Does membrane curvature elastic energy play a role in mediating oxidative stress in lipid membranes?

Julia Bahja¹ and Marcus K. Dymond¹∗
¹Centre for Stress and Age-Related Disease, University of Brighton, Lewes Rd. Brighton, BN2 4GL, UK.

∗ Author for correspondence: M.Dymond@brighton.ac.uk

Abstract

The effects of oxidative stress on cells are associated with a wide range of pathologies. Oxidative stress is predominantly initiated by the action of reactive oxygen species and/or lipoxygenases on polyunsaturated fatty acid containing lipids. The downstream products are oxidised phospholipids, bioactive aldehydes and a range of Schiff base by-products between aldehydes and lipids, or other biomacromolecules. In this review we assess the impact of oxidative stress on lipid membranes, focusing on the changes that occur to the curvature preference (lipid spontaneous curvature) and elastic properties of membranes, since these biophysical properties modulate phospholipid homeostasis. Studies show that the lipid products of oxidative stress reduce stored curvature elastic energy in membranes. Based upon this observation, we hypothesize that the effects of oxidative stress on lipid membranes will be reduced by compounds that increase stored curvature elastic energy. We find a strong correlation appears across literature studies that we have reviewed, such that many compounds like vitamin E, Curcumin, Coenzyme Q10 and vitamin A show behaviour consistent with this hypothesis. Finally, we consider whether age-related changes in lipid composition represent the homeostatic response of cells to compensate for the accumulation of in vivo lipid oxidation products.

Keywords: Oxidative stress; intrinsic curvature hypothesis, lipid oxidation, membrane curvature, curvature elastic energy, curvature elastic stress
1.0 Context

The cumulative effects of high levels of oxidative stress are thought to negatively impact many biological processes and facilitate pathogenesis such as neurodegeneration and age related diseases [1–3]. Mechanistically, oxidative stress refers to a complicated set of chemical reactions but unsaturated compounds such as lipids are particularly susceptible to attack [4,5]. Lipid oxidation or peroxidation is the process where unsaturated and polyunsaturated lipids undergo oxidative degradation. This can be due to oxidative stress, via reactive oxygen species (ROS), free radical independent non-enzymatic oxidation, or though enzymatic oxidation by lipoxygenases [6] and typically results in the formation of toxic ‘bioactive’ aldehydes. Two of the most studied bioactive aldehydes are 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), which can be formed by oxidation of omega 6 (n=6) polyunsaturated lipids [7]. However, a range of other aldehydes can form through the oxidation of plasmalogen lipids [8] as well as via omega 3 (n=3) polyunsaturated lipids [9]. These aldehyde products react with the amine groups on biomolecules like DNA and proteins, or with lipids like phosphatidylethanolamine (PE) and form Schiff bases [8,10], adding a further level of complexity to toxic mechanisms behind oxidative stress. In this manuscript, taking into account recent discoveries that fatty aldehydes (products of plasmalogen oxidation) destabilise bilayer lipid structures [11,12], the effects of the molecular products of lipid oxidation on cellular membranes are collated. By casting this discussion within the context of the elastic theory of membranes [13], we ask if membrane curvature elastic energy plays a role in mediating oxidative stress in lipid membranes. And in turn ask if age-related lipid compositional changes are consistent with this hypothesis. To contextualise these questions, we first overview the elastic theory of membranes.

1.1 Stored elastic energy in membranes

Lipids are amphipathic molecules that self-assemble, due to the hydrophobic effect, into a range of different mesophases dependent on the level of hydration and temperature [14], chemical structure of the lipid [15], chemical composition of the lipid mixture [16] and the presence/ absence of additives like divalent cations [12], DNA or RNA [17–19]. Figure 1 provides a basic overview of the terminology used to describe the phase behaviour of lipids, which are lyotropic liquid crystals (LLCs).
Lyotropic liquid crystals:
Lipids are amphiphilic molecules, comprising of hydrophilic headgroups and hydrophobic tails. The hydrophobic effect drives the aggregation of lipids into lyotropic liquid crystal (LLC) phases. The geometry of the LLC phase formed can be flat (lamellar), curve towards water (hexagonal) or curve away from water (inverse hexagonal).

**Figure 1 Overview of the terminology used to describe the lyotropic liquid crystal properties of lipids.** Fig. 1A the organisation of common lyotropic liquid crystal mesophases. Fig. 1B the concept of lipid spontaneous curvature. Fig. 1C stored curvature elastic energy in membranes and its effect on membrane interacting proteins.

One of the key definitions in Figure 1 is that of lipid topology (Fig 1A), such that type I lipids form phases with positive curvature i.e. micelles, micellar cubic and hexagonal phases. Type 0 lipids form liposomes or lamellar (Lα) phases and type II lipids form inverse hexagonal (HII) phases, inverse micellar cubic phases (fd3m) or inverse micelles. Fig. 1B summarises the lipid spontaneous curvature (cₒ), which is measured using small angle X-ray scattering (SAXS) and is defined as 1/Rₒ, where Rₒ is the radius of curvature of an unstressed aggregate of the pure lipid, see Kozlov for a detailed explanation [20]. Type I lipids have positive spontaneous curvature...
values and type II lipids have negative values. A reliable guide to determine the
relative lipid type can be made by assessing the impact of lipids on the temperature
of the $L_α$ to $H_{II}$ phase transition of a known type II lipid like 1,2-dielaidoyl-$sn$-glycero-
3-phosphoethanolamine (DEPE) or 1,2-dioleoyl-$sn$-glycero-3-
phosphoethanolamine (DOPE). Figure 1C gives a brief overview of how stored
curvature elastic energy arises in bilayer membranes and how it can influence the
activity of membrane interacting proteins [21].

Stored curvature elastic energy arises from the frustration of the spontaneous
curvature of lipids and can be quantified using the elastic theory of membranes
proposed by Helfrich [13]. However, it should be noted that earlier work by Canham
[22] linking the biconcave shape of red blood cells to the minimum energy of
membrane bending was an important step in the development of the elastic model.
Equation 1 shows the Helfrich Hamiltonian which determines the elastic free energy
of bending ($g_c$) of a lipid monolayer with surface area $A$, confined to different
geometric curvatures.

$$g_c = \frac{1}{2} \kappa_M A (c_1 + c_2 - c_o)^2 + \kappa_G A c_1 c_2$$

Eq. 1

where, $c_1 (=1/R_1)$ and $c_2 (=1/R_2)$ are the principal curvatures of the interface (such
that a monolayer is defined as having negative curvature if it curves towards water),
$c_o (=1/R_o)$ is the spontaneous curvature of the monolayer, $\kappa_M$ is the mean curvature
bending rigidity and $\kappa_G$ is the Gaussian curvature modulus. Helfrich [13] also
described the elastic free energy of monolayer stretching ($g_s$), Equation 2, valid for
small deformations that do not result in rupture, such that

$$g_s = \frac{1}{2} \kappa_A (\Delta A - A)$$

Eq. 2

where $A$ is the surface area and $\kappa_A$ is the area compressibility modulus. The power
of Equations 1 and 2 is that they relate the free energy of membranes to the material
parameters of lipids, since $c_o$, $K_M$ and $K_A$ are measurable experimentally [20,23–25]
for known lipid compositions. Determinations of $K_G$ are less robust, $K_G$ is not
generally considered to vary significantly between lipids however it does have a geometric dependence on spontaneous curvature [26].

As presented in Figure 1C, stored curvature elastic energy can regulate the activity of some membrane proteins [21,27–29], notably proteins involved in lipid biosynthesis [29,30]. However, the sensitivity of intrinsic and extrinsic proteins to curvature elastic energy varies. For example, for intrinsic proteins like rhodopsin the equilibrium constant ($K_{eq}$) of metarhodopsin I to metarhodopsin II is increased by curvature elastic energy [31], although significant lipid compositional changes (> 20 mol%) are required to double $\ln K_{eq}$. Similarly, studies that assessed the impact of anaesthetics (theorised to act on membrane stored elastic energy by Cantor [32]) have concluded that bulk bilayer properties such as membrane curvature elastic energy and hence their impact on intrinsic proteins, specifically sodium channels, are only significant at very high concentrations [33,34]. Extrinsic proteins are arguably more sensitive to stored curvature elastic energy. For example, phage shock protein and vesicle-inducing protein are recruited to the membrane in response to elevated curvature elastic energy [35]. Notably, CTP: phosphocholine cytidylyltransferase (CCT), the rate determining enzyme in phosphatidylcholine biosynthesis is particularly sensitive to membrane curvature elastic energy, such that a twofold decrease in activity is induced by circa 3 mol% of lysolipids [30]. Similarly, other proteins involved in lipid synthesis such as phospholipase A$_2$ [36] and phosphoinositide-specific phospholipase C are regulated by stored curvature elastic energy [37]. Such observations have led to the suggestion that cells regulate membrane stored curvature elastic energy and use it to control membrane protein activity [38–42]. Furthermore, the apparent sensitivity of many proteins involved in lipid biosynthesis to membrane curvature elastic energy suggests this biophysical feedback process might be behind the regulation of phospholipid homeostasis/ membrane lipid composition in vivo [43,44]. In such a regulatory process, membrane interacting/ sensing proteins modify the lipid composition of cells and maintain stored curvature elastic energy within certain boundaries, rather than a specific lipid composition. As a result, endogenous or exogenous substances that decrease curvature elastic energy in cells cause a homeostatic response, usually the biosynthesis of a type II lipid, to restore the optimal membrane stored elastic energy.
Aside from its role in regulating protein activity in vivo, membrane curvature elastic energy also plays a role in the wider curvature landscape of cellular processes, which are also lipid composition dependent. These are secretory processes such as endocytosis and exocytosis [45] and the resultant membrane fusion/fission processes, such as occurs in synaptic vesicle release [46]. Or the complex cubic membrane geometries sometimes observed in organelles like the endoplasmic reticulum and mitochondria [47]. Within this context it appears critical, for many membrane dependent cellular processes, that cells control membrane composition and leaflet asymmetry, by retaining membrane curvature elastic energy at optimal levels. Studies by Aref et al. [48] show this relationship very clearly, direct nano-injection of phospholipids with different curvature preferences into the inner membrane leaflets of chromaffin cells impacted the exocytosis process, monitored through catecholamine release. Phosphatidylethanolamine (type II) lipids increased catecholamine release, type I and type 0 lipids decreased catecholamine release. These findings and those of other studies [45,49,50] have led to the emerging idea that membrane remodelling can occur through secretory pathways, as well as through biosynthetic pathways [51]. From the perspective of lipid oxidative stress this is an interesting hypothesis as it makes the link between studies showing cells control both membrane lipid asymmetry [52] and membrane curvature elastic energy [38], relating these directly to secretory processes important in learning and memory [53], which are also impacted by age and oxidative stress [54].

To understand how the products of lipid oxidation might impact stored elastic energy in cells, we first review the main molecular products of lipid oxidation and signpost their significance as biomarkers of age-related disease.

1.2 Endogenous products of lipid oxidation: mode of formation and pathological significance

Polyunsaturated fatty acids (PUFAs), PUFA containing lipids, plasmalogens and cholesterol are oxidised by lipoxygenases (LOXes), non-enzymatic pathways via ROS or a non-radical alternative [5,55,56]. LOXes are a group of enzymes found in prokaryotes and eukaryotes, which oxidise PUFAs, often but not exclusively when the PUFAs are attached to lipids [57,58]. There are a wide range of different LOX enzymes each able to specifically peroxidise PUFA containing lipids at different chain positions [59]. LOX enzymes play an important role in the generation of lipid
mediators [60,61] and in plants they are involved in the generation of oxylipins (bioactive aldehydes) [62,63]. LOXes have been implicated in a large range of age-related diseases [64,65], since overexpression or upregulation of LOXes can lead to high levels of oxidative stress in cells [66–68].

ROS are produced in vivo via enzymatic oxidation reactions by enzymes like mono amine oxidase (MAO) [69] in the mitochondria, peroxisomes and endoplasmic reticula [5,70], or through autooxidation of compounds like catecholamines, hydroquinone and sterols [56,71]. Exogenous stimuli like ionising radiation [72], UV light, alcohol and tobacco smoke are also sources of in vivo ROS production [1,73–75]. ROS like the hydroxyl radical (HO•) and the hydroperoxyl radical (HO2•) react with lipids to form oxidised lipids or peroxidised lipids, respectively [76,77]. ROS reactions with lipids go through an initiation stage, where the radical ROS species combines with a hydrogen atom adjacent to an unsaturation, resulting in a molecule of H2O (or H2O2) and an unstable fatty acid radical. Next propagation occurs and the fatty acid radical reacts with molecular oxygen, resulting in a lipid peroxyl radical that can abstract hydrogen from a water molecule, generating a further hydroxyl radical that is free to react with another PUFA, a process that can repeat many times. Termination occurs when there is a high concentration of radical species, resulting in high probability of a collision between two free radicals. Antioxidants such as vitamin E, vitamin C, and enzymes like superoxide dismutase, catalase, and peroxidase can also terminate radical reactions. Hence the consumption of antioxidants is thought to play an important role in mitigating the negative health effects of ROS-derived oxidative stress [78,79].

The complexity of the many different chemical species caused by ROS type reactions i.e. oxidised lipid, peroxidised lipids, epoxidised lipids, aldehydes etc. [5,55,80] arises from the nonspecific nature of radical reaction mechanisms. Monounsaturated fatty acid (MUFA) containing lipids are susceptible to oxidation too [81,82] but to a much lesser extent than PUFA containing lipids [83]. Figure 2 shows some of the most common molecules associated with lipid oxidation and summarises their mode of production.
Figure 2 Examples of commonly occurring molecular products of lipid oxidation. Where (1) is a PC lipid containing both n=3 and n=6 fatty acids (PUFAPC); (2) is the plasmalogen PC lipid 1-(1Z-hexadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine; (3) 1-Palmitoyl-2-(9-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PoxnoPC) and (4) 1-Palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PazePC) are oxidised lipids; (5) 4-oxo-trans-2-nonenal (4-ONE), (6) 4-oxo-trans-2-hexenal (4-OHE), (7) 4-hydroxy-2-hexenal (4-HHE), (8) 4-hydroxy-2-hexenal- (4-HHE), (9) malondialdehyde (MDA) (9), (10) pentadecanal and (11) α-hydroxyhexadecanal are common aldehyde products of lipid peroxidation. Compounds (13) and (14) show the potential Schiff base
compounds formed when aldehydes such as (10) and (11) react with the generic PE lipid (12).

These are the oxidised phospholipids (OxPL), example compounds being the aldehydophospholipids1-palmitoyl-2-(9-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PoxnoPC) (3) and 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PazePC) (4) generated from n=3 or n=6 PUFA containing a lipid (a PC lipid in this instance in Figure 1) (PUFAPC) (1). OxPL analogues derived from the other lipid headgroup classes such as PE, PS and PG also exist [84–86], with a range of different alkyl chain lengths, oxidation states and oxidation positions. Elevated levels of OxPLs, often termed inflammatory lipids [87], are associated with age-related diseases like atherosclerosis [88] and widely used as biomarkers of human disease as reviewed [89,90]. Estimates of the overall amount OxPLs in membranes varies [90], largely as it not clear what the baseline levels of OxPLs are, or because individual studies have quantified specific OXPLs, rather than the total OxPLs. Levels of some oxidised phosphatidylcholine lipids (OxPC) have been detected as high as 6.5 µM in the plasma of hyperlipidemic patients [91], with an estimated total phospholipid concentration of circa 2 mM.

The aldehydes 4-oxo-trans-2-nonenal (4-ONE) (5) and 4-HNE (7) are products of n=6 fatty acid containing substrates. Similarly, 4-oxo-trans-2-hexenal (4-OHE) (6) and 4-hydroxy-2-hexenal (4-HHE) (8) are products of n=3 fatty acid containing substrates. Elevated levels of 4-HNE are associated with age-related diseases [1,92–96], with estimates suggesting it reaches a concentration of up to 5 mM under oxidative stress [97]. MDA (9) is another product of ROS action on PUFAs, which is used as a biomarker of oxidative stress and associated health issues [98–101]. Fatty (i.e. long alkyl chain) aldehydes like pentadecanal (10) or α-hydroxyhexadecanal (11) are usually generated by oxidation or peroxidation of plasmalogen lipids [8] like 1-(1Z-hexadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine (2), concomitantly producing a lyso lipid, or through catabolism of fatty alcohols and sphingolipids [102]. Elevated levels of fatty aldehydes and α-hydroxyaldehydes are associated with Sjögren-Larsson syndrome [103], atherosclerosis [104] and coronary heart disease [105]. Quantification of the precise amounts of the bioactive aldehydes in membranes is complicated due to their high chemical reactivity, although recent progress has been made in this area [106].
The aldehyde products of lipid oxidation (5 – 9), have high chemical reactivity towards the amine (-NH₂) functional group, which is widespread on DNA, proteins and some lipid molecules. Compounds (13) and (14) are the Schiff bases formed when (10) and (11) react with the generic PE lipid (12). 4-HNE protein Schiff bases have been identified and related to the progression of multiple pathologies [107] and MDA-lysine (MDALys) adducts have been identified as a biomarker for ageing [108]. MDA, a dialdehyde, can also crosslink amine groups [109] through two Schiff base adducts on a DNA or protein molecule, or between two lipid macromolecules [110]. Crosslinking can also occur between OxPLs like (3) and (4), since the aldehyde groups on the oxidised chains can also form Schiff bases with amino groups, such as those on PE lipids [111].

2.0 Lipid oxidation in the context of phospholipid homeostasis

As summarised in Section 1.2, a wide range of molecular lipid oxidation products can result from oxidative stress of lipids. Quantification of the individual amounts of these lipid oxidation products is an ongoing challenge, due to the chemical diversity of the many different chemical species formed. The emerging area of oxidative lipidomics is beginning to enable this although quantitative data across individual lipid species is limited by the availability of stable internal standards [90,106]. Currently very little is known about the collective amount and hence collective effect of lipid oxidation products in in vivo membranes. However, many of these lipid derived products are amphipathic or hydrophobic molecules that will associate with lipid membranes and will impact the elastic energy in lipid membranes. This raises an important question in the context of phospholipid homeostasis i.e. how does the cell adapt to lipid oxidation? One suggestion is that age-related changes in lipid membrane composition occur in response to higher levels of the molecular products of oxidative stress [80]. This might be due to their accumulation over a lifetime, or that the older body cannot remove them as efficiently. Due to the dynamic nature of cellular phospholipid composition this is a difficult hypothesis to test and studies that have explored the links between lipid metabolism and ageing show this is a complex process. However a number of trends appear in the data [112]. For example, increases in the PUFA to MUFA ratio are observed with ageing and higher MUFA/ PUFA ratios correlate with longevity in
humans, as reviewed [113]. Plasmalogen lipids of the type shown in Figure 2 (2) decrease after reaching a maximum level in humans at around 30 years of age [113,114]. Studies also show correlated elevations of some lipid species of cholesterol esters, diacylglycerols, triacylglycerols and phospholipids with human longevity in plasma [115] and increases in OxPL species are observed in a range of age-related diseases [116], over a lifetime.

One of the reasons that lipid compositional changes due to lipid oxidation are complex to understand is that such changes occur within the already complex process of phospholipid homeostasis. Lipid membranes are asymmetric and control the lipid composition (at headgroup and chain level) of the inner and outer membrane leaflets, with studies suggesting each leaflet might have finely tuned physical properties [52]. Lipids in cells undergo rapid turnover and lipid composition varies between cell type and organelle, changing in response to environmental contaminants [117,118] and growth conditions [119–122]. Furthermore, since dietary fatty acids are biosynthetic precursors for lipids and play a vital role in homeostatic control of lipid composition [123], change to the supply fatty acid composition also modifies in vivo lipid composition, both in cultured cells and humans [120,121,124–128]. Therefore, the complexity of disentangling the effects of lipid oxidation from the homeostatic response of cells to oxidative stress makes it preferential to study lipid oxidation in model lipid membranes.

2.1 Lipid oxidation decreases curvature elastic energy in model membranes

Studies that assess the impact of oxidative stress on model membranes use preformed liposomes [129] or preformed lyotropic liquid crystal phases [77]. Lipid oxidation/ peroxidation is initiated by a range of different methods such as through UV irradiation [81,130,131], heating [77], enzyme action [132] or via chemical methods utilising peroxides [133–135]. An overall picture emerges whereby liposomes containing lipids susceptible to oxidation, often PC derived lipids since these readily form liposomes, increase in oxidised lipid and aldehyde content [136,137]. Depending on the duration and intensity of oxidative stress the accumulation of lipid oxidation products initially causes an increase in the mean area per lipid (ΔA +ve) [130], as shown by a number of experimental and simulation studies [131,138–143]. At a molecular level increases in the mean area per lipid are attributed to the reduced hydrophobicity of oxidised lipid chains, which move away
from the hydrophobic membrane interior to the aqueous interface [138]. This model, termed the whisker model [144,145], because the oxidised/ peroxidised lipid chains protrude, like whiskers, from the membrane surface, is consistent with measurements of surface charge density through the zeta potential, observed for liposomes under oxidative stress [130,146,147].

Lipid oxidation also induces membrane lateral phase separation [148,149] and as a result of the mechanical changes to the membrane increases in membrane permeability are also observed [139,150,151]. Eventually high levels of lipid oxidation products are detrimental to the continued formation of stable bilayer structures, pores form through the membrane and the membrane disintegrates [152]. Pore formation in bilayers and membrane disintegration into micelles are behaviours typically seen when lipids with positive spontaneous curvature are incorporated into membranes, hence the evidence suggests that overall lipid oxidation products have a net positive curvature preference [153].

Concurrent with these changes the bilayer thickness reduces, caused by the remaining alkyl chains moving to fill voids left by oxidised alkyl chains migrating to the interfacial region of the membrane. As a result changes in lipid packing or the order parameter are observed, often rationalised as membrane fluidity changes [82,135]. A number of studies have linked membrane oxidative stress to changes in the mechanical properties of the oxidised membrane. So-called bilayer thinning has been observed in experimental [108] and simulated [139,150] lipid oxidation studies, and confirmed in SAXS studies of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) mixed with POPC-OOH (the hydroperoxide of POPC) and PazePC containing model membranes [154]. Overall $\kappa_A$ decreases as the fraction of oxidised membrane lipids increases [140,142] and a result of these changes to $A$ and $\kappa_A$, as is clear from Eq. 2, lipid oxidation/ peroxidation impacts elastic strain energy in the membrane.

To understand the impact of oxidative stress on the spontaneous curvatures of lipids and the stored curvature elastic energy of membranes, Eq. 1, some studies have looked at oxidation in preformed lyototropic liquid crystal phases. Sankhagowit et al. [77] showed that oxidation of the HII phase forming lipid DOPE resulted in the formation of a lipid mixture that preferentially formed a bicontinuous cubic phase. Utilising SAXS the study was not able to quantify $c_o$ and $\kappa_M$ however the propensity
of the oxidised mixture of lipids (3:2 molar ratio of non-oxidised to oxidised DOPE) to form a less tightly curved structure is consistent with a mixture of less negative $c_0$. This finding is also consistent with studies that have suggested oxidation of PC lipids decreases curvature elastic energy in membranes. However, in contrast to studies that have used PC lipids the PE lipid utilised raises the possibility that Schiff base compounds like (13) and (14) are formed by aldehyde products of peroxidation, making it difficult to discern which chemical component (Schiff base, aldehyde or oxidised lipid) contributed most to the phase behaviour of the lipid mixture. Hence the complexity of such oxidised mixtures makes it desirable to assess the effects of individual lipid oxidation products on $\kappa_A, \kappa_M, \kappa_G$ and $c_0$ to enable a more detailed energetic treatment to be made. Currently few studies have been carried out in this area.

2.2 The impact of oxidised lipids, aldehydes and PE Schiff bases on the elastic properties of model membranes

Slatter et al. [132] incorporated increasing amounts of the hydroxy fatty acid hydroxyeicosatetraenoic acid containing PE lipids (HETE-PE) in Lα phases composed of 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS, 10%), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 45%), 1-stearoyl-2-arachidonyl-sn-glycero-3-phosphoethanolamine (SAPE, 35% – 45%) and HETE-PE (0% to 10%) by SAXS. The data showed that replacing SAPE with HETE-PE increased the Lα d-spacing and bilayer thickness, which contrasts with the bilayer thinning observed when preformed membranes are subject to oxidation.

Burrell et al. [11] found that incorporating increasing amounts of the aldehydes trans-trans-2,4-decanedienal (DD) and cis-11-hexadecenal (HD) into DOPE promoted the formation of tightly curved fd3m phases. HD is a fatty aldehyde of the type formed by the oxidation of plasmalogen lipids (2) and DD is a byproduct of lipid oxidation, often found in cooking oil fumes [155]. The $c_0$ values obtained from this data were -0.63 ± 0.05 and -0.52 ± 0.04 for DD and HD, respectively, which indicates that both HD and DD will increase stored curvature elastic energy in membranes as predicted by Eq. 1. Plasmalogen lipid oxidation also results in the formation of lyso lipids, which are type I lipids with positive spontaneous curvatures [156]. There are few studies in the literature that look at the effect of aldehydes like
4-HNE and MDA on membrane lipid phase behaviour, so linking these compounds directly to the changes they cause in stored elastic energy is less straightforward. The spontaneous curvature of aldehydes like cis and trans retinal have been determined and these also have negative curvature preferences [12], although structurally they are dissimilar to the other bioactive aldehydes like 4-HNE etc., so it is not obvious that all the aldehyde products of oxidative stress will have the same effects on membrane-stored elastic energy. It is, however, worth pointing out similarities between the interactions of fatty aldehydes, fatty alcohols and fatty acids with model membranes [11], such that all these molecules have tight negative spontaneous curvature preferences.

Huang et al. [157] studied the effects of a homologous series of even chain alcohols from C\textsubscript{12} to C\textsubscript{20} on PC lipids. Overall, they found that C\textsubscript{12} alcohols induced H\textsubscript{II} phases in PC lipids but as the alcohol chain length increased fd3m phases were formed. Short chain alcohols increase the phase transition temperature for L\textsubscript{α} to H\textsubscript{II} [158,159] which demonstrates that these alcohols decrease stored curvature elastic energy in the membrane. In part this behaviour is driven by the hydrophobicity of the alkyl chain in the alcohol molecule and hence is one reason to expect similar behaviour in the aldehydes with alkyl chain length. Long chain alcohols sit deeper in the bilayer and short chain alcohols sit closer to the aqueous interface, an observation that can be rationalized by their octanol: water partition coefficients [34].

4-HNE (7) has a log P (octanol: water distribution coefficient) of 2.45 [160] indicating that it has a considerable affinity for hydrophobic regions of membranes. 4-HNE (8) has one less carbon than DD and an extra OH group, it is less hydrophobic than DD (DD has a log P of 3.42) but still therefore likely to impart negative curvature on a membrane depending on where the molecule is located. Studies show 4-HNE locates to the hydrophobic region of the bilayer [161] where values up to 10 mol % marginally increase the average area per headgroup. Overall this suggests a small negative effect on membrane curvature. 4-HHE has a log P (octanol: water distribution coefficient) of 0.89 [160] but the shorter alkyl chain compared to 4-HNE suggests it is unlikely to impart negative curvature on a membrane. MDA has a log P of around -1.0 and interacts with membrane hydrophilic part of the membrane [162].

Lipid oxidation products like PazePC (1) and PoxnoPC (2) are expected to have positive spontaneous curvatures [163] as demonstrated by elevation of the L\textsubscript{α}
to \( \text{H}_{\text{II}} \) phase transition of DEPE by 1-palmitoyl-2-(5-keto-6-octene-dioyl)phosphatidylcholine (KODiA-PC) [164]. There are currently few studies that enable the spontaneous curvature of PE Schiff base adducts to be determined but it has been suggested that these also have positive spontaneous curvatures since they have been detected in high amounts when PE \( \text{H}_{\text{II}} \) phases are oxidised [77]. However, it is difficult to disentangle the effect of the PE Schiff base from the oxidised PE in these studies but ONE-PE adducts have been reported to impact a range of other membrane physical properties as reviewed [165,166]. More focused studies looking at the effects of a range of PE Schiff bases have shown the \( \text{L}_\alpha \) to \( \text{H}_{\text{II}} \) transition temperature of DEPE is increased by 1,2-dipalmitoyl-\text{sn}-glycero-3-phosphoethanolamine-N-(glutaryl) (glt-PE), which suggests it is a type I lipid. Glt-PE is a PE lipid modified by glutaraldehyde, a dialdehyde like MDA, with two extra methylene units, therefore this observation strongly suggests MDA-PE Schiff bases are also type I lipids.

3.0 Lipid derived oxidative stress products in cells: the protective effects of type II lipids

As established in Section 2.1, the molecular changes that occur during lipid oxidation, and the accumulation of these molecules in a model membrane leads to a net decrease in stored curvature elastic energy. \textit{In vivo} these observations suggest that, to combat the effects of lipid oxidation, cells are required to take steps to mitigate decreases in the stored curvature elastic energy of their membranes. Increasing the synthesis/composition of lipids that increase stored curvature elastic energy, i.e. type II lipids is a likely way to achieve this. This observation suggests two testable outcomes. The first is that endogenous treatment with type II lipids will ameliorate the effects of oxidative stress on cell membranes. The second is that age-related changes in lipid composition, in keeping with the oxidative stress theory of ageing, will favour increases in type II lipids (to overcome the accumulation of the type I lipid oxidation products). These mechanisms are summarised in Figure 3.
Figure 3 Summary of oxidative stress effects on membranes and a possible remediation pathway. Figure 3A shows a stable bilayer membrane maintaining stored curvature elastic energy (SCE) constant with a balance of type II and type 0 lipids. Figure 3B shows the effect of oxidative stress, lipid hydroxides, hydroperoxides, aldehydes and lysolipids are formed. Bilayer thinning occurs, type I lipids accumulate and lipid whiskers form. Figure 3C shows the effects of the extended accumulation of oxidative stress products, local areas of positive curvature occur leading to pore formation and membrane disintegration, if unchecked. Figure 3D shows a route to remediate the effect of high levels of oxidative stress products, lipid homeostasis increases the synthesis of type II lipids to combat high levels of type I oxidative stress products. Remediation can also occur by exogenous supplementation of type II lipids.

Looking though the existing literature we were able to identify a significant number of examples that support the suggestion that type II lipids will have protective effects against the products of oxidative stress. Table 1 summarises the spontaneous curvature or curvature preference of some common eukaryotic lipids, their lipid oxidation products, and a selection of compounds commonly linked with combating oxidative stress.
Table 1 summary of lipid types for commonly found lipids, lipid oxidation products and compounds used to ameliorate oxidative stress

<table>
<thead>
<tr>
<th>Lipid Species</th>
<th>Evidence (ΔΔnm) Lα-HII</th>
<th>Ref</th>
<th>Type I/II</th>
<th>Lipid oxidation products</th>
<th>Evidence (ΔΔnm) Lα-HII</th>
<th>Ref</th>
<th>Type I/II</th>
<th>Ameliorating compounds</th>
<th>Evidence (ΔΔnm) Lα-HII</th>
<th>Ref</th>
<th>Type I/II</th>
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<tr>
<td>DOPC</td>
<td>-0.091 ± 0.008</td>
<td>[25]</td>
<td>II, weak</td>
<td>OxPL</td>
<td>Lα - HII increased</td>
<td>[164]</td>
<td>I</td>
<td>Vitamin E</td>
<td>-0.75</td>
<td>[167]</td>
<td>II</td>
</tr>
<tr>
<td>DOPE</td>
<td>-0.399 ± 0.005</td>
<td>[25]</td>
<td>II</td>
<td>Fatty aldehydes</td>
<td>HD</td>
<td>-0.52 ± 0.04 -0.63 ± 0.05</td>
<td>[11]</td>
<td>II</td>
<td>Vitamin A (t-retinoic acid)</td>
<td>-0.63</td>
<td>[12]</td>
</tr>
<tr>
<td>DOPS</td>
<td>-0.35 ± 0.03</td>
<td>[12]</td>
<td>II</td>
<td>4-HNE</td>
<td>Structural similarity</td>
<td>-</td>
<td>II</td>
<td>CoQ10</td>
<td>Lα-t-HII reduced</td>
<td>[168]</td>
<td>II</td>
</tr>
<tr>
<td>DOPA</td>
<td>-0.42 ± 0.03</td>
<td>[12]</td>
<td>II</td>
<td>PE Schiff base</td>
<td>glt-PE</td>
<td>Lα - HII increased</td>
<td>[165]</td>
<td>I</td>
<td>Cholesterol</td>
<td>-0.36 ± 0.03</td>
<td>[156]</td>
</tr>
<tr>
<td>DOG</td>
<td>-0.99</td>
<td>[169]</td>
<td>II</td>
<td>Lyso OPE</td>
<td>&gt; +0.025</td>
<td>[156]</td>
<td>I</td>
<td>Vitamin K</td>
<td>Lα - HII reduced</td>
<td>[170]</td>
<td>II</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.36 ± 0.03</td>
<td>[156]</td>
<td>II</td>
<td>Lyso OPC</td>
<td>+0.26</td>
<td>[156]</td>
<td>I</td>
<td>Curcumin</td>
<td>Lα - HII reduced</td>
<td>[171]</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>18:0,22:5ω6PE</td>
<td>-0.364</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18:0,22:5ω6PE</td>
<td>-0.364</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PUFAs</td>
<td>Lα - HII reduced</td>
<td>[173]</td>
</tr>
</tbody>
</table>
As shown in Table 1, a number of compounds associated with reducing oxidative stress are classifiable as type II lipids and increase stored curvature elastic energy in model membranes. We now discuss these studies in more detail. Mosca et al. [146], showed cholesterol provides a protective effect against oxidative stress, which they attributed to the rigidity that cholesterol confers on the membrane, above the chain melting temperature $T_m$. This rigidifying role of cholesterol can be understood by the effect that cholesterol has on the mixed membrane material parameters $c_o$ and $\kappa_M$ [174], Eq. 1. Cholesterol is a type II lipid with negative spontaneous curvature $\text{circa} -0.36 \, \text{nm}^{-1}$. Similarly, it has been noted that the fatty aldehydes produced by lipid oxidation, which are type II lipids [11], might mitigate some of the membrane destabilising effects of oxidised lipids [149].

Vitamin E and other tocopherols have been widely shown to prevent oxidative damage [2,79,145,175,176], and whilst it is very clear that Vitamin E has an antioxidant effect, via a radical quenching mechanism [5], a significant number of studies have established that it has a negative spontaneous curvature (circa $-0.75 \, \text{nm}^{-1}$) and significant effects on the physical properties of lipid membranes [167,177].

Vitamin A analogues like retinoic acid, $\text{cis}$-retinal and $\text{trans}$-retinal are established antioxidants which prevent lipid oxidation [178] and remediate the effects of oxidative stress in a significant number of studies [179–181]. Vitamin A is hydrophobic and associates with membranes. X-ray diffraction studies show that the vitamin A analogues retinoic acid, $\text{cis}$-retinal and $\text{trans}$-retinal are all type II lipids with spontaneous curvature around $-0.61 \, \text{nm}^{-1}$ [12].

Vitamin K has become increasingly widely used to combat oxidative stress [182–185], both vitamin K1 and K2 associate with membranes [186] and vitamin K1 forms $\text{H}_{\text{II}}$ phases in combination with DEPE in water, lowering the $\text{L}_{\alpha}$ to $\text{H}_{\text{II}}$ transition temperature of DEPE [170]. This demonstrates that vitamin K1 and K2 (due to the similarity of their structures) are type II lipids, although no estimate of spontaneous curvature is available in the literature.

Coenzyme Q10 (CoQ10) is a fat-soluble antioxidant which inhibits lipid peroxidation in cell membranes [187–189]. A number of studies have assessed the
effect of CoQ\textsubscript{10} on lipid membranes showing that it promotes negative curvature by reducing the L\textsubscript{α} to H\textsubscript{II} of DEPE [168] and POPE [190].

Curcumin is an increasingly widely used compound that has protective effects against oxidative stress [175,191–193]. Studies of curcumin in DEPE have shown that it promotes the formation of H\textsubscript{II} phases from L\textsubscript{α} phases [171].

Endogenous treatments of cells with PUFAs or PUFA lipids is another strategy widely used to reduce the effects of oxidative stress [194–197]. PUFAs and PUFA lipids are almost exclusively type II amphiphiles due to their inverse conical shape. PUFA PE lipids (18:0, 22:6 PE and 18:0, 22:5 PE) form H\textsubscript{II} phases in water (c\textsubscript{0} ~ - 0.37 nm\textsuperscript{-1}) PUFA (n=3) containing mono acyl glycerol lipids form H\textsubscript{II} structures [198] and finally free PUFAs like linoleic acid promote H\textsubscript{II} phase formation [173].

In the context of the oxidative stress theory of ageing and given widespread observations that age related changes in lipid composition occur, it is interesting to consider if stored curvature elastic energy has a role to play in age related lipid compositional changes. If this is the case then, as levels of lipid oxidation products build up in a cell, due to either their increased production, or decreases in the cell’s ability to remove them, then one would expect age related increases in type II lipids in cells as a compensatory mechanism. A number of studies support this, albeit in a qualitative way. For example, polyunsaturated fatty acids restore ageing neuronal membranes as discussed [199]. Oxidative stress in young animals raises the level of cholesterol (type II lipid) to that seen in older animals [200]. Similarly the decrease seen in the MUFA:PUFA ratio widely reported as decreasing (i.e. increases type II lipids) with age [113] and age-related accumulation of free fatty acids (type II lipids) [16] in membranes [201] could also represent compensatory mechanisms whereby cells attempt to restore stored curvature elastic energy to ‘normal’ levels.

4.0 Conclusions and future perspective

There is a clear consensus in the literature showing that the accumulation of lipid derived products of oxidative stress leads to significant changes in the physical properties of membranes. Overall a mechanism has emerged from studies in recent years whereby the net positive curvature of lipid oxidation products destabilizes the bilayer configuration of lipid membranes, which can be related back to the elastic free energy of membranes, as we have presented. Interestingly, this observation
raises the possibility that type II lipids (with negative curvature) might show a protective role against lipid derived oxidative stress. Further investigation revealed a significant number of cases that support this idea, suggesting that providing cells with a mechanism to increase the stored curvature elastic energy of their membranes can combat against oxidative stress. Such a mechanism should of course be considered as occurring alongside many of the established effects of antioxidants on reducing oxidative stress. However, given the enormous complexity of the glycerolipid and glycerophospholipid biosynthetic pathways, the vast number of different mechanisms of lipid oxidation/ peroxidation, and the complex downstream effects of lipid oxidation products, it is interesting that an observation linked to the free energy of biological membranes can cut through this complexity.

One important area for development, which likely provides a test of this hypothesis, requires the development of new methodology to quantify the global effects of oxidative stress on in vivo lipid membranes. This is big challenge that requires further developments in the field of oxidative lipidomics [90], but once achieved will enable the net effect of lipid oxidation on the physical properties of membrane to be determined. Since it will establish whether the amount of positive curvature generating lipid species in the in vivo membrane is enough to significantly impact the landscape of curvature dependent processes in cells. As a guideline, a few mol % of lysolipids can modulate the activity of CCT [30], discussed in Section 1.1, therefore compositional changes of a few mol %, corresponding to the net increase in lyso lipids, OxPLs and PE Schiff base lipids would need to be detected, since these are shown to be type I lipids, see Table 1.

In the future it will be important to understand the roles of proteins in this process since key enzymes that remove the oxidative stress products of lipids will likely be sensitive to stored curvature elastic energy. There are already many examples of proteins involved in lipid biosynthesis that are regulated by stored curvature elastic energy in cells, and many of these like phospholipase A\(_1\), A\(_2\), D and C have been implicated as playing roles in oxidative stress and age-related disease as discussed [138,202].

Finally, our observation that stored curvature elastic energy might play a role in mediating oxidative stress in membranes, could prove a useful tool in designing antioxidant analogues, since the addition of long unsaturated or branched alkyl chains will impart negative curvature and increase membrane stored elastic energy.
in addition to any antioxidant effects. There is some evidence to support this since long chain fatty acid analogues of quercetin showed enhanced cellular antioxidant activity [203].

**CRediT statement.**

JB: Writing-original draft preparation, Writing - Review & Editing; MKD: Conceptualization, Writing-original draft preparation, Supervision, Writing - Review & Editing, Visualisation.
References


enzymatic activity of 6-phosphofructo-1-kinase from B. stearothermophilus.,
https://doi.org/10.1016/j.chemphyslip.2011.08.003.

Oras, S. Collier, M.M. Hussain, L. Dong, S. Patel, A. Alvarez-Guaita, V.
Saudek, B.J. Jenkins, A. Koulman, M.K. Dymond, R.C. Hardie, S.
Siniossoglou, D.B. Savage, PCYT1A Regulates Phosphatidylcholine
Homeostasis from the Inner Nuclear Membrane in Response to Membrane

CTP:phosphocholine cytidylyltransferase by membrane curvature elastic
https://doi.org/10.1073/pnas.160260697.

of membrane elastic energy to rhodopsin function, Biophys. J. 99 (2010) 817–

https://doi.org/10.1021/bi9627323.

Andersen, H.C. Hemmings, Volatile anesthetics inhibit sodium channels
without altering bulk lipid bilayer properties, J. Gen. Physiol. 144 (2014) 545–

[34] H.I. Ingólfsson, O.S. Andersen, Alcohol’s effects on lipid bilayer properties,

[35] C. McDonald, G. Jovanovic, O. Ces, M. Buck, Membrane stored curvature
elastic stress modulates recruitment of maintenance proteins pspa and vipp1,

[36] A. Sen, T. V. Isac, S.W. Hui, Bilayer Packing Stress and Defects in Mixed
Dilinoleoylphosphatidylethanolamine and Palmitoyloleoylphosphatidylcholine
and Their Susceptibility to Phospholipase A2, Biochemistry. 30 (1991) 4516–
4521. https://doi.org/10.1021/bi00232a021.

[37] A. Arduin, P.R.J. Gaffney, O. Ces, Regulation of PLCβ2 by the electrostatic


https://doi.org/10.1016/j.chemphyslip.2019.05.005.


https://doi.org/10.1093/gerona/glw048.


[121] K.A. Ferguson, M. Glaser, W.H. Bayer, P.R. Vagelos, Alteration of fatty acid

https://doi.org/10.1016/0163-7827(90)90002-3.

https://doi.org/10.1016/j.cbpa.2017.06.002.


https://doi.org/10.1016/j.plefa.2017.06.001.


https://doi.org/10.1159/000012729.


Browning, M. Malmsten, Oxidation of Polyunsaturated Lipid Membranes by
Photocatalytic Titanium Dioxide Nanoparticles: Role of pH and Salinity, ACS
https://doi.org/10.1021/acsami.0c08642.

Clayton, V.J. Tyrrell, M. Rosas, S.N. Lauder, A. Watson, M. Dul, Y. Garcia-
Díaz, M. Aldrovandi, M. Heurich, J. Hall, J.H. Morrissey, S. Lacroix-Desmazes,
S. Delignat, P.V. Jenkins, P.W. Collins, V.B. O’Donnell, Enzymatically oxidized
phospholipids restore thrombin generation in coagulation factor deficiencies,

[133] A. Sevanian, M. Lou Wratten, L.L. McLeod, E. Kim, Lipid peroxidation and
phospholipase A2 activity in liposomes composed of unsaturated
phospholipids: a structural basis for enzyme activation, Biochim. Biophys. Acta
(BBA)/Lipids Lipid Metab. 961 (1988) 316–327. https://doi.org/10.1016/0005-
2760(88)90079-3.

Phosphatidylethanolamine and Increases Oxidation in Lipid Membranes, J.

I. Liau, Interplay between structure and fluidity of model lipid membranes
https://doi.org/10.1021/jp1014719.

as model membranes to study lipid peroxidation photoinduced by pterin,
https://doi.org/10.1016/j.bbamem.2015.11.002.

[137] M.R.M. Domingues, A. Reis, P. Domingues, Mass spectrometry analysis of

initiated by photosensitized lipid oxidation, Biophys. Chem. 254 (2019)

[139] M. Lis, A. Wizert, M. Przybylo, M. Langner, J. Swiatek, P. Jungwirth, L. Cwiklik,


[148] C.K. Haluska, M.S. Baptista, A.U. Fernandes, A.P. Schroder, C.M. Marques,


[166] E.E. Pohl, O. Jovanovic, The role of phosphatidylethanolamine adducts in modification of the activity of membrane proteins under oxidative stress,


