

## Thematic Review Series: Lipids and Lipid Metabolism in the Eye

## Lipids and the ocular lens

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**Abstract** The unusually high levels of saturation and thus order contribute to the uniqueness of human lens membranes. In addition, and unlike in most biomembranes, most of the lens lipids are associated with proteins, thus reducing their mobility. The major phospholipid of the human lens is dihydrosphingomyelin. Found in significant quantities only in primate lenses, particularly human ones, this lipid is so extremely stable that it was reported to be the only lipid remaining in a frozen mammoth 40,000 years after its death. Unusually high levels of cholesterol add peculiarity to the composition of lens membranes. Beyond the lateral segregation of lipids into dynamic domains known as rafts, the high abundance of cholesterol in the human lens leads to the formation of patches of pure cholesterol. Changes in human lens lipid composition with age and disease as well as differences among species are greater than those observed for any other biomembrane. The relationships among lens membrane composition, structure, and lipid conformation reviewed in this article are unique to the mammalian lens and offer exciting insights into lens membrane function. This review focuses on findings reported over the last two decades that demonstrate the uniqueness of mammalian lens membranes regarding their morphology and composition. Because the membranes of human lenses do undergo the most dramatic changes with age and cataractogenesis, the final sections of this review address our current knowledge of the unusual composition and organization of adult human lens membranes with and without opacification. Finally, the questions that still remain to be answered are presented.—Borchman, D., and M. C. Yappert. *Lipids and the ocular lens*. *J. Lipid Res.* 51: 2473–2488.

**Supplementary key words** cataract • cholesterol • membrane • phospholipids

## UNIQUE PROPERTIES OF LENS MEMBRANE MORPHOLOGY AND FUNCTION

The purpose of the lens is to focus light onto the retina in the back of the eye. The lens is avascular, thus avoiding light scattering, and is in a hypoxic environment, containing less

oxygen than any organ in the human body ( $pO_2$  of 11 mm Hg) (1). Light passing through a human lens traverses through approximately 2,800 cellular membranes. These membranes serve as impermeable barriers to cations and as a matrix for the major lens membrane proteins, aquaporin (AQPO), plasma membrane  $Ca^{2+}$ -ATPase (PMCA), and Na, K-ATPase that are necessary for the control of lens water, calcium, sodium, and potassium homeostasis, respectively, all of which are required for maintenance of lens clarity (see “Lipid-Protein and Lipid-Calcium Interactions”).

Human lens membranes are also unusual in their phospholipid composition. The most abundant phospholipid is dihydrosphingomyelin, found in significant quantities only in primate lenses, particularly human ones (Fig. 1 and “Lipid compositional changes in human lens membranes with age and cataract”). Membranes of adult human lenses are some of the most saturated, ordered membranes in the human body, and their high level of cholesterol leads to the formation of patches of pure cholesterol bilayers (Fig. 2 and “Cholesterol” section). Furthermore, most of the lipids are associated with proteins, thus limiting their mobility (Fig. 2, right). In contrast, a typical biomembrane, as represented by the Singer fluid-mosaic model, contains proteins floating in a sea of fluid phospholipids with lateral mobility within the bilayer (Fig. 2, left). Epithelial cells in the equatorial region differentiate into fiber cells (Fig. 3). Some of these fibers can reach lengths of 1 cm. The younger fibers comprise the cortical region (Fig. 3). With age, the cortical fibers are compressed toward the central nuclear region (Fig. 3).

The lens is a unique syncytium of interconnected cells through which cations deep within the lens find their way to the epithelial layer on the anterior surface, where they are transported out of the lens, predominantly in the equatorial region (3). The cortical fibers exhibit hexagonal cross-sections and pack uniformly (Fig. 4). However, deeper within the lens, the uniformity of their

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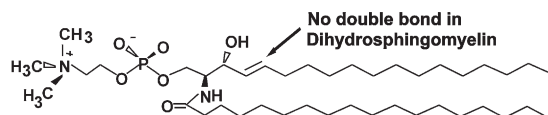
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Abbreviations: AQPO, aquaporin; Le<sup>x</sup>, Lewis glycolipid; MLB, multilamellar body; PMCA, plasma membrane  $Ca^{2+}$ -ATPase; ROS, reactive oxygen species; SERCA, sarcoplasmic/endoplasmic reticulum  $Ca^{2+}$ -ATPase; TOF, time-of-flight.

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### Sphingomyelin



**Fig. 1.** Structure of sphingomyelin and dihydrosphingomyelin, the major phospholipids in the human lens.

packing diminishes, and intracellular organelles are lost (Fig. 4, blue dots) for cells in the remodeling zone and the adjacent transition region. At these zones, compounds such as red-dextran cannot permeate. As a consequence of the loss of organelles, turnover of cell membranes and proteins does not take place in the deeper fibers (nucleus). Therefore, lipids in the core of the nuclear region are as old as the lens itself. The lack of cell turnover contributes to the increase in size and weight of the lens with age.

### Morphological changes in membranes of cataractous tissues

Numerous studies have addressed this topic. Indeed, the number of reports is so high that a fair and thorough review will not be attempted. Instead, only a few representative studies are cited herein. Morphological (5–16) studies have shown that membrane structural derangement occurs in human cataractous lenses in all regions and types of cataract. Convoluted, undulating membranes, vacuoles, and lamellar bodies were noted with cataract in most morphological studies. Many studies suggest that the globular bodies are deteriorated fiber plasma membranes that contain AQPO (11). Gilliland et al. (16) describe multilamellar bodies (MLBs) as crystalline proteins surrounded by a shell of lipid. The lipid originates from lipid-rich membranes that do not contain integral membrane proteins. Only a small, almost immeasurable change in lens membrane morphology is necessary for a lens to become cataractous. For instance, Gilliland et al. (16) estimate that in

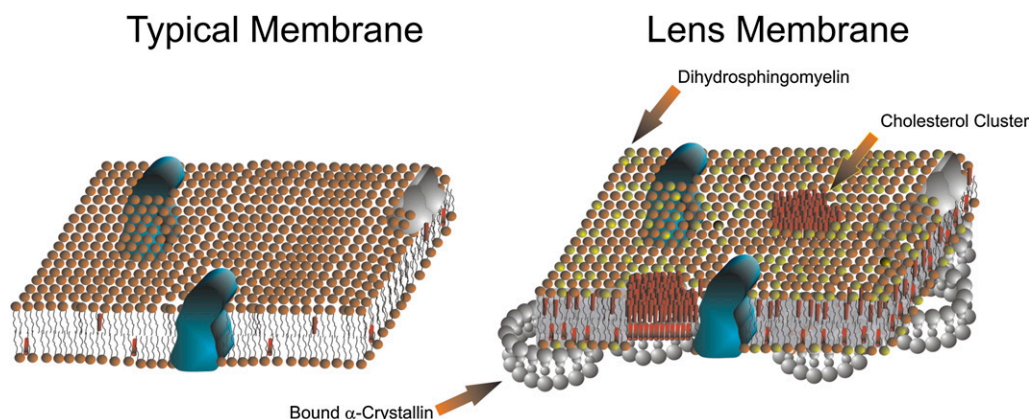
a  $160 \times 160 \times 160 \mu\text{m}$  cube of lens tissue, only 14 MLBs are necessary to cause opacification. In contrast, only two of such MLBs are present in a comparable volume of clear lens tissue (Fig. 5). To find and quantify the small amount MLBs in histological slices is a challenge.

### LIPID COMPOSITIONAL CHANGES IN HUMAN LENS MEMBRANES WITH AGE AND CATARACT

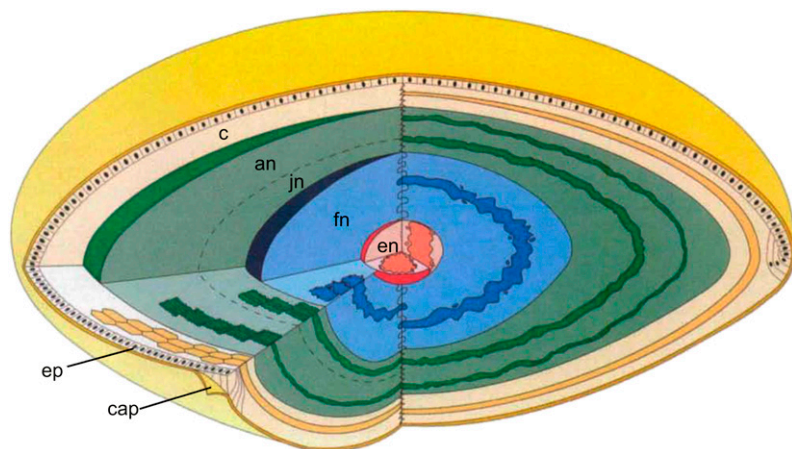
#### Historical perspective

The study of lens lipids began almost two centuries ago in 1825 (17). It is remarkable given the technology of the time that the major phospholipid in the human lens was correctly described in 1857 as a myelin-like lipid (18). We now know that sphingomyelin is a major phospholipid in myelin. The first comparison between lipids from human cataractous and clear lenses was published in 1881 and reported elevated levels of cholesterol in human cataractous lenses (19). At the turn of the century, in 1914, it was reported that, compared with clear lenses, traumatic human cataractous lenses had elevated levels of sphingolipids (20). In 1922, lens cholesterol levels were reported to increase with age (21). Since then, lens lipid studies proliferated enough to warrant their review in 1935 (22). The results reported in 1965 by Feldman and Feldman (23), Broekhuysen (24), and others were last reviewed in 1982 (25).

Developments in both P-31 and H-1 NMR spectroscopy facilitated the identification and characterization of dihydrosphingomyelin (Fig. 1), a lipid found in significant amounts only in primate lenses, particularly in adult human lens membranes (26–33). The biological significance of sphingolipids in lens membranes was reviewed by Yappert and Borchman (29). As discussed in the following sections, sphingolipid compositional changes are related to the lens membrane's organization (34), structure (35–38), and function (29, 39–45).



**Fig. 2.** Left: A typical membrane. Right: Human lens membrane. Typical membranes contain fluid lipids with relatively few cholesterol molecules (red cylinders). Human lens membranes are unique. Most of the lipid is associated with proteins such as  $\alpha$ -crystallin ( $\alpha$ -crystallin assembly shown as gray balls, one large ball and one small ball for each  $\alpha$ -crystallin) and AQPO, which limits their mobility. Human lens membranes are some of the most saturated, ordered (stiff) membranes in the human body. The major lipid of the human lens is dihydrosphingomyelin (green shaded balls). Found in quantity only in the human lens.



**Fig. 3.** Diagram of an aged normal human lens. Fiber cell regions are approximately to scale; the embryonic nucleus is enlarged  $\times 4$  to show detail. Fiber cells are approximately to scale in relation to each other but not with respect to the lens regions. Cross-sections are shown on the left, longitudinal sections on the right. The complex suture pattern is not shown. The epithelium (ep) and capsule (cap) are enlarged for clarity. c = cortex; an = adult nucleus; jn = juvenile nucleus; fn = fetal nucleus; en = embryonic nucleus. Text and caption from (2) with permission.

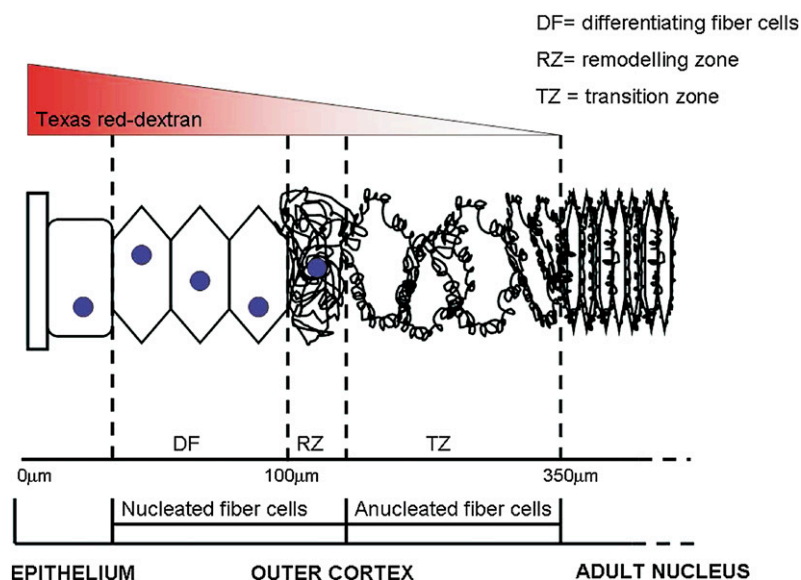
### Lipid oxidation

Despite the low levels of  $O_2$  in the lens, photo- and/or chemical oxidation can and do take place in the lens and affect lipids and proteins. Malondialdehyde is a major secondary product of lipid oxidation. The concentration of malondialdehyde in the human lens increases with age (33) and cataract (5, 33, 46–52). The association between lens opacity and lipid oxidation was so convincing that Babizhayev et al. (5), Bhuyan et al. (46–48), Micelli-Ferrari et al. (49), and Simonelli et al. (50) boldly proclaimed that lipid oxidation may be an initiating step in the pathogenesis of human cataract. Over a human lifetime, more than 40% of the lens phospholipids are degraded, forming deleterious oxidation products (53). Even more degradation occurs with cataract (53). It has been shown in other systems that products of lipid oxidation compromise membrane function and alter relevant cellular processes, including inhibition of growth, respiration, ATPase and phosphate transport, receptors, and DNA, RNA, and protein synthesis, to name a few (54).

Lipid oxidation is obviously deleterious to the lens, and inter-species differences in phospholipid composition support the idea that humans have adapted so that their

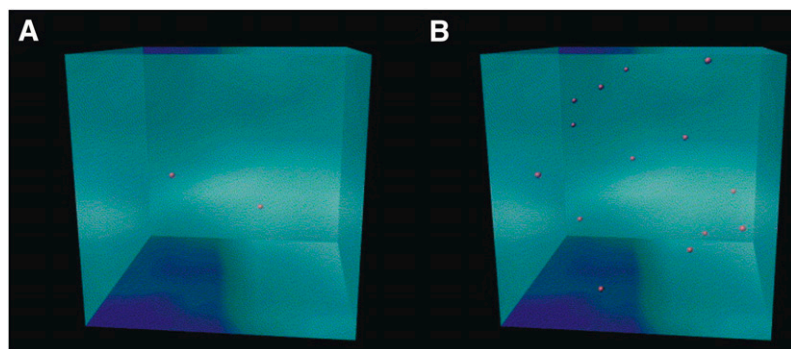
lens membranes have a high sphingolipid content that confers resistance to oxidation, allowing these membranes to stay clear for a relatively longer time than is the case in many other species (45). Furthermore, age-related changes in human lens lipid composition may serve as a marker for oxidative stress and may reflect systemic oxidative insult, providing a window into the health of an individual (45).

Lens lipid composition changes dramatically with cataract. Compared with normal lenses of similar age, the total amount of glycerophospholipids is much less in cataractous lenses (Fig. 6). Interestingly, the total amount of sphingolipids also decreases in cataractous lenses but to a much lesser extent (Fig. 6) (53). These changes are thought to be due to the preferential oxidation of glycerophospholipids. Lens sphingolipids are three to four times more saturated than glycerolipids, and consequently they resist oxidation more effectively than unsaturated lipids (31, 55). Indeed, the rate constant for the propagation step of lipid oxidation decreases sharply when the number of lipid double bonds is reduced (56). Sphingolipids are so stable that they were the only phospholipids found to



**Fig. 4.** Schematic diagram (not drawn to scale) summarizing changes in the morphology of differentiating fiber cells in the outer cortex of the human lens. Blue circles: nuclei. Figures and caption copied with permission from (4).





**Fig. 5.** A diagrammatic representation of MLBs in the aged transparent lens nucleus versus the cataractous nucleus. It is probable the MLBs contain lipids. A: A model cube with dimensions of  $160\ \mu\text{m} \times 160\ \mu\text{m} \times 160\ \mu\text{m}$  is used to demonstrate the approximate volume of lens tissue in the embryonic nucleus ( $4 \times 10^6\ \mu\text{m}^3$ ). Approximately two MLBs are found in this volume of tissue in the aged transparent human lens. B: A similar cube is shown, displaying approximately 14 MLBs in the same volume of tissue in the human age-related cataract. Imagine histological sections through the cubes and the distributions of rare MLBs in the sections. Figures and caption copied with permission from (16).

be present after 40,000 years of exposure in a frozen mammoth (57). With sphingomyelin in the membrane, the degree and rate of oxidation of a polyunsaturated phosphatidylcholine were less than when a saturated phosphatidylcholine, rather than sphingomyelin, was present in the membrane (58), thus suggesting that in addition to their high degree of saturation, sphingolipids form better interfacial barriers than glycerophospholipids. This is possible due to the formation of H-bond networks that connect the interfacial regions between the nonpolar tails and the polar headgroups of neighboring sphingolipids (58). These data support the idea that lens sphingolipids do retard the oxidation of unsaturated glycerolipids. However, the loss of unsaturated phosphatidylcholines and phosphatidylethanolamines with age and cataract is still observed and could be attributed to oxidation (Fig. 7A). As the relative rates of biosynthesis of phospholipid and cholesterol appear not to change significantly within the range of ages studied (59), the relative enrichment of dihydrosphingomyelin (Fig. 7B) (53) and other dramatic variations in lipid composition with age and cataract are a consequence of selective degradation.

#### Phospholipase A2, oxidation, and changes in lipid composition

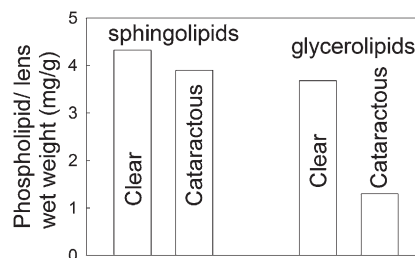
It has been suggested (45) that lipases eliminate oxidized unsaturated glycerolipids so that the membrane is increasingly composed of more saturated sphingolipids. Oxidized lipids contain hydrophilic groups such as hydroxyl and hydroperoxyl moieties located on the hydrophobic hydrocarbon chains that disrupt lipid-lipid interactions, causing structural irregularities in the membrane (60). Phospholipase A2 degrades lipids at points in the membrane that exhibit such structural irregularities (61). Indeed, phospholipase A2 is much more active when oxidized membranes are the substrate (62). Phospholipase A2 is present in the lens (63–66), so it is possible that sphingolipid content, and hence lipid order (38, 67), could increase with age and cataract due to increased oxidation and subsequent degradation of glycerolipids by phospholipase A2.

#### Lens lipid composition, conformation, and function

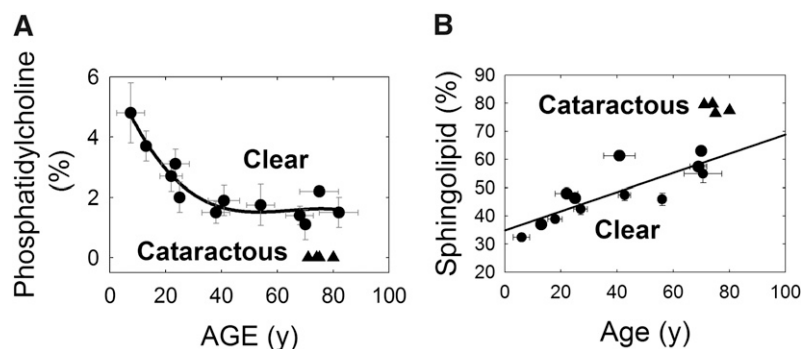
Sphingolipids may be essential to the transparency of human lenses. Broekhuysen (68) over 30 years ago and other investigators recently working with modern NMR

(30) and mass spectroscopic techniques (55) have reported a wide diversity of phospholipids found in lenses from a variety of species. For instance, the relative content of sphingolipids ranges from 3% in the roach lens to more than 60% in human and 77% in camel lenses (Fig. 8B) (30, 45, 68). Borchman et al.'s (45) data suggest that humans have adapted so that their lens membranes have a high sphingolipid content to confer resistance to oxidation, allowing these membranes to stay clear for a relatively longer time than is the case in many other species (45). A higher amount of sphingolipid prolongs lens transparency. Paradoxically, an increase in sphingolipid with age (26, 30, 32, 68) and cataract (53) may indicate phospholipid oxidation, deleterious to lens transparency.

The functional significance of lipid changes with age and cataract has yet to be explored. Plausible functional relationships are provided throughout the following sections. It is around 40–45 years of age that the rates of changes in human lens lipid composition begin to reach a plateau (Fig. 9), the accommodative amplitude of the lens is challenged (69, 70) (Fig. 9), and cation passive permeability increases (71). Although correlations are not necessarily indicative of causation, plausible scenarios can be envisioned in which lens membrane stiffness induced by phospholipid compositional changes could contribute directly or indirectly to presbyopia or passive membrane permeability of cations. The changes in lipid composition



**Fig. 6.** Plot of the estimated differences in the amounts of the two major phospholipid groups in cataractous (seven pools between 46 and 87 years old) compared with clear human lenses (seven pools between 30 and 86 years old). Data were calculated from extraction yields and relative phospholipid composition data in (53). It was assumed that the cholesterol-phospholipid molar ratio was 3:1 in both clear and cataractous human lenses. The relative amount of sphingolipid and glycerolipids was averaged from four pools (55, Table 1).



**Fig. 7.** Changes in the relative levels of human lens phospholipids with age and cataract. A: Compared with clear lenses (closed circle), the relative content of phosphatidylcholine (A) decreased in cataractous lenses (closed triangle). B: In contrast, the relative amount of sphingolipid was higher in cataractous lenses (relative to clear ones). Data compiled from (30, 39, 53).

and conformation with cataract appear to be an exacerbation of the aging process (Fig. 7) (53). A threshold of lipid oxidation may be crossed above which a cascade of lipid and protein changes could be triggered and lead to opacification. If this hypothesis were proven to be true, therapies to keep that threshold from being crossed could be developed to inhibit cataract development.

#### Lens lipid diversity and changes with age and cataract

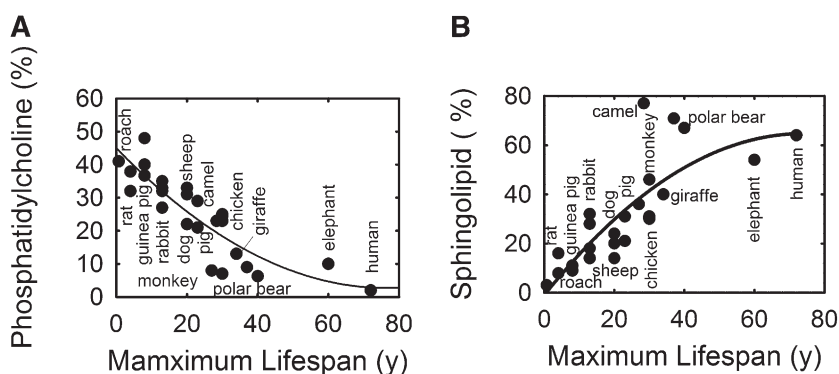
In every lens of the various species examined using infrared spectroscopy, the presence of sphingolipids resulted in more ordered membranes (Fig. 11) (53). The ordering of sphingolipids may result from tight interfacial H-bonding interactions (72, 73) as well as the high level of saturation in the variable acyl chain. In human lens membranes, nearly 60% of the acyl chain of dihydrosphingomyelin is due to the palmitoyl tail with 16 carbons and no unsaturation sites (16:0) and about 25% is due to the nervonoyl chain (24:1) in which the double bond is in the 15th position of the chain and creates a kink that allows the packing of this lipid as if it were saturated but shorter (55). In addition, the inherent saturation of the dihydrosphingosine backbone enables strong inter-lipid interactions.

A recent reevaluation of the phospholipid composition of adult human lens membranes has revealed the presence of ceramide-1-phosphate and dihydroceramide-1-phosphate (74). These two phospholipids had been misassigned in previous P-31 NMR reports. The role of these species in the architecture and function of lens

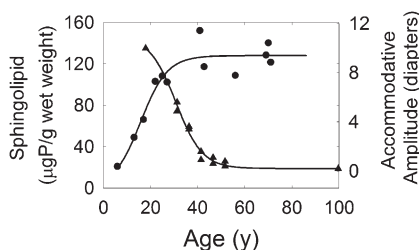
membranes is yet to be explored. Another interesting finding is the presence of the lyso form of phosphatidylethanolamine ether (55, 74). Even though the acyl chain has 18 carbons and one double bond, the fact that there is only one chain, an ether, rather than an ester, linkage and a nonbulky headgroup suggests that this species can pack well despite the single site of unsaturation. However, this lipid was found to be absent in the cholesterol-enriched domains (34) extracted from adult human lenses, where the degree of order exceeds that of the rest of the membrane (74).

The revised composition also reveals the presence of relatively small amounts of other glycerolipids and, importantly, they have an ether linkage in the sn-1 chain (74). About 20% of the phospholipids found in humans possess 1-*O*-alkyl or 1-*O*-alkenyl ether linkages, and inactivation of their synthesis results in numerous pathologies (75–78). The 1-*O*-alkenyl ether linked phospholipids are often called plasmalogens. Plasmalogens serve multiple functions (76), including their role as potent antioxidants (79). In addition, these plasmalogens contain significant amounts of the highly unsaturated arachidonoyl acyl chain (20 carbons long and four sites of unsaturation). Upon enzymatic cleavage of this chain, arachidonic acid, a critical lipid second messenger, is released (79).

Recent studies (74) have clarified previous observations (53) to show that about 35% of the phospholipids in the human lens are predominantly ether-linked (*O*-alkyl but not *O*-alkenyl) phosphatidylethanolamines and phosphati-



**Fig. 8.** The relationship between species differences in maximum lifespan and the relative amount of lens phospholipids (A) and sphingolipids (B). Sphingolipids include dihydrosphingomyelin and sphingomyelin. Adapted from (45). Line indicates the linear regression curve fit with an order of 2.



**Fig. 9.** The relative amount of sphingolipid (closed circle) was calculated from the relative amounts of phospholipid and the total phospholipid data extrapolated from Broekhuysse (24), where P is phospholipid phosphorus (closed triangle). Accommodative amplitude data was adapted from data in (69, 70).

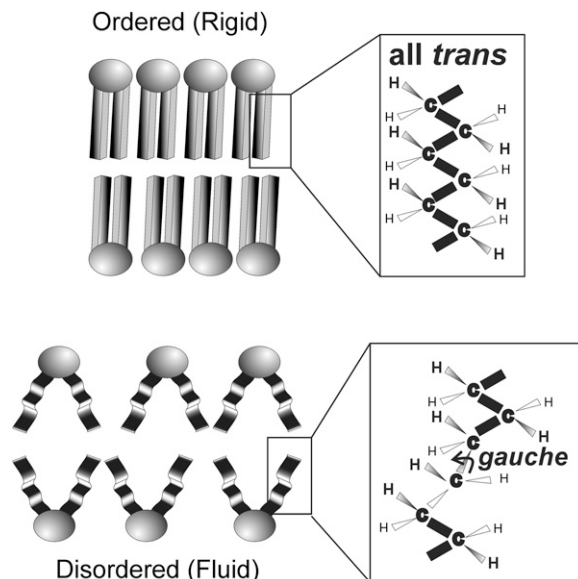
dylserines. Phosphatidylethanolamine (18:1e/18:1) is the most abundant ether-linked phospholipid in the human lens (55). Synthesis of ether-linked glycerophospholipids has been followed in lens epithelial cells (80). In the human lens, over two-thirds of the phosphatidylethanolamine and phosphatidylserine (55), if not all (74), are ether-linked.

Phosphatidylethanolamine 1-*O*-alkyl ether lipid decreases with age and cataract (identified as PE-I in citation 55). The function of ether-linked lipids in the lens is speculative, but they may be important to lens transparency, because in a mouse model, when synthesis of these lipids is inhibited, cataracts develop (77, 78). The cataracts are characterized by many histological observations: vacuole formation, loss of cell-cell adhesion, and poorly differentiated epithelial cells. It has been suggested that ether-linked lipids are involved in the formation of distinct membrane domains that are required for the regulation of growth and, hence, lens transparency (76). It appears that phospholipids with either ester- or vinyl-linked chains are preferentially degraded, leaving the more stable ether-linked species in the membrane. The role of ether-linked phospholipids in human lenses is not known.

#### Hydrocarbon-chain conformation and changes with age and cataract

Lipid structural order may be quantified as the relative amount of *trans* rotomers in the hydrocarbon chains (Fig. 10). When lipids are completely ordered, with all *trans* rotomers, they pack more tightly together, and van der Waal's interactions among adjacent lipids are maximal. When they are disordered, the number of *gauche* rotomers increases, the lipids pack more loosely, and van der Waal's interactions decrease (Fig. 10). Except for the camel lens nucleus, the degree of lipid order increased linearly with sphingolipid content (Fig. 11) (53). Lipid order increased in the human lens with age (37) and cataract (35, 39, 53).

In vitro studies demonstrated that ordered lipids scatter more light than disordered ones (81). Studies suggest that with cataract, light scattering increased by 20% just from the increase in lipid order of the lens membrane (81). It is plausible that the increase in lipid-lipid interactions may contribute to myopia by causing greater compaction and overall stiffness of the lens. In addition, lipid order influences the activity of two abundant lens proteins, PMCA

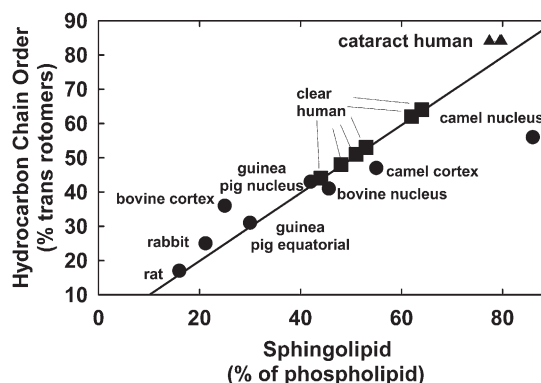


**Fig. 10.** Schematic of lipid conformation that define lipid order. The more *trans* rotomers, the tighter the packing the greater the van der Waal's interactions between lipids and the greater the lipid order (stiffness). The opposite is true for *gauche* rotomers.

and sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA) activity. Interestingly the activity of Na, K-ATPase is not affected by changes in hydrocarbon-chain order ("Lipid-Protein and Lipid-Calcium Interactions"). It has been postulated that lipid order may affect AQPO function, structure, quaternary assembly, and stability (82).

#### LIPID-PROTEIN AND LIPID-CALCIUM INTERACTIONS

Calcium homeostasis is made possible through a balance between the passive permeability of calcium across



**Fig. 11.** The relationship between lens sphingolipid content and hydrocarbon chain order. Hydrocarbon chain order reflects the structural stiffness of the hydrocarbon chain region of lipids in membranes. Clear human lens cortex and nucleus (closed square); cataractous human lenses (closed triangle). This figure has been adapted from Figure 5 in (53). All the data except those related to cataractous lens lipid are from Borchman et al. (45). Cataractous lipid order information is extracted from Paterson et al. (39).



the lens cell membrane and the active transport of calcium that is controlled by proteins at various levels: the protein cell membrane (PMCA) and the Na/Ca exchanger; endoplasmic reticulum (SERCA); and mitochondria (mitochondrial Ca uniporter). Ultimately, the level of lens calcium is determined by PMCA and/or the Na/Ca exchanger, the only two calcium exporters located on the cell membrane that pump cations out of the lens. As discussed below, lens lipids have been shown to influence the activity of PMCA and SERCA but have no effect on lens Na, K-ATPase activity (83).

### SERCA/PMCA-lipid interactions

Thirty lipids have been reported to surround and be in direct contact with SERCA (84). The composition and structure of the lipids in the annulus (84) affect SERCA activity (85). For instance, when the hydrocarbon chains of the lipids are ordered, the rate of calcium pumping by SERCA is lower. This is due to the reduced rate of phosphorylation at the catalytic site of the enzyme (86–89). SERCA pump function is also influenced by bilayer thickness (90, 91), phospholipid composition (92), fluidity (88, 89, 93, 94), and hydrocarbon-chain saturation (94, 95). When the hydrocarbon chains of lipids are disordered (fluid), cholesterol inhibited SERCA activity (96), especially when cholesterol was forced into the lipid annulus surrounding the protein (97). However, cholesterol had no effect on SERCA activity when the lipid matrix was composed of bovine ocular lens lipids that are highly ordered (42).

Changes in lipid composition such as those observed with age or disease could potentially influence SERCA/PMCA function. For example, acidic phospholipids activate PMCA (98, 99). As mentioned above, lipids are essential for maximal SERCA activity (85, 97) and also for PMCA (40). The sarcoplasmic reticulum membranes where SERCA is located contain mostly glycerophospholipids that are relatively unsaturated, providing a fluid lipid environment for the SERCA isoforms (38, 100). On the other hand, plasma membranes contain more cholesterol and saturated lipids such as sphingomyelins (108) that are more ordered. Thus, the natural lipid environment of PMCA in the lens is generally more ordered than that surrounding SERCA. Interestingly, PMCA activity is highest when reconstituted into ordered lipids (such as lens lipids) (40). Conversely, the more fluid and disordered natural environment of SERCA leads to greater activity. Therefore, the differences in the natural lipid environment of SERCA (42) and PMCA (40) contribute to the activity of these different enzymes in the lens and thus to calcium homeostasis.

### AQPO

AQPO is the most abundant protein in lens fiber cell membranes. It forms water pores and thin lens junctions (101, 102). The boundary lipids detected in purified AQPO junctions from bovine lens fiber cells were enriched with cholesterol, ethanolamine glycerophospholipids, and sphingomyelin (102).

Crystallographic studies indicate that a hydrated network of hydrogen bonds and salt bridges holds the lipid phosphate groups in place (103). The headgroup interactions were adjustable, and the protein could accommodate C<sub>16</sub> or C<sub>18</sub> chains, or even unsaturated chains. AQPO probably binds to its annular lipids (104). An annulus of 28 lipids surrounds AQPO, and these lipids are tightly integrated into the protein's architecture where they can affect its function, structure, quaternary assembly, and stability (82). It is unclear if or how changes in lens lipid composition with age and cataract influence AQPO function, because reconstitution studies that include lipids native to the human lens, such as sphingolipids and cholesterol, have yet to be done.

### Mitochondrial protein-lipid interactions

When human lens epithelial membranes contained more cardiolipin and more sphingolipids with higher levels of saturation, they were found to be more resistant to oxidation (105–108). Interestingly, cardiolipin is found only in the mitochondria of cells in the lens epithelium and superficial cortical fibers. It is present at almost undetectable levels in total human lens extracts and makes up only 4% of the total lens epithelial phospholipids. Despite its low level in the lens, cardiolipin is important to mitochondrial function (113, 114). For instance, cardiolipin binds to cytochrome c, keeping it from being released from the membrane. When cardiolipin is oxidized, it no longer binds to cytochrome c, causing it to be released from the membrane (115). This step is critical to mitochondrion-mediated apoptosis (116). In another system, replenishment of cardiolipin restored the activity of the mitochondrial enzyme cytochrome oxidase (117), suggesting that cardiolipin plays an active role in supporting the enzyme activity. In lens-hypoxia studies, the amount of cardiolipin was related to cell viability and mitochondrial membrane potential and inversely related with reactive oxygen species (ROS) production (105–108).

It is reasonable to speculate that cardiolipin is directly involved in the correlations mentioned above, but to test for causality, one would need to alter cardiolipin levels directly by inactivation or activation of cardiolipin synthesis. Thyroxine was used to double the amount of cardiolipin in human lens epithelial cells. Compared with untreated cells (107), thyroxine stimulated the level of three key enzymes involved with cardiolipin synthase (109, 110, 114–117). In lens epithelial cells grown in a hypoxic atmosphere, the amount of cardiolipin was inversely related to the amount of lipid oxidation products (108). This correlation was valid whether cardiolipin and the mitochondrial membrane potential were decreased as a result of oxygen or increased as a result of thyroxine treatment (108). Therapies designed to increase mitochondrial cardiolipin content could protect mitochondria from damage and reduce damaging ROS generation that may potentially ameliorate the effects of oxidation that occur in aging tissues and in diseases such as cataract. Almost all of the ROS generated in hyperoxic lenses came from the mitochondria (105). Because cardiolipin and ROS generation are inversely related, it would be interesting to determine if cardiolipin

levels do decrease with age and cataractogenesis, causing an increase in oxidation.

### Raft protein-lipid interactions

Rafts are ordered segregated domains of proteins, sphingolipids, and cholesterol. They are involved with cell signaling and endocytosis. Several reviews have focused on the function and the nature of the forces that lead to their formation (118–125). Rafts have been reported in human lens membranes (34). Phospholipid compositional differences alone could not account for the substantial enrichment of cholesterol in rafts (34). Specific proteins, such as caveolin-1, must recruit cholesterol and induce clustering. Raft domains were not detected in cataractous membranes, which may possibly be relevant to lens transparency (see glycolipids and rafts, “Glycolipids”) (34). The rafts may have been disrupted and/or their density increased due to protein-induced raft aggregation that would interfere with their isolation and detection (34). In lens epithelia from rabbits and guinea pigs, depletion of cholesterol abolished the majority of caveolae, suggesting that these cholesterol-rich domains are likely to play important roles in the lens (126). Indeed, in the bovine lens, the interactions of protein kinase C  $\gamma$  with caveolin-1 and connexin43 present in lipid rafts may regulate their distribution into or out of rafts and gap junction plaques (127). Rafts could be important to our understanding of cataractogenesis if it were found that compositional changes of these domains in cataractous lenses contributed to the disruption of gap junctional regulation and derangement.

### $\alpha$ -Crystallin lipid binding

$\alpha$ -Crystallin is the only crystallin that binds noncovalently to bovine lens lipid membranes (128–140) that are devoid of protein and synthetic lipid membranes (43, 129–131). Lens membranes have both a high-affinity saturable and a low-affinity nonsaturable  $\alpha$ -crystallin binding site (135–137). The binding of  $\alpha$ -crystallin to lens membranes in vitro may not involve proteins (135), because  $\alpha$ -crystallin binding to native membranes is enhanced when extrinsic proteins are stripped from the membrane surface (128, 129, 138), exposing the lipid moiety. These results contradict an earlier study indicating that  $\alpha$ -crystallin interacts mostly with MP26 (139). It may be relevant to cataractogenesis that as  $\alpha$ -crystallin becomes denatured, it binds more deeply into the membrane (128, 134). The association of  $\alpha$ -crystallin with the membrane increases with age and cataract, as does light scattering (130, 140, 141). The increased association is thought not to be due to posttranslational modifications of  $\alpha$ -crystallin. Indeed, in vitro studies have shown that  $\alpha$ -crystallins from older (129, 139, 142) or cataractous lenses (143) that have undergone posttranslational modifications do not bind effectively to lens membranes. In vitro binding studies show that the binding capacity of  $\alpha$ -crystallin to lipids from older lenses increases with age and decreases in diabetic donors who were treated with insulin (144). This supports the idea that with age and perhaps certain types of diabetes, more  $\alpha$ -crystallin is bound to the membrane and that age-related increased association

of  $\alpha$ -crystallin with membranes may be due to age-related lipid compositional changes (144). Bound  $\alpha$ -crystallin could serve as a condensation point to which other crystallins bind and then become oxidized.

### Calcium-lens lipid interactions

Maintenance of calcium homeostasis is critical to lens clarity (145–147), and every type of cataractous lens has elevated calcium levels [see nine references in (148)]. There is a 150-fold difference between free and bound calcium levels in the lens, and the location of bound calcium in the lens is not known. Infrared spectroscopic studies of the head group region of sphingolipids, the major lipid in human lenses, revealed that calcium binds to select phosphate groups and partially dehydrates others (144). An in vitro binding study indicates that human lens lipids have the capacity to bind nearly all the calcium present in the human lens and that age and cataract diminished the capacity of lens lipids to bind calcium (148). It is possible that increased intracellular calcium concentrations and a diminished capacity of lens lipids to bind to calcium initiate a cascade of events that culminates in increased light-scattering from lipids and especially proteins. The intracellular calcium concentration is too low for calcium to bind to the inner leaflet of the membrane. However, it was suggested that most of the diffusible calcium in the lens is in the intercellular spaces where the calcium concentration would be high enough so that lens lipids in the outer leaflet of the bilayer bind it (148).

## GLYCOLIPIDS

Glycolipids are composed of 4-sphingenine (sphingosine) or ceramide moiety to which carbohydrates are bound. Glycolipids compose <1% of the total human lens lipid but are critical for lens differentiation and maturation of lens epithelial cells to lens fibers (149, 150). They are distributed in the outer leaflet of biomembranes, and their carbohydrate moieties are known to change in association with cellular differentiation and transformation (151). Glycolipids associate with rafts together with sphingomyelin, cholesterol, and several membrane proteins such as caveolin (“Lipid-Protein and Lipid-Calcium Interactions”), Src, Rac, and Rho, where they are involved in signal transduction cascades (152, 153). Glycolipids are also important to the control of cell growth, oncogenic transformation, cellular interaction, cell differentiation, and immune recognition. They have been extensively characterized and quantified in clear human lenses with age (154–156) and cataract (156–158). Among glycolipids, gangliosides are more complex and exhibit oligosaccharide chains containing N-acetylneuraminic acid, a sialic acid, that are attached to ceramide or dihydroceramide. The content of lens gangliosides has been reported to increase with age (155, 156, 159) and cataract progression (156). Such a relative increase in these species could modify cell-to-cell interactions and lead to the initiation and



progression of cataract (154). Unique Lewis glycolipids were found to be elevated in cataractous lenses (160), but their function in human cataracts is still unknown. These lipids are denoted as Le lipids and contain a trisaccharide headgroup composed of lacto-N-fucopentaose-3 having the carbohydrate sequence, galactose  $\beta(1\rightarrow4)$  [Fuca(1 $\rightarrow$ 3)]N-acetylglucosamine  $\beta$ . This trisaccharide is a determinant found on glycolipids or glycoproteins and is known as 'Lewis' saccharide after observing that Mrs. HDG Lewis' serum caused the agglutination of red blood cells of approximately 25% of normal blood donors (161).

Extensive studies of Ogiso et al. (162–165) on rhesus monkey and human lenses revealed that both species had Lewis glycolipid (Le<sup>x</sup>) and sialylated Le<sup>x</sup> epitopes on neolacto-series of glycolipids. Sialyl Le<sup>x</sup> are glycolipids containing a tetrasaccharide carbohydrate, the same carbohydrate that is usually attached to O-glycans on the surface of the cells. Primary cultures of lens epithelial cells made it possible to examine the roles of carbohydrate epitopes in the cell-to-cell adhesion of lens cells. Prolonged cultures of rhesus monkey lens cells showed morphological alterations, depending on cell-to-substratum adhesion, and expressed sialyl-Le<sup>x</sup> gangliosides on the vitronectin and laminin surfaces (166). Ariga et al. (158) explored the glycolipid composition of cataractous lenses and found a very high contribution of sphinganine (or dihydrosphingosine) long chains to the glycolipids, with the major fatty acids being palmitic (C16:0), nervonic (24:1), and lignoceric acid (24:0). It appears then that the compositional trends reported for the hydrophobic tails of sphingophospholipids are also observed in lens glycolipids.

In light of the important functions of glycolipids in other cells, the investigation of their distribution and function in the human lens is of utmost relevance and has yet to be pursued. The association of glycolipids with rafts may be important to cataractogenesis, because the physicochemical nature of these domains appears to be different in clear human lenses and cataractous ones (34) ("Lipid-Protein and Lipid-Calcium Interactions").

## SYSTEMIC FATTY ACIDS AND CATARACT

Fatty acids are carried by albumin in the vitreous humor. Lens cells actively transport albumin from the apical to the basolateral compartment in a process that involves caveolae and clathrin-coated vesicles (167–169). Micromolar concentrations of unsaturated fatty acids are cytotoxic to cultured bovine and human lens cells (170–175). The aqueous humor of elderly patients who had cataracts contained micromolar levels of fatty acids and raises the possible involvement of these fatty acids in cataract formation. In a number of pathophysiologic states related to cataract formation, fatty acid is elevated in blood [see references in (175, 176)]. Fatty acid exposure results in bleb formation where fatty acid molecules accumulate in the cells (174). Bleb formation, disruption of the actin cortical network, and the redistribution of actin are related (177, 178) and contribute to lens opacity.

## PHOSPHOLIPID SYNTHESIS

As discussed in "Lipid Compositional Changes in Human Lens Membranes with Age and Cataract", changes in lens lipid composition can be explained by lipid degradation. However, phospholipid synthesis is necessary for repair in response to aging or cataract as well as growth and fiber elongation. Lens lipid synthesis was reviewed in 1984 (25). Fatty acids carried by albumin are precursors for glycerol- and sphingo-phospholipids ("Glycolipids" and 168)]. Changes in the synthesis of phospholipids and their precursors during cataractogenesis have been investigated, but further studies are necessary to determine if the changes are related to natural differences in phospholipid metabolism or if these alterations are the result of damage-induced pathways that lead to membrane repair (179). The concentrations and rates of synthesis of phosphorylcholine and phosphoethanolamine, precursors of phospholipid synthesis decrease in cataractogenesis and cataractogenic stress (180–191), and the kinetic properties and substrate specificity of choline/ethanolamine kinase change during galactosemic cataractogenesis (192). Increased phospholipid synthesis was reported to occur in an aldose-reductase inhibitor cataract model (185). The response of the lens to galactose insult is complicated by an initial decrease in phosphorylcholine synthesis followed by a recovery period (179). This recovery may be part of a broader response that controls phospholipid biosynthetic pathways and protects lenses against stresses.

In animal and human lenses, higher dihydrosphingolipids correlated with lower lens growth rates. This was also reflected in correlations based on the regional phospholipid composition reported for bovine and human lenses (30). Diacylglycerol, a metabolite of phosphatidylcholine promotes growth, whereas ceramide and sphingosine, metabolites of sphingolipids, function to retard or stop growth. Interestingly, the analogs without the *trans* double bond (dihydroceramide and sphinganine) do not have biological activity. The same group used MALDI time-of-flight (TOF)-MS simultaneously track the biosynthesis of multiple phospholipid classes while providing details on their acyl chains at the picomolar level (193). Faster growth in lenses with higher levels of phosphatidylcholines and lower levels of sphingomyelins was observed in the MALDI TOF-MS study (193). Inhibition of sphingomyelin synthesis was expected to increase the relative levels of phosphatidylcholines and thus enhance cell growth (30). To elucidate this issue, future studies using MALDI TOF-MS could be used to systematically study the inhibition of different steps in the *de novo* synthesis and catabolism of sphingolipids to understand the possible effect(s) of each metabolite on the rate of epithelial cell growth.

## CHOLESTEROL

The relationships between lens cholesterol and human lens cataract (194) and direct evidence for cholesterol crystalline domains (195) have been reviewed. The lens

contains more cholesterol than any other organ in the human body, reaching a cholesterol-phospholipid ratio as high as 8:1 in the center of the lens (34, 196). Such a high ratio leads to the formation of pure cholesterol domains (Fig. 12) (195, 197). These domains are actually cholesterol bilayers, different from the rafts that contain laterally segregated phospholipids and are enriched in cholesterol (Figs. 2 and 12). Phospholipid oxidation promotes cholesterol-domain formation (198), and these domains are distinct from those formed with nonoxidized membranes (199). The relative amount of cholesterol is lowest in the outer cortical region of the lens where the molar cholesterol-phospholipid ratio is 1:2 (197, 200, 201). This is a very high ratio considering a typical membrane such as that of the sarcoplasmic reticulum of muscles contains 0.08 mol of cholesterol/mole phospholipid (100). Spectroscopic studies show that cholesterol increases the structural order of cortical membrane lipid and decreases the order of nuclear lipids so that the two membranes have a similar order (36). The physiological function of cholesterol in the lens is uncertain. Cholesterol is relatively stable and impervious to oxidation compared with unsaturated lipids, so it could add stability to the membrane.

Electron spin resonance probe studies have shown that cholesterol contributes to the impermeability of oxygen across lens membranes (202, 203). Maintaining low levels of oxygen is critical to the lens to prevent oxidation and opacification. One of the roles of the mitochondria, located in the epithelium and outer cortical fibers (204–208), is to degrade oxygen (209). The cholesterol-related impermeability of the membranes to

oxygen may serve to keep oxygen in the outer regions of the lens long enough for the mitochondria to degrade it.

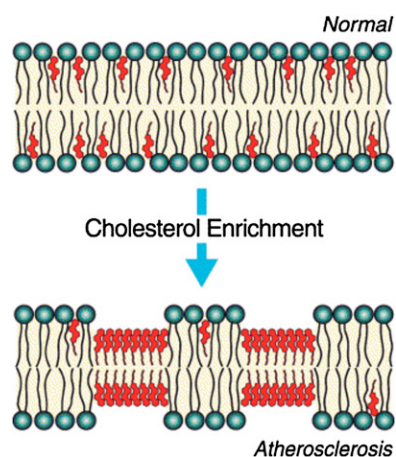
Clinical trials (1–5 years in duration) have shown that hypocholesterolemic drugs are safe to the eye. However, the long-term safety of these drugs is unknown (194). Cholesterol synthesis has been studied extensively since some inhibitors of cholesterol biosynthesis, such as statins, were reported to produce cataracts in dogs (194). The cholesterol-synthesis inhibitor U18666A has a profound effect on lens cells that could be independent of restricting the availability of cholesterol for lens membrane growth (210). U18666A segregates into lens membranes and alters their physical properties. These changes could contribute to cataract formation by both causing membranes to scatter more light and inducing apoptosis of epithelial cells (210).

The physiological functions of cholesterol as well as the regulation of its synthesis in clear, and cataractous lenses should and will be the focus of many future studies.

#### CATARACT LENS LIPIDS, LIFESPAN, AND MORTALITY

Podgor et al. (211) have suggested that in general, senile cataracts may reflect systemic factors associated with overall health status such as heart disease, cancer, and diabetes and advanced age. In the context of lipid changes, cataract appears to be an exacerbation of the aging process and a marker of it (53). Aside from aging and lifespan (45), as discussed in “Lipid Compositional Changes in Human Lens Membranes with Age and Cataract,” there is a two-fold increase in mortality risk associated with both lens opacity (212–214) and cataract surgery (215–219). The authors noted that lens opacity was found to be a marker of mortality rather than the cause (218, 220). The highest mortality risk was observed in patients with nuclear sclerotic (211, 220, 221) mixed cortical and nuclear cataract (221, 224) and posterior subcapsular cataract (211, 220, 221). Pure cortical cataract showed very little (211, 223) or no association (218, 220–223) with mortality. These studies do not imply that the medical treatments for diseases unrelated to the lens cause lens opacity. Indeed, the association between mortality and cataract is also seen even where such medical treatments are not readily available (214). There is no evidence relating specific cellular processes to the observed mortality trends; however, lens lipids may serve as markers for accelerated mortality, because lens sphingolipids were related to maximum life span and both species-dependent and age-related lens lipid compositional differences (45) (Fig. 8).

Is this association coincidental or are there biochemical bases that may begin to address these trends? “Lipid Compositional Changes in Human Lens Membranes with Age and Cataract” discusses studies that show a high sphingolipid content that confers resistance to oxi-



**Fig. 12.** Membrane cholesterol domains were identified using small angle X-ray diffraction approaches. Schematic representation of the effects of lipid peroxidation on membrane structure and cholesterol organization. Based on data collected in this study, cholesterol at relatively low concentrations is fully miscible in normal or nonperoxidized membranes and contributes to overall bilayer width. Exposure to oxidative stress induces segregation of cholesterol into distinct domain regions (34 Å) within the liquid crystalline membrane bilayer. This transfer of cholesterol from the phospholipid bilayer into separate cholesterol domains is also marked by a decrease in overall membrane bilayer width. Captions and figure are from (197).

dation allows membranes to stay clear for a relatively longer time than is the case in many other species. Age-related changes in human lens lipid composition may be related to mortality, because they may serve as a marker for oxidative stress. Changes in lens lipid composition may reflect systemic oxidative insult and could serve as markers of the health of an individual.

## LENS LIPIDS AND CATARACT: CONCLUSION AND FUTURE PERSPECTIVES


The etiology of human cataract in relationship to lens lipids is complicated, because the lens lipid composition of nonprimate animals is very species dependent and also very different from that of human lenses, thus making it difficult to relate the results from animal models to human cataract. Another difficulty in studying human cataracts is that only very minute, almost immeasurable changes in lens membrane morphology are necessary for a lens to become cataractous. Gilliland et al. (16) estimates that in a  $160 \times 160 \times 160 \mu\text{m}$  cube of lens tissue, only 14 MLBs are necessary to cause opacification. In contrast, only two of such MLBs are present in a comparable volume of clear lens tissue (Fig. 5). A wide range of membrane morphological changes has been observed with cataract ("Unique Properties of Lens Membrane Morphology and Function"). Scientists have been aware of lipid compositional changes associated with human cataracts for well over a century, yet, the relationships among lens membrane morphological changes, lipid composition, structure, function and lens clarity, have been elusive. Phospholipid oxidation and subsequent degradation could account for the dramatic changes observed in human lens lipid composition with age and cataract ("Lipid Compositional Changes in Human Lens Membranes with Age and Cataract").

A decrease in  $<1,000$ th of the total lens lipid could account for all of the membrane changes and protein oxidation/aggregation observed with human cataract. For instance, a decrease in the amount of cardiolipin that makes up  $<1,000$ th of the total lipid in the lens could cause the mitochondria to generate all of the ROS necessary to cause the lens to become opaque. A decrease in the amount of glycolipids that make up  $<1\%$  of the total lens lipid could disrupt the cell signal transduction cascade, influencing factors that could contribute to cataractogenesis such as cell growth, cellular interactions, and differentiation ("Glycolipids"). Loss of ether lipids with cataract could influence lens growth, cell-cell adhesion, and lens transparency ("Lipid Compositional Changes in Human Lens Membranes with Age and Cataract").

Major changes in lens lipid composition could also cause cataract, influencing membrane permeability properties, and the function of membrane proteins such as PMCA, SERCA, and AQPO. Costello et al. (15) speculate that dihydrosphingomyelin could laterally associate with AQPO arrays producing stable curved membranes. Glyc-

erol-phospholipids, excluded from the arrays together with cholesterol, may form the protein-poor lipid bilayers of undulating membranes. Because the lipids in the undulating membranes are more susceptible to oxidation, with age they could become damaged and lost. There is no source to replenish the lipids deep within the lens nucleus, so cell integrity would be lost while the remaining gap junctional plaques and rigid sheets of crystalline AQPO could preserve the cell shape.

Not only is the degradation of membrane lipids significant to cataractogenesis, but lipid synthesis, necessary for growth and repair, could also be compromised in cataractous lenses ("Phospholipid Synthesis").

Systemic factors such as fatty acids ("Systemic Fatty Acids and Cataract") in the aqueous humor or drugs that decrease cholesterol synthesis ("Cholesterol") could contribute to cataractogenesis. Indeed, age-related changes in human lens lipid composition may serve as markers for systemic stresses, including those related to oxidation ("Cataract Lens Lipids, Lifespan, and Mortality"). There is considerable support for the idea that changes in lens lipid composition could contribute to cataract, enough to warrant further investigations. A careful systematic investigation of the relationships between lens membrane lipid composition, conformation, morphology, membrane function, and lens clarity would undoubtedly provide insight into the etiology of cataract, myopia, and possibly other diseases. 

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