential effects on the health of animals [9].

In this review, the main effects of natural and synthetic fatty acids as modulators of membrane structure, microdomain organization and cellular signaling are described, focusing on the human health benefits and the new therapeutic approaches that have been developed.

2. Effects of natural and synthetic fatty acids on membrane structure

Fatty acids may exert structural effects on membranes, either as free entities (i.e. FFA) or as part of other molecules such as phospholipids and triacylglycerides. The interaction of FFA with membranes and their incorporation in more complex molecules occurs within minutes. Thus, it is observed that externally added OA in phosphatidylcholine (PC) giant unilamellar vesicle solutions induces vesicle swelling after 3 min [10], indicating that the insertion of the FFA within the membrane structure takes place within that period of time. Another study also described a destabilization of giant unilamellar vesicles composed of palmitoyl-oleoyl-phosphatidylcholine:phosphatidylethanolamine:sphingomyelin:cholesterol (POPC:PE:SM:Cho; 1:1:1:1; mol ratio) after 3-min incubation with OA, arachidonic acid (ARA) and DHA [11]. Interestingly, the same effect was observed when using their 2-hydroxylated, synthetic analogs 2-hydroxyoleic (2OHOA), 2-hydroxyarachidonic (2OHARA) and 2-hydroxydocosahexaenoic (2OHDHA) acid [11]. Concerning the esterification of natural FFA, it is known that the omega−3 eicosapentanoic acid may be detected as part of phospholipids and triacylglycerols in rat liver, brain and heart within 5 min after intravenous infusion [12]. Moreover, the synthetic FFA, 2OHOA, has also been detected in PC, PE, phosphatidylinositol and phosphatidylserine from U118 human, glioma cells, 2–24 h after incubation with this lipid [13]. All in all, the rapid insertion of FFA into membranes and their incorporation in more complex molecules enable these acyl chains to induce changes in the structure of lipid bilayers.

Since the seventies, the effects of fatty acids on model membrane structure have been studied using a variety of techniques, including differential scanning calorimetry (DSC) [14–17], fluorescence spectroscopy [18–20], electron spin resonance [21,22], light scattering [23], electrophoresis [24], nuclear magnetic resonance [25], scanning densitometry [26] and differential thermal analysis [17]. Together, these studies have shown that long-chain saturated fatty acids increase the gel-to-fluid phase (L_{β}-to-L_{α}) transition temperature (also known as melting temperature, Tm) of phospholipid bilayers, whereas short-chain or *cis*-unsaturated fatty acids decrease the Tm. Thus, the length and degree of unsaturation of natural FFAs affect their impact on membrane lipid structure [27]. The perturbations induced by these fatty acids on membrane lipid structure involve changes in membrane fluidity, phase behavior, permeability, membrane fusion, lateral pressure and flip-flop dynamics. For instance, it has been proposed that FFAs perturb the lipid bilayer, and that they disturb the protein–lipid interactions in human erythrocyte membranes [28].

The addition of unsaturated FFAs to liposomes formed by DPPC has also been studied; DHA and EPA particularly producing a broadening and a shift of the Tm values of DPPC to lower temperatures (Table 1). The phase transition temperature and 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence anisotropy values at 37 °C decrease progressively with increasing amounts of unsaturated fatty acids, while Triton X-100 solubilization is facilitated by the presence of unsaturated fatty acids. These data suggest that the tightly packed DPPC bilayer becomes more disordered and fluid when it contains DHA and EPA [29].

Membrane fluidity plays an important role in cellular functions since the activity of membrane proteins is modulated by the surrounding lipid environment. In this case, lipids may influence the optimal conformation for the catalytic activity of proteins by changing the membrane's biophysical properties [30]. For instance, it was proposed that FFAs affect non-specific interactions with the lipid bilayer,

Table 1
Changes in the phase transition temperature of phospholipids upon incubation with different hydroxylated and non-hydroxylated fatty acids.

Lipid under study	Fatty acid	Effect	Technique	Reference
DPPC	30 mol% SA	41.3 °C	[a]DSC, FL	[29]
L_{β}-to-L_{α} 40.8 °C	30 mol% OA	37.5 °C		
	30 mol% EPA	36.3 °C		
	30 mol% DHA	36.2 °C		
DEPE	5 mol% SA	66 °C	XRD	[34]
L_{α}-to-H_{II} 65.5 °C	5 mol% OA	53 °C	XRD, ^{31}P NMR	[34,35]
	5 mol% EA	59 °C	XRD	[34,36]
	5 mol% 2OHOA	55 °C	DSC, XRD	
POPE	2.5 mol% OA	51 °C	XRD	[34]
L_{α}-to-H_{II} 70 °C				
DMPC	5 mol% SA	24.3 °C	DSC	[38]
L_{β}-to-L_{α} 23.4 °C	5 mol% OA	22.6 °C		
	5 mol% (+)-Ricinoleic acid	22.0 °C		
	5 mol% R/S-2-OH octadecanoic acid	24.3 °C		
	5 mol% R/S-2-OH hexadecanoic acid	24.5 °C		
	5 mol% R/S-3-OH hexadecanoic acid	24.3 °C		

[a] DSC, differential scanning calorimetry; FL, fluorescence spectroscopy; XRD, X-ray diffraction; and NMR, nuclear magnetic resonance.

inducing changes in the morphology of the membrane and its fluidity, finally leading to changes in the activity of the human erythrocyte membrane sodium pump [31,32]. It has also been shown that human neutral sphingomyelinase activity in red blood cells is modified by the surface tension of the membrane, which is in turn modulated by the lipid composition of the bilayer [33].

The effect of FFAs on the phase behavior of fully-hydrated lipid mixtures has been measured by a variety of methods (see Table 1). X-ray diffraction experiments show that OA, LA and alpha linolenic acid (α-LNA) significantly decrease the Lα-to-H_{II} phase transition temperature (T_H) of ethanolamine-containing phospholipids 1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine (DEPE) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) [34,35]. Unlike OA, its *trans*-double bonded analog elaidic acid (EA) and the saturated stearic acid (SA) induce smaller changes in the structural properties of DEPE. Experiments done with the synthetic *cis*-monounsaturated fatty acid, 2OHOA, show that this FFA reduces the temperature at which DEPE forms the H_{II} phase and that it destabilizes the lamellar phase [36]. Molecular dynamics simulations confirm the effect of 2OHOA on the structural parameters of membranes composed of DEPE [37]. Interestingly, unsaturated fatty acids have a greater effect on the T_H than on Tm. For instance, 30 mol% OA induces a 3 °C reduction in the Tm of DPPC, while 2.5 mol% induces an ~20 °C reduction in the T_H (Table 1). This suggests that surface packing and lateral pressure are more affected by MUFAs than fluidity.

DSC of 1,2-dimirystoyl-sn-glycero-3-phosphocholine (DMPC) vesicles was performed in the presence of 5 mol% saturated, monounsaturated and hydroxylated FFAs. SA slightly elevated the Tm by about 1 °C, whereas OA decreased it by 1 °C compared to pure DMPC vesicles. The addition of a hydroxyl group to the carbon 12 of OA (ricinoleic acid) further stabilized the fluid phase by decreasing the Tm value. When using the 12-hydroxylated SA derivative, the Tm value was not modified compared to the pure DMPC, although the stabilization of the –OH group on the L_α phase was confirmed by a reduction in the enthalpy of the phase transition. A different pattern is observed in the presence of hydroxylated fatty acids containing the hydroxyl group near the hydrophilic carboxyl end. Here, 2-hydroxystearic acid, 2-hydroxypalmitic acid and 3-hydroxypalmitic acid increased the Tm value in a similar way as SA [38] (Table 1).

The effect of FFAs on membrane permeability has been associated with membrane disorder [39]. It is known that at the gel-to-fluid phase transition of DMPC, when the largest number of defects in the lipid matrix appears, membrane permeability reaches its maximum [40]. Unsaturated FFA prevents iodide and dithionite ion permeabilization without modifying the thermodynamic properties of DMPC bilayers, whereas saturated FFAs show little effect. The postulated mechanism of action suggests that unsaturated FFA affects defected areas within the membrane and prevents ions from crossing the lipid membrane [41]. It has also been demonstrated that DHA more effectively increases permeability than its metabolic precursor, LA or OA [42]. DHA can increase the permeability of phospholipid vesicles and T27A tumor cells, as monitored by vesicle swelling in isomolar erythritol and leakage of sequestered carboxylfluorescein. DHA is incorporated into lipid vesicles either as a FFA or as part of a phospholipid in 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine [43].

Due to their inverted conical shape, FFAs have been postulated to participate in fusion processes [44]. The idea that amphipathic molecules might act as intermediates in membrane fusion was first proposed some years ago [45]. For instance, FFAs containing 10 to 22 carbon atoms were shown to induce the fusion of erythrocytes [46] or model membranes [16]. In model systems, an increase in the size of vesicles composed of saturated PC was observed above a threshold concentration of saturated FFA at temperatures slightly below the lipid phase transition temperature [47]. When isolated chromaffin granules (the secretory vesicles of the adrenal medulla) are aggregated by synexin (a calcium-binding protein present in chromaffin cells and other secretory tissues) and then exposed to *cis*-unsaturated fatty acids at 37 °C, they fuse together to form large vesicles, as determined by phase and electron microscopy, and by turbidity measurements. ARA and OA are the most effective fusogens, whereas saturated and *trans*-unsaturated fatty acids are inactive [48]. FFAs have also been implicated in membrane fusion mediated by proteins of the SNARE family since they can act on syntaxin, a plasma membrane protein directly involved in vesicle fusion [49–51].

Regarding the effect of fatty acids as part of phospholipids, DHA is an example of a fatty acid that affects biophysical parameters of the membrane bilayer, such as lipid packing, the membrane transition temperature, curvature, inter-leaflet lipid flip-flop rate, lipid phase separations and permeability [52,53]. For instance, DHA can provoke the phase separation of bilayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-oleoyl-2-docosahexanoyl-sn-glycero-3-phosphocholine [43,54]. Although DHA is not likely to greatly change the spontaneous curvature of the membrane, the high flexibility of this molecule [53,55] causes a marked reduction in the bending and saddle splay moduli [56].

3. Effects of natural and synthetic fatty acids on membrane microdomain organization

Biological membranes are not homogeneous lipid mixtures but rather, they are composed of dynamic lipid and protein clusters referred to as microdomains that differ in composition from their flanking regions [57]. It is likely that lipid microdomains form within cell membranes, at least in part, as a consequence of the distinct affinities between lipids [58], and that these localized lipid patches have unique compositions that establish environments that favor the activity of specific proteins. Recently, there has been great interest in one particular type of lipid domain, the lipid raft. Rafts are sphingolipid- and Cho-enriched liquid-ordered (Lo) domains floating in "a sea" of liquid-disordered (Ld) phospholipids [59]. Their biological interest stems from the close association of lipid rafts with essential cell signaling proteins, including lipid-linked proteins, such as the src kinase family in the inner leaflet and GPI-anchored proteins in the outer plasma membrane leaflet [60].

While there are compelling reasons to believe that lipid domains exist, and they have been clearly demonstrated in model lipid monolayers and bilayers, their precise nature, composition, size and lifetime/dynamics in biological membranes still remains controversial [61]. The problem resides in the process used to isolate lipid rafts. Traditional methods to isolate rafts from cultured cells and tissue samples have exploited the biochemical properties of these microdomains: their relative resistance to solubilization by non-ionic detergents at 4 °C; and their light buoyant density attributable to their high Cho and sphingolipid content. Thus, raft microdomains are commonly isolated by density gradient separation in the presence of 0.5–1% Triton X-100. This and other detergent-based methods have been discussed extensively, and new methods to isolate rafts from cultured cells without the use of detergents have also been proposed [62]. Techniques that allow researchers to track and manipulate single molecules or small groups of molecules in the cell membranes of living cells have become widely available. These methods include single fluorescent molecule video imaging using fluorescent probes, single particle tracking using colloidal-gold probes, and optical trapping, which allows researchers to move gold particle-conjugated molecules in living cell membranes [63–65]. These methods that enable researchers to see and grab single molecules in living cells will greatly advance our understanding about the cell membrane functions. Although such studies have only been initiated recently, they have already started to yield new significant insights that could not have been obtained without observations at the level of each individual molecule [66]. Although the idea of membrane domains seems relatively novel, lipid domains in biological membranes and their implication in cell function were first

described in 1979 [67]. From the very outset, it was postulated that partitioning of FFAs into membranes could selectively perturb lipid domains in membranes, and that such perturbation could disturb the behavior of proteins embedded in such lipid domains [19,68,69].

Studies using free 2OHOA in model membranes showed that the packing of ordered domains is enhanced while the global order of the membrane decreases [13]. In addition, 15 mol% of OA, ARA, DHA and their 2-hydroxylated analogs 2OHOA, 2OHARA and 2OHDHA were recently shown to increase cell membrane fluidity when incorporated into lipid bilayers (palmitoyl-oleoyl PC:PE:SM:Cho; 1:1:1:1; mol ratio), reducing the Lo/Ld ratio from ca. 50 to ca. 40%. In addition, the insertion of both natural and synthetic FFAs into model membranes induces a transformation of the lipid structures into new microdomains that differ in size and composition from their initial situation (Fig. 1). This reorganization of lipid microdomains is thought to modulate the localization of signaling proteins, thereby controlling their intracellular cascades [11].

The mouse fibroblast plasma membrane is an excellent model system to study how fatty acids influence the membrane, since it contains no PUFAs when cultured in serum-free, chemically defined medium [70]. The fatty acyl composition of phospholipids from mouse fibroblast cells is dramatically altered by supplementing the culture medium with LA and LNA. It has been shown that the addition of these two fatty acids decreases the molecular order of the membrane and changes the organization of different domains by transbilayer sterol redistribution [70,71]. Thus, in plasma membrane from mouse fibroblasts without FA in the cell medium, the cytosolic leaflet is enriched in sterol, while the presence of PUFA the transbilayer sterol gradient is reversed. In smooth muscle cells, Cho efflux increased on exposure to EPA and DHA, whereas OA, LA or ARA has no effect on this process [72].

Alzheimer's disease is a neurodegenerative disorder characterized by the accumulation of amyloid β (Aβ) in perivascular deposits and senile plaques within the brain. The intake of the DHA has been associated with decreased amyloid deposition and reduced risk of AD in several but not all, epidemiological studies [8]. However the exact molecular mechanism underlying this effect of DHA remains unknown. DHA is a major component of fish oils and it is required for the adequate development of the human central nervous system, as well as for the continued maintenance of brain cell functions. It has been shown that DHA-containing phospholipids affect the organization of plasma membranes, inducing changes in raft and non-raft domains [73]. Isothermal titration calorimetry and molecular dynamics simulations have shown that monomeric Aβ42 binds more tightly to Ld regions than to Lo ones to model membranes [74]. Meanwhile, Aβ peptide dimers accumulate in Lo, lipid raft domains in mice [75]. Together, it appears that the organization of membrane microdomains is implicated in the binding of Aβ to the membrane, and that DHA can control the accumulation of these deposits by modulating the distribution of Lo and Ld domains.

Although it has not been demonstrated, the mechanism of action of DHA could be similar to other fatty acid derivatives such as the synthetic alkylphospholipids edelfosine and miltefosine. These molecules modify the plasma membrane lipid composition, namely Cho and SM content, inducing raft coalescence and provoking apoptosis in several cancer cells [76]. Fluorescence lifetime imaging microscopy measurements in Jurkat cells show that edelfosine and miltefosine do not significantly affect the membrane biophysical properties of both model and cell membranes. However, a slight disordering of the membrane was detected in model membrane systems [77].

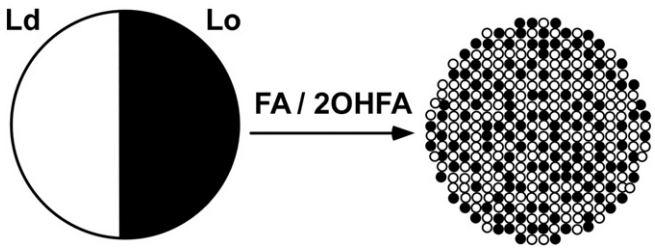

Fig. 1. Model of lipid domain reorganization induced by natural and 2-hydroxylated FA derivatives upon insertion into artificial membranes. The Lo/Ld domain ratio is reduced from *ca.* 50% to *ca.* 40% of the total membrane surface upon insertion of fatty acid and 2-hydroxylated fatty acid derivatives into a lipid membrane. The size of the Lo and Ld domains decreases and the lipid composition is modified.
Taken from [11], with permission.

4. Effects of natural and synthetic fatty acids on cell signaling and pathophysiology

The effects of FFAs on membrane structure are of particular interest as they influence membrane protein function and signal propagation. FFAs have been shown to act both as modulators and messengers, particularly of signals triggered at the level of cell membranes. The multiple effects of FFAs appear to involve the positive or negative regulation of a wide range of signaling cascades (e.g., inositol phosphate metabolism, inflammation and calcium-dependent signaling).

The relationship between membrane fatty acid composition and inositol phosphate metabolism has been studied in human colon tumor cells cultured in media with different concentrations of saturated and unsaturated fatty acids. This study showed that PUFAs increase the response of the inositol phosphate cycle to deoxycholic acid stimulation [78]. In addition, enrichment in omega − 3 α-LNA increases the basal turnover of inositol phosphate. All in all, alterations in membrane fatty acids have a profound effect on the inositol phosphate cycle. Fatty acids are also closely related to inflammation since eicosanoids, mediators and regulators of inflammation, are generated from 20-carbon PUFAs. Indeed, the mechanism by which non-steroidal anti-inflammatory drugs induce apoptosis in human colorectal cancer cells seems to be related to the levels of fatty acids. These drugs inhibit the activity of cyclooxygenases, which increases the cellular pool of ARA. At the same time, ARA stimulates neutral sphingomyelinase activity [79], which catalyzes the hydrolysis of SM to generate ceramide, a pro-apoptotic second messenger [80]. While ARA is considered as a pro-inflammatory lipid, EPA and DHA have been studied as anti-inflammatory molecules due to their capacity to reduce the production of pro-inflammatory cytokines via the NF-κB signaling pathway [81]. Moreover, EPA is itself a substrate for cyclooxygenase and lipoxygenase, giving rise to mediators that often have opposite biological effects to those of ARA.

Concerning calcium-dependent signaling pathways, it is well known that calcium is a vital intracellular second messenger that governs a wide array of cellular processes. There is considerable evidence that the accumulation of membrane-derived FFAs is important in the pathogenic events caused by myocardial ischemia [82]. Low concentrations of long-chain unsaturated and saturated fatty acids induce multifold increases in voltage-dependent calcium currents in cardiac myocytes, which causes irreversible cardiac damage. By contrast, neither short-chain fatty acids nor fatty acid esters have any effect on voltage dependent calcium currents, indicating that activation of calcium channels depends on chain length and requires a free carboxyl group. Moreover, inhibition of protein kinase C (PKC), PKA, G proteins, eicosanoid production, or non-enzymatic oxidation does not block the fatty acid-induced increases in intracellular calcium. Thus, long-chain fatty acids appear to be activators of calcium channels by possibly acting at lipid specific sites near the channel or directly on the protein channel itself [82]. In human keratinocytes, triglycerides and saturated fatty acids do not affect the intracellular calcium concentration, whereas unsaturated fatty acids increase intracellular calcium, altering the calcium dynamics in epidermal keratinocytes and inducing abnormal follicular keratinization [83]. LA has also been related to calcium signaling since it binds to mouse CD36-positive gustatory cells, it induces Src-PTK phosphorylation, it triggers calcium signaling, and it evokes the release of 5-

hydroxytryptamine and noradrenalin, which in turn may be implicated in the downstream signaling to the afferent nerve fibers that transmits the output signal from taste buds to the central nervous system (CNS) [84].

It has been shown that several signal transduction systems are involved in the regulation of cell proliferation, including the PKC system, which may play an important role in fast growing cells [85]. A key enzyme in this system is phospholipase C, a membrane bound enzyme that produces the second messenger DAG for PKC activation. Phospholipase C is regulated by G proteins, which are in turn sensitive to the presence of omega 6 γ-LNA in the membrane [86].

High olive oil consumption reduces the incidence of cardiovascular disease and several types of cancer [9]. Olive oil intake increases the levels of OA, which can modulate the membrane structure and concomitantly, the localization and/or activity of signaling proteins (G-proteins, PKC and adenylyl cyclase) [87,88]. Regulation of adenylyl cyclase activity by G proteins controls blood pressure [89], consistent with the hypotensive effects of OA derivatives [4,90,91]. 2OHOA, is a potent anti-cancer drug that induces cell cycle arrest in human lung cancer cells [92] and apoptosis in human leukemia cells [93]. Its anti-cancer activity has been associated with different cell effects. On the one hand, this molecule augments the propensity of membrane lipids to organize into non-lamellar, H_{II} structures, promoting the subsequent recruitment of PKC to the cell membrane after 15–60 min [92]. This event is followed by activation of $p21^{CIP1}$, cyclin-cdk inhibition, pRb hypophosphorylation and E2F1 knockdown. E2F1 is a critical transcription factor that regulates several genes involved in cell proliferation, including DHFR. The down-regulation of DHFR impairs DNA synthesis, which results in either cell cycle arrest or apoptosis [94,95]. On the other hand, treatment with 2OHOA induces translocation of Ras from the membrane to the cytoplasm in the first 10 min, which inhibits the MAP kinase pathway and dampens the activity of the PI3K/Akt pathway, downregulating cyclin D-cdk4/6 expression and provoking hypophosphorylation of the retinoblastoma protein [96] (Fig. 2). 2OHOA also causes a deregulation of phospholipid metabolism, which includes a dramatic increase of SM, ceramide, cerebrosides and lactosyl-ceramide after 6 h in U118 human glioma cells [97]. Besides, the increase in SM content, due to activation of sphingomyelin synthases, correlates with a decrease of PC and PE cell levels [98].

The gray matter of the frontal lobe and hippocampus in the brain of AD patients has a lower DHA content than that of healthy individuals [99]. Fish oil enriched in DHA has been shown to reduce Aβ42 production and to have a strong neuroprotective effect on primary cultures of human neurons [100]. DHA promotes neuronal survival by facilitating the membrane translocation and activation of Akt through its capacity to increase phosphatidylserine levels, the main acidic phospholipid in the cell membrane [101]. Indeed, it is known that high phosphatidylserine content increases hippocampal synaptic efficacy [102].

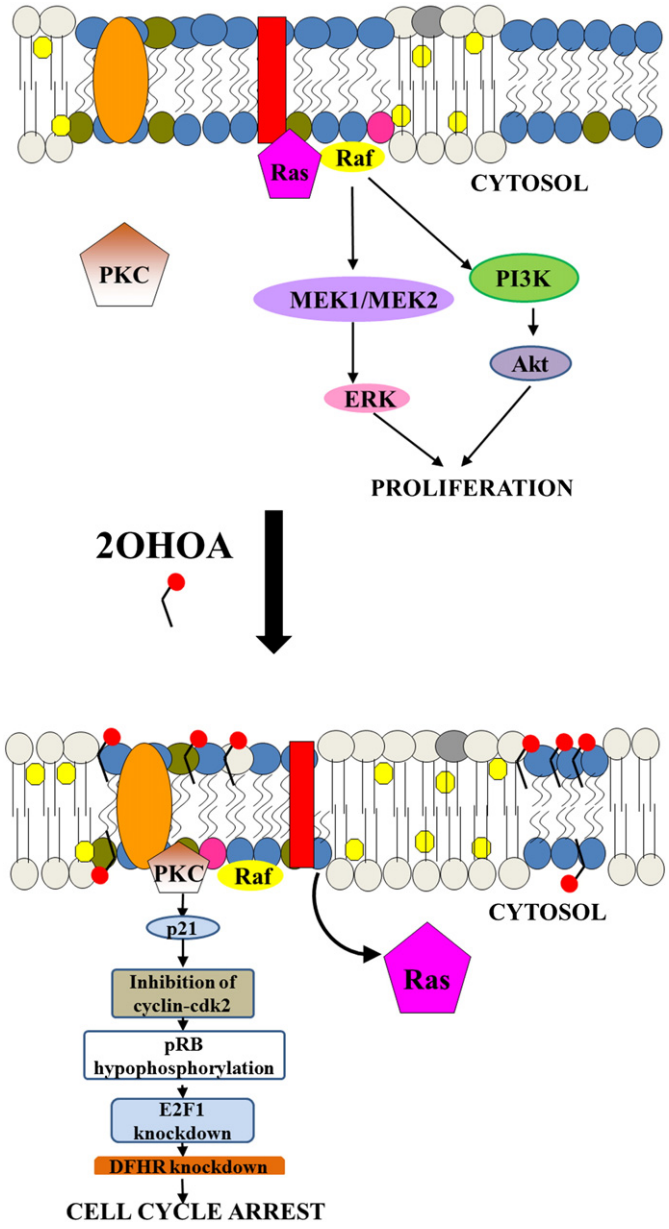

Fig. 2. Mechanism of action of 2OHOA. 2OHOA induces the translocation of PKC to the membrane and its subsequent activation. This activation leads to p21 overexpression, which is associated with the hypophosphorylation of pRB, and the ensuing inhibition of E2F-1 and silencing of DHFR. Conversely, Ras is translocated to the cytoplasm in the presence of 2OHOA, which would inhibit the PI3K/Akt and ERK pathway and the proliferation machinery. This leads to cell cycle arrest, apoptosis, differentiation and autophagy.

5. Natural fatty acids in human health

Fatty acids are part of the fat contained in food products and they contribute to their flavor and consistency, and lead to the feeling of fullness when eating. Moreover, fat, and fatty acids in particular, are a major source of energy and they aid the absorption of lipophilic substances like vitamins A, D, E and K. Humans lack the ability to synthesize omega − 3 from omega − 6 fatty acids, and vice versa, and they lack Δ^{12}- and Δ^{15}-desaturase activities, which are responsible for the formation of a double bond in the carbon 12 and 15 of an acyl chain, respectively. Thus, the omega − 3 fatty acid α-LNA ($18{:}3\Delta^{c9,12,15}$) and the omega − 6 fatty acid LA ($18{:}2\Delta^{c9,12}$) are the two essential fatty acids in humans (i.e., they must be incorporated into the metabolism through the diet) [103,104]. From these, other fatty acids can be produced by human cells through different metabolic pathways (Fig. 3). For instance, through desaturation and chain elongation reactions, LA serves as the precursor for omega − 6 γ-LNA ($18{:}2\Delta^{c6,9,12}$) and ARA ($20{:}4\Delta^{c5,8,11,14}$). On the other hand, omega − 3 α-LNA can give rise to other omega − 3 counterparts such as EPA ($20{:}5\Delta^{c5,8,11,14,17}$) and subsequently, DHA ($22{:}6\Delta^{c4,7,10,13,16,19}$). However, the capacity to convert LA to ARA and α-LNA to DHA is costly in metabolic terms and their dietary absorption may be required for the correct development of brain and retinal tissue in infants [105]. In fact, studies have shown that breast-fed infants have a greater mean percentage of DHA (by weight) than formula-fed infants. Moreover, supplementation of maternal diet during pregnancy and breastfeeding with cod liver oil, which is enriched in DHA and EPA, improve children's intelligence quotient at 4 years of age compared with corn-oil supplementation [106].

In recent years, much attention has been paid to fatty acids due to their implications in human health. The amount and type of fatty acids consumed are directly involved in the etiology of various diseases,

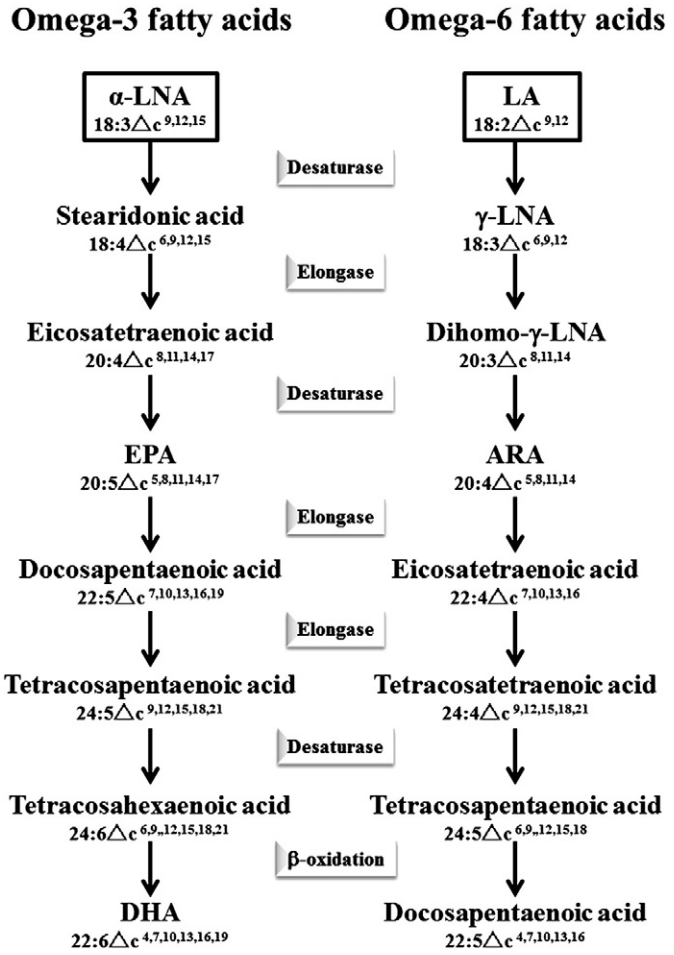

Fig. 3. Metabolism of omega−3 and omega−6 fatty acids. Different omega−3 and omega−6 fatty acids are produced through elongation and desaturation of the essential α-LNA and LA fatty acids, respectively. The addition of double bonds and the elongation of the acyl chains occur in the endoplasmic reticulum, while the final step in the synthesis of the omega−3 DHA and the omega−6 docosopentaenoic acid consists of a single reaction from β-oxidation in the peroxisome.

such as diabetes, cancer, neuromuscular disorders, visual dysfunction, cardiovascular disease, psychiatric disorders, immunity and inflammatory disease, kidney disease, liver disease and aging [107]. Dietary fatty acids are essential for normal growth, development and homeostasis, and they fulfill a number of important functions. Increasing evidence indicates that fatty acids and their derived substances may mediate critical cellular events, including gene activation and expression, and the regulation of cell signaling [108]. Understanding the mechanisms by which fatty acids exert their biological effects is important in unraveling the pathogenesis of these disorders, and it may help provide effective preventive measures. For instance, a reduced risk of coronary heart disease has been described in people who follow a diet enriched in the omega−3 fatty acids EPA and DHA [109]. Indeed, the interest in these types of lipids arose from observation that the mortality of Greenland Eskimos related to coronary heart disease was only 10% that of Caucasians. Although the amount of body fat in both populations was similar, the diet of native Eskimos was rich in omega−3 PUFAs. In addition to the lower occurrence of coronary heart disease, the Eskimo population also had a lower prevalence of inflammatory and immune diseases like asthma, psoriasis or rheumatoid arthritis [110].

The beneficial effect of these lipids has become so popular that dietary supplements containing omega−3 fatty acids have become available as nutraceutics. By contrast, consumption of saturated and *trans* unsaturated fatty acids favors the increase of low-density lipoprotein Cho, which increases the risk of coronary heart disease [111]. Moreover, many countries have adopted guidelines indicating the amount of *trans* fat per serving on the nutritional labels of food products. Interestingly, few reports have demonstrated definitive relationships between dietary *trans* fatty acids and obesity [112], although in recent decades, the prevalence of obesity has increased steadily, mainly in developed countries, and it has been considered as an important risk factor contributing to the development of the three leading causes of death—cardiovascular disease, cancer and diabetes—as well as other disorders worldwide [113].

It has been proposed that elevated omega−6 fatty acid intake may promote inflammation, while omega−3 fatty acids help reduce it. For instance, omega−6 ARA is the precursor of the pro-inflammatory molecules prostaglandins, thromboxanes, prostacyclins and leukotrienes [114]. However, there are several different types of omega−6 fatty acids and not all promote inflammation. In fact, γ-LNA is known to possess anti-inflammatory properties [6]. Indeed, a healthy diet may need to establish a balance between omega−3 and omega−6 fatty acids to equilibrate cell activity [115]. It has been estimated that humans have evolved on a diet based on an omega−6/omega−3 ratio of approximately 1, whereas this ratio has nowadays increased to 15–20/1, indicating that Western diets are deficient in omega−3 fatty acids [116]. However, differences can also be found between current diets in different regions and, interestingly, the omega−6/omega−3 fatty acid plasma ratio is substantially lower in the Mediterranean (2.60 ± 0.19) than in the Swedish diet (4.72 ± 0.19, $p < 0.0001$) [117].

In 2010, the Mediterranean diet was recognized by the UNESCO as an Intangible Cultural Heritage of Humanity. This dietary pattern is characterized by a high consumption of plant foods (i.e. vegetables, fruits, beans and cereals), a high intake of olive oil, enriched in *cis* omega−9 OA (C18:1Δ^{c9}), moderate fish intake, low-to-moderate intake of dairy products, low meat consumption and the consumption of wine in moderate amounts during meals. A study carried out over 5 years in 6174 volunteers led to the conclusion that this type of diet, with a higher monounsaturated-to-saturated fatty acid ratio, is related to more favorable cognitive capacities in older age [118]. In line with this, the Mediterranean diet is associated with a blood pressure reduction and lower incidence of hypertension, which was directly linked to OA intake [4,119]. It has been proposed that the most abundant fatty acid in olive oil, OA, is responsible for the decrease of blood pressure associated with high olive oil consumption. The intake of olive oil increases OA levels in membranes, which regulates membrane lipid structure by increasing the propensity to form H_{II} phases in the lipid bilayers [35]. The subsequent reduction in blood pressure is modulated by the regulatory effects on G protein-associated signaling cascades ($\alpha_{2A/D}$-adrenoreceptor/G protein/adenylyl cyclase-cAMP/PKA) [88]. Interestingly, elaidic (C18:1Δ^{t9}) and stearic (C18:0) acids, the respective *trans*-doubled-bonded and saturated OA analogs, have no hypotensive activity, indicating that the molecular mechanisms that link membrane lipid structure and blood pressure regulation are very specific. This is most likely due to the divergent structural effects on the lipid bilayer (see below). Similarly, soybean oil does not reduce blood pressure and given its low percentage of OA, this favors the hypothesis that the hypotensive effect of olive oil is due to OA [4].

OA is also known for its beneficial effects on cancer. Thus, OA can down-regulate the overexpression of erbB-2, an oncogene involved in the metastasis of several human cancers and it can also induce apoptosis in carcinoma cells, possibly related to an increase of ROS levels and caspase-3 activity [120]. Protein:OA complexes, such as the so-called HAMLET (human α-lactalbumin made lethal to tumor cells) and BAMLET (bovine α-lactalbumin made lethal to tumor cells), have generated great interest in the antitumoral research field [[121] and references therein]. HAMLET and BAMLET are formed by the binding of OA to a partially unfolded conformation of human and bovine α-lactalbumin, respectively, and they have been shown to possess cytotoxic effects both in vitro [122] and in vivo [123]. This cytotoxic effect

was patent not only with OA but also, in complexes with other unsaturated fatty acids, and it diminishes when they were coupled to the saturated SA. The mechanisms of action of these complexes seem to be related to apoptotic processes like caspase activation, loss of mitochondrial potential and chromatin destabilization. HAMLET was also observed to bind to and induce membrane permeabilization in artificial model membranes and living cells [124], which may be consistent with the previously described effects.

Natural lipid molecules derived from free fatty acids are also known for their influence on cell function. Thus, resolvins and protectins are natural hydroxylated derivatives of omega−3 EPA and DHA that are known to possess anti-inflammatory activity [125]. DHA is hydroxylated at carbon 17 by the enzyme 15-lipoxygenase and by acetylated COX-2, producing 17S- and 17R-hydroxydocosahexaenoic acid (17OHDHA), respectively. These compounds are further hydroxylated to give rise to the trihydroxy derivatives 17-(S/R)-resolvins D1, D2, D3 and D4, and the dihydroxy compound 17-(S/R)-protectin (Fig. 4A). On the other hand, EPA is hydroxylated to 18-(S/R)-hydroxyeicosapentaenoic acid (18OHEPA), which is further processed to form 18-(S/R)-resolvins E1 and E2 (Fig. 4B). Given the importance of 17HDHA and 18OHEPA as indicators of the presence of resolvins and protectins, it is important to note that these metabolites can be found in human and mice blood [126] and that their presence is directly related to the intake of omega−3 in transgenic fat-1 mice [127].

Protectin D1 offers protection against AD by regulating secretase-mediated production of the Aβ peptide, by downregulating pro-inflammatory gene expression and by promoting cell survival. In human neural cells overexpressing beta-amyloid precursor protein, the lipid mediator suppressed Aβ42 shedding by downregulating β-secretase while activating α-secretase, thereby shifting amyloid precursor protein cleavage from the cytotoxic amyloidogenic pathway towards the non-amyloidogenic, neurotrophic pathway [128].

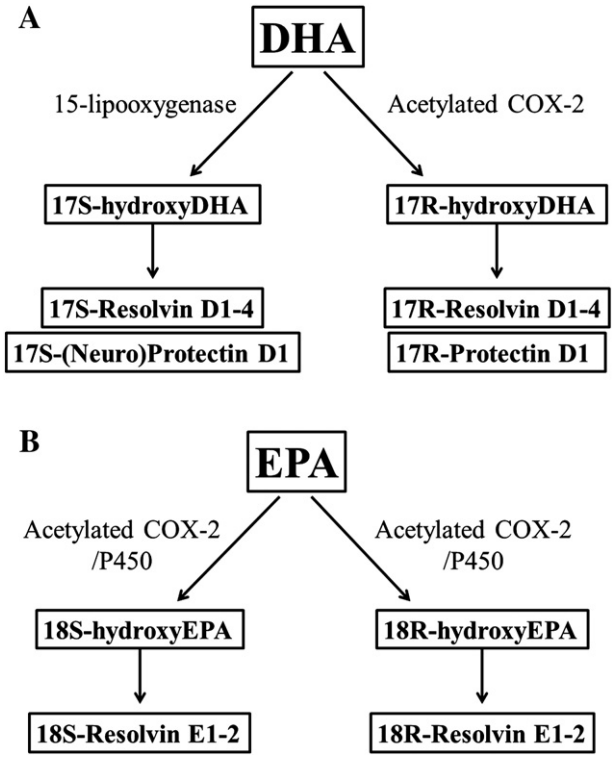

Fig. 4. Resolvin and protectin synthetic pathways. (A) Formation of the D-series of resolvins and protectins commences with the hydroxylation of DHA (D nomenclature derives from DHA) that gives rise to the S- and R-isomers of 17-hydroxydocosahexaenoic acid. Subsequent hydroxylation reactions produce the different isomers of resolvins D1-4 and protectin D1. (B) EPA is the precursor of the E-series of resolvins (name derived from EPA) that begins with the production of the 18S- and 18R-hydroxyeicosapentaenoic acid.
Adapted from [125].

Inflammation may have beneficial effect for host defense, although a resolution of inflammation is necessary in order to avoid chronic inflammatory situations. The term resolvins or 'resolution-phase interaction products' was coined by Serhan and colleagues, because these compounds were first encountered in resolving inflammatory exudates of murine models [129]. These compounds were shown to offer protection from acute ischemic kidney injury [130] and they alleviate induced colitis by reducing the levels of pro-inflammatory cytokines [131]. They are also effective against asthma, since they regulate the migration and recruitment of immune cells and they decrease the levels of pro-inflammatory interleukins [132]. Another DHA-derived monohydroxy lipid that is produced by 5-lipoxygenase and that possesses potent biological activity is 4-hydroxydocosahexaenoic acid. This molecule directly inhibits endothelial cell proliferation and angiogenesis via peroxisome proliferator-activated receptor γ (PPARγ), a mechanism that is independent of its anti-inflammatory effects [133]. However, not all hydroxylated lipid derivatives are beneficial for human health. Indeed, *trans*-4-hydroxy-2-hexenal is an aldehyde side product of DHA peroxidation that is accumulated in the CNS of patients with AD and it is thought to induce neurotoxicity [134].

Another interesting group of fatty acids is that formed by the conjugated linoleic acid. This is a family of 28 naturally occurring isomers of LA, with both *trans* and *cis* double bonds produced by bio-hydrogenation in ruminants or by bacteria from the gastrointestinal tract of humans. The consumption of ruminant meat (beef and lamb) and dairy products (milk and cheese) is the main source of dietary conjugated linoleic acids. In this case, the predominant isomer formed is 9-*cis*, 11-*trans*, also known as rumenic acid, and unlike other *trans* double bond-containing fatty acids, it may have beneficial effects on human health [135]. However, other conjugated linoleic acids are also available, mostly a mixture of 9-*cis*, 11-*trans*, 10-*trans*, and 12-*cis* isomers. These fatty acids have been shown to produce effects against cancer and inflammation, as well as changes in cell metabolism that lead to weight loss, although their true effect and the mechanism of action in such processes remain unclear [136,137]. Interestingly, conjugated linolenic acids, which are more abundant than conjugated linoleic acids in natural sources, have also been recently described as inhibitors of colorectal tumorigenesis [138].

Finally, naturally occurring omega−9 fatty acid derivatives also exist. Oleamide is an amide derivative of the OA that can be found endogenously in cerebrospinal fluid when animals are sleep deprived. This compound induces sleep in rats when injected at doses above 5 mg [139] and its mechanism of action seems to be related to the modulation of receptors that activate G proteins, playing a role in alertness and sleep [140]. Oleamide is structurally related to endogenous cannabinoids and it has the ability to bind to the cannabinoid receptor, as well as possessing significant hypnotic, analgesic and anxiolytic effects but only producing very weak dependence [141].

The regulation of membrane structure, at least partially, explains the modulation exerted by some natural fatty acids on membrane and cell function. Indeed, the unsaturated fatty acids OA, LA and LNA stabilize H_{II} structures in PE membranes, and OA increases fluidity in the lamellar phase [35], although neither EA nor SA alter the phospholipid mesomorphism. Interestingly, membrane-modifying natural fatty acids are effective in certain therapeutic applications. For instance, blood pressure is decreased in rats upon treatment with OA but not with EA or SA [4]. Similarly, OA but not EA induces weight loss in rats [142]. Concerning their antitumoral properties, *cis*-unsaturated OA has a moderate antiproliferative effect on tumor cells [93], while its *trans*-unsaturated congener EA does not inhibit cell growth at all.

6. Therapeutic approaches using synthetic fatty acids and related lipid derivatives

The seven-carbon heptanoic acid and the corresponding triacylglyceride triheptanoin have proven useful to treat glycogen metabolic syndrome. These seven-carbon acyl chain moieties are metabolized by β-oxidation to produce propionyl-CoA, which is subsequently carboxylated to succinyl-CoA. The latter is one of the metabolites of the Krebs cycle and its incorporation in the metabolic pathway results in anaplerosis (refilling of the tricarboxylic acid cycle). Heptanoate is also transformed in the liver to the C5 ketones, β-ketopentanoate and/or β-hydroxypentanoate, which are thought to cross the blood–brain barrier and enter the brain [143]. This compound has been shown to be effective in certain therapeutic processes, such as in glycogen metabolic syndrome IV, also known as Adult Polyglucosan Body Disease (APBD). APBD is a rare progressive neurodegenerative disorder, most often affecting adults of Ashkenazi Jewish origin and characterized by neurogenic bladder, progressive difficulty with walking and sensory abnormalities in the lower extremities typically developed in the 4th or 5th decade of life. The pathogenesis of the disease includes the deposition of intracellular polyglucosan bodies (amylopectin-like polysaccharides) in the peripheral and CNS cells, which is often associated with a partial deficiency of glycogen branching enzyme, a protein that catalyzes the α(1 → 6)glycosidic bond during the synthesis of glycogen. It is hypothesized that decreased glycogen degradation leads to an energy deficit in cells of the nervous system. Therefore, anaplerotic therapy may supply the substrates required for the citric acid cycle in order to correct the energy deficiency. In fact, a study has been performed with APBD patients consisting in dietary supplementation with the synthetic triglyceride triheptanoin, concluding that patients with APBD experienced a stabilization of disease progression and in most cases, limited functional recovery [144]. Besides APBD, oral triheptanoin has recently been discovered to be effective as anticonvulsant in acute and chronic mouse seizure models, representing a potential treatment for epilepsy [145].

Bacterial lipids have also been studied for their potential therapeutic use. Thus, ONO-4007 is a synthetic lipid A derivative with antitumoral effects and with low endotoxic activities [146]. Lipid A is a lipid component of an endotoxin responsible for toxicity in Gram-negative bacteria, which consists of two glucosamine units with attached acyl chains, and normally one phosphate group on each carbohydrate. Its synthetic derivative, ONO-4007 was effective against KDH-8, a tumor necrosis factor (TNF)-sensitive rat hepatoma cell line, although interestingly it showed no antitumor activity in any TNF-resistant cell line. These results suggest that ONO-4007 may be therapeutically useful for the treatment of TNFα-sensitive tumors and its mechanism of action seems to be related to enhanced production of TNFα in tumor tissues [7].

The beneficial effects of OA for human health and that of other fatty acids has been proven, however, their efficiency is limited due to their metabolic use through β-oxidation in the mitochondria. However, the 2-hydroxylated OA analog (2OHOA) is not degraded by β-oxidation but by the α-oxidation pathway [142]. The 2-hydroxy-phytanoyl-CoA lyase is the rate-limiting enzyme of the α-pathway [147] and it may become rapidly saturated at low substrate concentrations, as it represents a secondary oxidation pathway for non-abundant natural fatty acids. As a consequence, 2OHOA's half-life is longer than its naturally occurring analog OA and therefore, its therapeutic effects last longer. The marked enhancement of the antiproliferative activity of 2OHOA with respect to OA in human cancer cells, in the absence of any apparent toxic effects, is evidence of the improved therapeutic efficiency of this hydroxylated fatty acid. Indeed, exposure to 2OHOA inhibits the growth of cancer cells and tumor growth in animal models [96].

Interestingly, naturally occurring 2-hydroxylated fatty acids exist. For instance, C22 to C26 saturated and monounsaturated 2-hydroxy fatty acids have been found as major lipid components of the cell wall in three marine chlorophytes [148], and detritus from the sea-grass *Zostera muelleri* is a source of 2-hydroxy acids (0.6 μg/g) that range from C_{18} to C_{28}, including different mono- and polyunsaturated derivatives [149]. Seed oils from *Thymus vulgaris* are enriched in 2-hydroxylinolenic acid (13%) [150], while the seed oil of *Salvia nilotica* contains 0.6% 2-hydroxyoleic, 4.2% 2-hydroxylinoleic and 5.4% 2-hydroxylinolenic acids [151]. Moreover, hydroxylated DHA derivatives may also be found among the aforementioned resolvins [125]. During the last few years, a number of 2-hydroxylated fatty acid derivatives other than 2OHOA have been rationally designed for the treatment of cancer, inflammation, AD, obesity, diabetes, spinal cord injury, etc.… Indeed, the data available indicate that the mechanism of action of these compounds is related to their capacity to modulate the lipid structure of the membrane [36,88,152]. In this context, 2OHOA has proved effective in reducing blood pressure in hypertensive rats through a mechanism that involves the modulation of membrane lipid composition, and of the membrane biophysical properties [153]. Also, 2OHOA is currently being studied in phase I/IIa clinical trials for the treatment of solid tumors (code NCT01792310) [154] and it is under preclinical development for the treatment of spinal cord injury. 2OHARA has been described as a new non-steroidal anti-inflammatory drug that can inhibit COX1 and COX2 activity, thereby reducing the synthesis of pro-inflammatory mediators. Molecular dynamics have predicted binding competition between 2OHARA and the proinflammatory fatty acid ARA to COX1 and COX2. Moreover, in addition to the in vitro inhibition of COX1 and COX2 activity, 2OHARA decreased plasma TNFα levels in vivo [155]. Finally, 2OHDHA has arisen as an interesting candidate to revert the cognitive deficiencies associated with neurodegeneration, such as in AD. This fatty acid derivative decreases Aβ accumulation in parallel with a recovery of cognitive scores in animal models. These results are consistent with the reduced binding of oligomeric and fibrillar Aβ lipid raft-like vesicles in the presence of 2OHDHA [156,157]. All these 2-hydroxylated compounds are thought to act by regulating signal transduction through Membrane Lipid Therapy, an approach that aims to regulate membrane lipid organization through structure–function principles [158]. Changes in the membrane's physico-chemical properties, such as the lateral pressure, membrane fluidity or phase behavior may regulate the localization and activity of relevant signaling proteins, resulting in the regulation of gene expression and a reversion of pathological states within cells.

7. Concluding remarks

Fatty acids are no longer considered as mere energy sources or constituents of complex lipids but rather, it is now clear that they may also exert important changes in cell membranes leading to modulation of cell functions. Both natural and synthetic fatty acids have been proven to modify biophysical parameters of membranes, such as their fluidity, permeability and domain formation, as well as cell processes involving cell fusion/fission (endo/exocytosis, cell division), signal transduction, membrane protein activities, and antiproliferative control. Thus, understanding how all these processes occur and considering the biophysical properties of lipid membranes as a drug target have led to the design of new therapeutic approaches that are proving to be effective against cell dysfunction, such as those events responsible for cancer, neurodegeneration or metabolic diseases.

Abbreviations

2OHARA	2-hydroxyarachidonic acid
2OHDHA	2-hydroxydocosahexaenoic acid
2OHOA	2-hydroxyoleic acid
α-LNA	alpha linolenic acid
γ-LNA	gamma linolenic acid
Aβ	amyloid-β
ARA	arachidonic acid

Cho	cholesterol
CNS	central nervous system
DEPE	1,2-dielaidoyl-sn-glycero-3-phosphoethanolamine
DHA	docosahexaenoic acid
DHFR	dihydrofolate reductase
DMPC	1,2-dimiristoyl-sn-3-phosphocholine
DOPE	1,2-dioleyl-sn-glycero-3-phosphoethanolamine
DPH	1,6-diphenyl-1,3,5-hexatriene
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSC	differential scanning calorimetry
EA	elaidic acid
EPA	eicosapentaenoic acid
FFA	free fatty acid
LA	linoleic acid
L_α	liquid crystalline or fluid phase
L_β	gel phase
Ld	liquid-disordered
Lo	liquid-ordered
MUFA	monounsaturated fatty acid
OA	oleic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
POPE	1-palmitoyl-2-oleoyl-sn-glycero-phosphoethanolamine
PPARγ	peroxisome proliferator activated receptor γ
PUFA	polyunsaturated fatty acid
SA	stearic acid
SM	sphingomyelin

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