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Age-related changes in the kinetics of human lenses: prevention of the cataract

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Abstract

• **The crystalline lens is a transparent, biconvex structure in the eye that, along with the cornea, helps to refract light to be focused on the retina and, by changing shape, it adjusts focal distance (accommodation). The three classes of structural proteins found in the lens are α, β, and γ crystallins. These proteins make up more than 90% of the total dry mass of the eye lens. Other components which can be found are sugars, lipids, water, several antioxidants and low weight molecules. When ageing changes occur in the lens, it causes a gradual reduction in transparency, presbyopia and an increase in the scattering and aberration of light waves as well as a degradation of the optical quality of the eye. The main changes that occur with aging are: 1) reduced diffusion of water from the outside to the inside of the lens and from its cortical to its nuclear zone; 2) crystalline change due to the accumulation of high molecular weight aggregates and insoluble proteins; 3) production of advanced glycation end products (AGEs), lipid accumulation, reduction of reduced glutathione content and destruction of ascorbic acid. Even if effective strategies in preventing cataract onset are not already known, good results have been reached in some cases with oral administration of antioxidant substances such as caffeine, pyruvic acid, epigallocatechin gallate (EGCG), α-lipoic acid and ascorbic acid. Furthermore, methionine sulfoxide reductase A (MSRA) over expression could protect lens cells both in presence and in absence of oxidative stress-induced damage. Nevertheless, promising results have been obtained by reducing ultraviolet-induced oxidative damage.**

• **KEYWORDS:** antioxidants; cataract; crystalline; eye lens; kynurenines; ultraviolet radiations

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INTRODUCTION

A cataract is a clouding of the eye lens that affects vision, and it is the major cause of blindness in the world. Recent figures from the World Health Organization in 2010 (http://www.who.int) estimated that 17.6 million people are blind from cataract, and it accounts for approximately 51% of the global blindness. Therefore cataract is a public health and social problem. At present, a 100% successful cataract prevention is not possible, and surgery [phacoemulsification and the insertion of an intraocular lens (IOL)] is the only course of treatment for cataract and optic correction.

Cataract surgery is the most frequently performed operation, thus it represents a significant public healthcare cost. Consequently, there is great interest in understanding molecular and biochemical mechanisms underlying cataract. For these reasons the knowledge of molecular basis of this process may allow researchers to develop more effective preventive treatments. We will go to evaluate if these observations could be a preventive measure to avoid the aging of the eye lens.

EYE LENS STRUCTURE AND FUNCTIONS

The eye lens is a transparent, biconvex structure and plays a crucial role in vision. It is located just behind the iris and in front of the vitreous humor, suspended in place by the ligaments of the lens, or Zinn's zonule, a ring of fibrous tissue that attaches to the lens at its equator and connects it to the ciliary body. The iris and the aqueous humor are anterior to the lens, while the vitreous body is posterior to it [1-2]. The eyeball tipically reaches its final size at puberty, while the lens continues to grow, even if the growth rate is substantially reduced after the second decade of life. The weight of the lens rapidly increases from 65 mg at birth to 125 mg by the end of the first year. Lens weight then increases at approximately 2.8 mg/y until the end of the first decade, by which time the lens has reached 150 mg. Thereafter, the mass of the lens increases at a slower rate

Table 1 Common crystallines and property of subunit

Property	α	β	γ
Subunit	αA, αA1, αB, αB1	βA1, βA2, βA3, βA4, βB1, βB2, βB3	γA, γF, γS
Number	30-45	0-8	2
Molecular weight	20 kDa	25-32 kDa	20-24 kDa
Native molecular	600-900 kDa	50-200 kDa	20-24 kDa
Thiols' contents	Low	High	High
Concentration	6%-21%	5%-14.3%	2.5%-15.5%
Activity	Chaperones, structural proteins, transparency's keeping	Structural proteins	Refraction

(1.4 mg/y) to reach about 260 mg by the age of 90 years old[1-3].

The human lens is very pale yellow to clear in colour; lens transparency is dependent on its highly organized structure. During the earlier part of the sixth decade the colour intensifies, and this is primarily confined to the nucleus, causing an effect on colour perception[4]. Since the change is gradual, it generally goes unnoticed. The lens absorption makes blue objects seem dull and grey unless they are very bright blues whereas green (made from mixing blue with yellow) appears yellow[4-5].

The adult lens consists of the nucleus, cortex, and capsule. The capsule is a smooth, transparent membrane and allows the passage of small molecules both into and out of the lens. It is composed of type IV collagen, laminin and fibronectin. The cortex is composed of cells formed continuously after sexual maturation from the germinal zone, which is a single layer of cells lining the anterior capsule and extends to the equatorial lens bow. The newly formed cells from the germinal zone are forced into a transitional zone where they elongate and differentiate in lenticular fibers to form the mass of the lens. The lens fibers are laid down in concentric layers, the outermost of which lie in the cortex of the lens and the innermost in the core of the nucleus which is already present at birth[5].

The crystalline lens is the second refractive medium after the cornea due to its position, shape and refractive properties which in humans, in its natural environment, is approximately 20 dioptres. In addition the lens helps to refract light to be focused on the retina and, by changing shape, it adjusts focal distance for the visual accommodation. This process is controlled by the autonomic nervous system innervation of to the ciliary muscle. It is estimated that about 80% of the refraction occurs in the cornea and the remaining 20% occurs in the inner crystalline lens[4-5].

When the eye is at rest and focuses on a distant object, the ciliary muscle is relaxed. On the contrary when the eye makes an effort to focus on a near object, the ciliary muscle contracts. This causes the bulk of the anterior ciliary body to move forward and towards the axis of the eye, resulting in a release in tension on the zonular fibres located around the lens equator.

When humans accommodate to a near object, they also converge their eyes and constrict their pupils. These three movements of accommodation, convergence, and miosis are necessary to prevent diplopia, and abnormalities which may lead to many binocular vision problems.

Unfortunately a dramatic change in focal power of the eye occurs as a consequence of a reduction in zonular tension induced by ciliary muscle contraction. The amplitude of accommodation declines with age. By the fifth decade of life the accommodative amplitude declines so that the near point of the eye is more remote than the reading distance. When this occurs, the patient is considered presbyopic and the accommodation decreases to essentially 0 dioptres at the age of 60 years old[3-5].

MOLECULAR STRUCTURE AND BIOCHEMICAL FUNCTIONS

The lens has the highest protein content of any other tissue in the body (about 38% of the total mass)[6]. Among these, there are crystallins which represent up to 90% of water-soluble proteins in the lenses of both vertebrates and invertebrates. Crystallins have long been considered as structural proteins, but to date crystallins are also known to protect cells from stress-induced apoptosis, regulate cell growth and enhance genomic stability[7].

The three main types of crystallins found in the human eye are α-, β-, and γ-crystallins (Table 1), characterized by sequence homology with other enzymes in the lens of vertebrates.

The α-crystallin, present in all vertebrates, has high molecular weight and consists of four bigger subunits (αA, αA1, αB, αB1) and up to 9 smaller subunits (Figure 1A). Physical analyses showed that the secondary structure of alpha-crystallin protein is predominately beta-pleated sheet, with a small amount of aminoacids arranged in a helix structure (less than 10%)[8-10].

Transparency in the eye lens has been hypothesized to result from spatial short-range ordering of crystallins that allows light diffusion [11]. In 1993 Horwitz provided direct evidence that α-crystallin can be considered a molecular chaperone which suppressed almost completely the heat-induced aggregation and precipitation of most enzymes. Moreover, it can assist in the refolding of unfolded protein at physiologic temperatures and can contribute to the mechanisms that maintain the lens in a transparent state under conditions of oxidative stress[12-14].

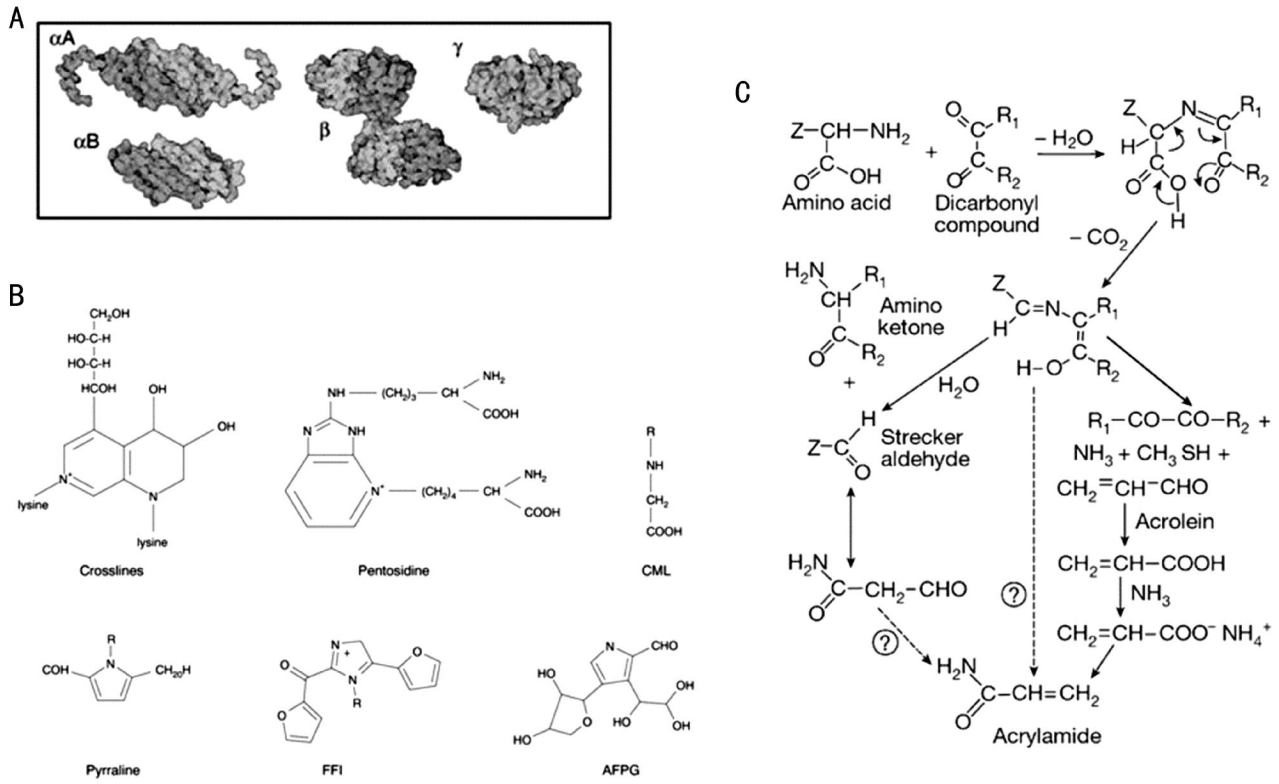

Figure 1 Age-related changes of the lens A: Common crystallines; B: The advanced glycation end products (AGEs); C: Maillard reaction.

Furthermore β-crystallins are characterized by a beta-pleated sheet secondary structure. The seven isoforms of β-crystallin are divided into four acidic (βA1, βA2, βA3, and βA4) and three basic (βB1, βB2, and βB3). Although their function is unknown, β-crystallins similarity with osmotic stress protein suggests that they may also act as stress proteins in the lens[8-9].

In addition, α-crystallins and β-crystallins are able to bind to cell membranes and cytoskeleton, whilst γ-crystallins, the smallest among the three crystallins, are localised into the nuclear lens, characterized by high protein concentration and low water content. Hardness of the lens is related to γ-crystallin content and, in addition, these proteins may play a role in electron transport chain and redox reactions [8]. The remainder of lenticular proteins are formed by insoluble proteins; the latter being primarily membrane proteins and cytoskeletal proteins. Cell membranes contain phospholipids and proteins that are binding sites for carbohydrates. Among these proteins there are the: MIP26/MIP22, MIP70/MIP38, EDTA/calpactin I, protein4.1, spectrin, and MIP17.

MIP26 is a proteolipidic channel (gap junction) that allows the passage of molecules up to 1500 Daltons (Da). *In vitro*, MIP26 proteolysis produces a core of 22 kDa, comparable to MIP22, a membrane intrinsic protein that increases with age[15].

MIP70 is the biggest membrane protein (approximately 70 kDa) and with its proteolysis product (MIP38) it is abundant in the cortical region of the lens. MIP 26 and MIP70 are both similar to gap junctions[16].

The cytoskeleton provides a structural framework for the cell, serving as a scaffold that determines cell shape and transport of organelles through the cytoplasm. The cytoskeleton is composed of three principal types of protein filaments: actin filaments, intermediate filaments (vimentin), and microtubules (tubulin).

About 80% of lipids in the lens include cholesterol and phospholipids such as lecithin, sphingomyelin and cephalin. Like all other organs, the lens needs energy. The main source of energy is adenosine triphosphate (ATP), at least 70% of lens ATP is derived from anaerobic glycolysis. Although an aerobic glycolysis produces only two molecules of ATP perglucose molecule, the low oxygen levels limit the use of aerobic glycolysis. However, although only a very small amount passes into the tricarboxylic acid cycle, approximately 3% of lens glucose, this aerobic metabolism generates 25% of lens ATP. An estimated 5% -10% of non-phosphorylated glucose 6-phosphate is converted in sorbitol by aldose-reductase, an epithelial enzyme which uses nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Furthermore, sorbitol is converted into fructose by polio-1-dehydrogenase. Sorbitol and fructose can increase the osmotic pressure, leading to the uptake of water and lens swelling[8]. Trace elements oroligo-elements, such as sodium and potassium, are present in the crystalline lens. In particular potassium reaches high amounts into the lens, when compared to other tissues. This is due to the presence

of a potassium pump in the epithelial cells membrane. Several low molecular weight components are present into the eye lens (Table 2), such as 3-hydroxykynurenine-O-β-D-glucoside (3-OHKYNG), L-kynurenine (KYN), and 3-hydroxykynurenine (3-OH-KYN), that result from by both enzymatic and non-enzymatic oxidation mechanisms of tryptophan[17].

These elements may be involved for the protection of the visual system against UV rays damage [10]. The lens of a young adult transmits 90% of visible light (400-700 nm) to the retina. The overall transmission of visible light decreases with the increasing of age[18].

The retina is prone to the dispersion and scattering phenomena (diffusion of a light wave caused by particles smaller than the wavelength of the wave itself), and it is protected against UV rays damage through an absorption mechanism of UV rays into the lens. The cornea absorbs all radiation with a wavelength less than 295 nm, while the lens absorbs UVB (295-315 nm) and UVA radiation (315-400 nm)[19]. The pigment 3-OHKYNG is responsible for over 80% of the total lens absorption, in fact more than 90% UV rays reaching the lens are UVA (315-400 nm), the wavelength with a maximum absorption for the 3-OHKYNG (295-445 nm, with a maximum at 365 nm). In addition, the reaction between 3-OH-KYN and the crystallins seems to affect the formation of the products leading to lens clouding, a phenomenon that involves proteins rich in lysine, cysteine and histidine residues[20]. The analysis of the lenticular tissue taken from patients at different ages proved that the kynurenine protein-related amount increases with age. However, 3-OH-KYN seems to be able to tan all of crystallins but it induces precipitation only in the case of alpha A-crystallin[17]. It has been studied the role of UV rays, pH, glutathione and oxygen in determining the binding of 3-OH-KYN to the lenticular crystallins, but only in the case of oxygen it has been proved to play a key role, to the extent that under hypoxic conditions it was possible to observe small changes. Although UV rays are not needed to activate the reaction, their presence is able to increase the cross-linking between 3-OH-KYN and lenticular proteins. In contrast, glutathione concentrations higher than 1 mmol/L were able to delay the reaction. Since glutathione concentration in the lens decreases with age, especially in the lenticular nucleus, this region could be particularly susceptible to modification. In 2007 Sotnikova *et al*[21] concluded that the 3-OH-KYN can modify crystallins also in hyperoxic conditions and in the absence of light, although the UV rays would accelerate this process.

In the eye lens, in addition to pigments, the main molecules involved in the UV rays absorption are the aromatic amino acids such as tryptophan and phosphoric groups. Tryptophan absorbs more than 95% of the radiation absorbed by all of the amino acids present in the human lens. Nevertheless, this value corresponds only to 5% of total amount of radiation absorbed by the crystalline[22].

Table 2 Common aminoacids and low-weight molecular solutes in human lenses, advanced glycation end products (AGEs) in human lenses and cataracts[10-20]

Component in lens	Proteins (μg/g)	AGEs in lens (nmol/L)	
Taurine	0.79	Pirraline	2-15
Serine	0.56	Pentosidine	1-4
Proline	0.16	Carboxymetillysine	350-1800
Glutammid acid	3.42	Crossline	-
Glicine	0.79	Argpiramidine	10-50
Glutathione (total)	1.5	Gliossallysine	1-7
Alanine	1.34		
Ammonium	0.01	AGEs in cataract (nmol/L)	
Urea	4.7	Pirraline	4-22
Ascorbic acid	302	Pentosidine	3-17
AHBG	7-20	Carboxymetillysine	2000-5000
3-OHKYNG	0.5-2	Crossline	-
3-OH-KYN	8-200	Argpiramidine	50-350
KYN	0.4-7	Gliossallysine	10-22

3-OHKYNG: 3-hydroxykynurenine-O-β-D-glucoside; KYN: L-kynurenine; 3-OH-KYN: 3-hydroxykynurenine.

With age, even the crystallins become darker and absorb UV rays. The wavelength absorbed by the aged lenses is about 500 nm. In summary, in lenses of aged patients 3-OHKYNG, 3-OH-KYN and the other filters involved in UV rays absorption are able to protect the retina and to reduce color aberrations, as well as to improve image definition.

ANTIOXIDANT LENTICULAR DEFENSE MECHANISMS

Glutathione Glutathione (L-γ-glutamyl-L-cysteinylglycine) is found at high concentrations in the lens especially in the epithelial layer (3.5-5.5 mmol/g wet weight). It has many important functions in the lens, including the following[8,10]: 1) maintenance of protein thiols in the reduced state, which helps to maintain lens transparency by preventing the formation of high-molecular-weight crystallin aggregates; 2) protection of thiol groups critically involved in cation transport and permeability *i.e.* oxidation of the -SH groups of the Na+/K+-ATPase pump, which results in an increased permeability of these ions; 3) removal of xenobiotics, the glutathione-S-transferase catalyzes the conjugation of glutathione to hydrophobic compounds with an electrophilic center; 4) protection against oxidative damage and amino acids transportation.

Glutathione has a half-life of 1/2d, therefore, glutathione synthesis and degradation rate are the same. It exists in both reduced (GSH) (95%) and oxidized states (GSSG) (5%). GSH can be regenerated from GSSG by the enzyme glutathione reductase (GSR) [8,10]. Additional GSH is transported into the lens from the aqueous humor by a transporter localized on the epithelial cells of the lens. Glutathione provides a hydrogen ion in a reaction catalyzed

by glutathione peroxidase. This reaction, catalyzed by glutathione peroxidase, neutralizes or eliminates H_2O_2 and protects against lipid peroxidation[8,10].

A study of human lenses ranging from birth to 92 years of age shows that over the years glutathione levels reduce up to 73%, and soluble oxidized glutathione levels increase from 2% to 18%[23].

Ascorbic Acid (Vitamin C) Vitamin C plays a major role in the antioxidant defense system of the human lens. It is present in large amounts in the outermost lenticular layers, while it is almost completely absent in the nucleus. In the presence of superoxide anions, superoxide radicals and hydroxyl radicals, ascorbate is oxidized in dehydroascorbate. Ascorbate also prevents lipid peroxidation and thiol groups reduction. Through the glutathione-ascorbate cycle, dehydroascorbate reacts with GSH generating GSSG and ascorbate[24-25]. Ascorbic acid degradation, occurring with the aging in the lens, produces advanced glycation end products (AGEs) (Figure 1B)[26]. On the other hand, through the dehydroascorbic acid pathway, ascorbic acid can covalently bind to lens proteins causing pigmentation, fluorescence, crosslinking and precipitation [27]. It seems that crosslinking occurs even if the free radicals rate is low, but it is inhibited by glutathione in any case.

AGE-RELATED CHANGES OF THE LENS

With aging, many biochemical processes in the lens are altered leading to changes in proteins, vitamins, glutathione, enzymes and water balance. In addition, it has been observed a reduced activity and/or amount of antioxidants especially in the nucleus of the lens. Consequently, proteins in this region are more susceptible to oxidative damage, and protection from it is provided by the cortical area[28]. Each of these changes is responsible for the clouding and cataract development in the lens.

Changes Related to Kinetics, Transport and Water Balance Modifications As well known, the lens grows throughout the course of life. The cells are not lost but rather deposited on pre-existing layers. Cellular fibers lose organelles and, so are repair mechanisms and membrane replacement mechanisms lost. Moreover, the cells found in the lens core are no longer capable of producing antioxidants such as GSH. Thus, fibers of the inner layers receive nutrients, water and GSH from the cortex and epithelial cells. The transportation of water and water-soluble metabolites into the lens is essential for the survival of the crystalline lens because of the lacking of a vascular system and the low amount of extracellular water. In 1999 Moffat *et al* [29] demonstrated that in aged lens the amount of water entering the lens nucleus *via* the epithelium and cortex is reduced. The reduction of water transportation could be due to both a reduction of the diffusion coefficient through the nucleus of the lens and the development of a barrier to water diffusion between the nucleus and the cortex. In particular, it seems that a reduction in water transportation could be due to the membrane itself and the high concentration of intracellular proteins. Moreover, any alteration in the transportation mechanism of nutrients, metabolic substances, antioxidants, and reactive molecules could lead to changes in the redox status (reactive species in the nucleus and a low rate of GSH). Both these events occur with aging and are able to damage lenticular proteins. These results are in agreement with other studies that showed a reduced transport rate of GSH from the cortex to the core [30]. They proposed that lower GSH levels may promote the onset of presbyopia and nuclear cataract.

Crystalline lens hydration during aging has been extensively studied by many authors. Pierscionek *et al* [31] have shown that the percentage of bound water is significantly reduced in all layers of the crystalline lens. Water molecules are able to interact through the H bounds with biopolymers surface, bound water reduction causes protein aggregation and packing.

This argues in favor of syneresis involvement in aging, where bound water is released from biopolymers hydration layer [32]. Concerning the eye, syneresis leads to the development of endovitreal fluid pockets [33]. Recently it has been hypothesized that reversible syneresis could be a response to the change in hydrostatic pressure, a phenomenon that also occurs during accommodation [32]. Free/bound water rate is inversely proportional to pressure, therefore it is possible that presbyopia and cataractogenesis, which are both age-related afflictions, may have synergetic components[32,34].

Glucose Metabolism –related Changes The activity of many enzymes involved in glucose metabolism decreases with age. Among these enzymes there are glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, aldolase, enolase, phosphoglycerate kinase, and phosphoglycerate mutase. Although all metabolic activities decrease, the ability to synthesize proteins, fatty acids and cholesterol in the lens is maintained at correct levels. The decrease in metabolic activity is therefore not a restrictive factor for new lenticular fibers production [8-9]. On the other hand the compounds deriving from carbohydrates such as glyceraldehyde are able to interact with the proteins of the lens *in vitro*, causing yellow coloring and loss of thiol groups in proteins[35]. In addition, also AGE development (Table 2), *via* non-enzymatic glycosylation of proteins in the lens, seems to be partly responsible for the clouding of the lens. In this regard, in 1999, Dillon *et al* [36] highlighted the possible role of the Maillard reaction in lenticular protein glycosylation (Figure 1C). This reaction starts with a sugar molecule which binds to an amino acid, usually being lysine. In lens from young patients, 1.3% of the lysine residues

present in crystallins are glycosylated, while at 50 years of age this value increases from 2.7% to about 4.2% in old people. In advanced stages of the Maillard reaction, sugar residues can further cross-link with other proteins of the lens. In fact, brown pigments isolated from cataract after proteolytic digestion have an UV rays absorption spectrum, a fluorescence excitation spectrum and a chromatographic retention timing similar to the products of the Maillard reaction. In addition, the non-enzymatic glycosylation of lysine residues in lens proteins is able to change conformation and to increase reactivity of thiol groups, as well as to increase disulfide bonds formation [37]. Besides structural changes, that affect lens transparency, AGEs are able to modify α- and β-crystallin chaperone properties[38-39].

Protein Metabolism-related Changes Lenticular proteins have long half-life and are characterized by long-term stability due to the inhibition of degradative enzymes. Nevertheless, several molecular modifications occur during the time. This, in turn, disturbs the short-range order of the crystallins and decreases lens transparency[28].

Crystallins modification due to accumulation of high molecular weight aggregates and insoluble protein Even in 2008, studying the biophysical laws of solids transparency, Linetsky suggested that lens remains transparent until the amount of high molecular weight aggregates does not exceed a certain level[40]. The oxidation and aggregation of lenticular native proteins into high molecular weight (HMW) complexes have been extensively studied and, probably, they are the leading cause for nuclear clouding. Aggregation is due to intermolecular cross-links, especially S-S bonds, resulting from sulfhydryl groups (-SH) and the oxidation of cysteine. The transparency of the lens depends on the optimum balance between oxidants and antioxidants. The antioxidant activity decreases with age. Reduced proteins keep lens transparency and the tertiary structure protects proteins against sulfhydryl groups oxidation. Tertiary structure changes result in protein unfolding and destabilization. In addition, aging is able to deaminate asparagine residues leading to the conversion from L-isomer to D-isomer [35]. These processes occur because several small molecules are able to interact *via* a non-enzymatic mechanism with the reactive groups of crystallins; among these molecules there are sugars, cyanate and thiocyanate, glucocorticoids, aldehydes and in particular malondialdehyde (MDA). The fluorescence and the absorption spectra generated from MDA in presence of lenticular structural proteins are similar to that generated from a Schiff base, moreover electrophoresis analysis highlighted the development of high molecular weight aggregates similar to those observed in aging [41]. Likewise the increase in the glycosylation rate of crystallins with the presence of glucose or ascorbic acid causes a cross-link of proteins and HMW aggregates. Levels of HMW aggregates increase from 0.16 mg in the lenses from donors between 16 and 19 years of age to 2.3 mg in 60 year-old patients [42]. This phenomenon is also due to the inhibition of proteolytic enzymes such as trypsin which is able to degrade these aggregates. HMW aggregates are localized in the nucleus of young lenses, and they consist of α-crystallins [28]. Higher concentration of HMW cause an increase in scattering light from the nucleus. When the crystalline lens ages, these aggregates become more complex because they result from the association of different crystallins, in particular αA, αB and γS crystallins. Below 20 years of age almost 6% of HMW is made of degraded polypeptides, while at 60 years of age this percentage rises up to 27%[42]. Many of the HMW aggregates are thought to be precursors of insoluble proteins. Below 50 years of age approximately 4% of the lenticular proteins are insoluble and at 80 years of age their percentage rises up to 40%-50%[8].

Before reaching 30 years of age, the insolubility rate is approximately the same as that found in the cortex and in the nucleus, although with aging this rate rises in the nucleus. Up to 80% of the nuclear proteins of an aged lens may be insoluble and most of the nuclear α-crystallins are insoluble after 45 years of age [8-9]. This phenomenon leads to transparency loss of the lens and actively contributes to senile cataract onset. Once again, the oxidation of sulfhydryl groups of the lens, resulting in the formation of disulfides, plays an important role in aging-dependent solubility decrease of lenticular proteins. Since γ-crystallins sulfhydryl groups are more exposed, they are one of the most susceptible crystallins to oxidative modification [9]. The reason of these changes is not completely clear, but some authors argue in favor of a transparency decrease that could be due to an altered interaction between cells[7,43].

Membrane and cytoskeletal proteins changes With aging cytoskeletal and membrane proteins are lost, some are reduced and others are forever lost. In fact, the fibers lose their gap junctions and hexagonal shape, whereas lens nuclei completely leak the cytoskeleton. It has been proposed that lenticular proteases such as calpain, are responsible for these events. In addition, at approximately 50 years of age there is a loss of membrane potential and an increase of Na^+ and Ca^{2+} ions [44]. In 2009 Chepelinsky produced polyclonal antisera against synthetic peptides corresponding to the C-terminal octapeptide and N-terminal nonapeptide of bovine MIP26K (the main component of gap junctions) in order to demonstrate that a covalent change in MIP26K occurs during human aging[45].

Lipid metabolism-related changes During aging there is an increase of lenticular lipid content. Its amount rises from 4.1 to 14.5 mg while the crystalline weight rises from 1 to 2.5 g. The age-related changes that affect lipid composition can be used as an oxidative stress marker, opening a window

onto the patient's health [46]. Biochemical studies have shown that cholesterol and phospholipids rates in the eye lens fiber-cell plasma membrane dramatically drops from 0.6-0.8 mg to 0.5 mg in the deeper layers of the nucleus and the cortex. The relative and absolute amount of sphingolipids, including dihydrosphingomyelin and sphingomyelin, increase with age, whereas glycerolipids, including phosphatidylcholine and two phosphatidylethanolamine-related phospholipids, decrease. These changes are exacerbated by the presence of cataract[47]. The changes in the amount of lipids with age and cataract support the idea that glycerolipids are selectively oxidized over lipids with fewer double bonds, such as sphingolipids.

Studies show that the relative amount of sphingolipids (dihydrosphingomyelin and sphingomyelin) increase from 48% at 22 years of age to 57% at 69 years of age [46-50]. However, an increase in sphingolipid content in the human lens together with aging and cataract may indicate deleterious phospholipid oxidation. In addition, lenticular plasma membranes contain a high level of gangliosides [51]. Changes concerning their content and composition can damage plasma membrane functions, such as ion transportation, cell interaction and cross-talkig and so on [52]. In this regard, in 1995 Ogiso *et al* [53] showed that human lenses accumulate gangliosides in conjunction with aging and senile cataract progression. Thus, age-related changes in lens glycolipids may modify the cell-to-cell interaction induced by cell surface sugar chains, leading to the onset and progression of cataract [54]. There are three types of lipid deposits: congenital deposits, crystals on the cortical and nuclear surface of young and old patients and deposits present in the nucleus of aged lens. In the nucleus it has been found the highest deposit concentration: about 50% more than in the cortex and three times more than in the capsule.

Antioxidant metabolism–related changes Photooxidative and oxidative stress induced by light radiation is limited by intralenticular low oxygen levels and by light-absorbed energy dissipation through a non-destructive photophysical way [55-56]. Since the crystalline lenses lack of both cell and protein turnover, during their lifetime, the ability to repair any damage decreases with age. Incident optical radiation and short wavelengths absorption (265-400 nm) make the crystalline particularly sensitive to photochemical reactions. Thus, continuous exposure of the lens to UV causes progressive photochemical damage. Photons reaching the eyes, characterized by wavelength similar to the UV rays, have a cataractogenic property, particularly UVB radiations. The main factors able to induce lenticular photo-alteration are electromagnetic radiations that cross the cornea and are absorbed by the crystalline lens and the susceptibility to photooxidative damage. In a more recent study, it has been observed that with aging the perilenticular oxygen concentration increases. In turn it is able to enhance the numbers of oxidative damage in aged nucleus, characterized by low antioxidant levels [57-58]. The increase of the oxygen concentration could be due to age-related degeneration of vitreous humor, *i.e.* to vitreous degeneration (liquefaction) and posterior vitreous detachment (DVP)[57].

Therefore, in the eye with an intact vitreous gel, soluble substances such as growth factors, ions and metabolites are redistributed by the relatively slow process of diffusion. The concentration of these molecules should be highest near the tissues that produce them or transport them into the eye and lowest near tissues that consume them or transport them out of the eye. The liquefaction or the removal of the vitreous body, which permits to intermix rapidly the fluid in different regions of the eye, would prevent the formation of these gradients and rapidly distribute solutes throughout the posterior segment of the eye. One solute that should be significantly altered in its distribution in the eye after the loss of the vitreous gel is oxygen.

Oxygen diffuses from the retinal arterioles into the vitreous body and the cells of the inner retinal layers consume oxygen, removing it from the vitreous body. These competing processes result in a steep, standing oxygen gradient within the narrow band of vitreous gel that is closest to the retinal arteries and arterioles. The oxygen concentration measured around the lens is remarkably low in the intact human eye. When the retina is not bounded by the vitreous gel, as occurs after vitrectomy or posterior vitreous detachment, oxygen from the retinal vessels would be carried away from the surface of the retina by fluid movements and distributed throughout the liquefied portion of the vitreous. This could increase the level of oxygen close to the lens[56]. The intact vitreous gel normally helps to maintain the low level of oxygen around the lens by preventing bulk flow of the vitreous fluid. Degeneration or destruction of the vitreous gel would allow a greater amount of oxygen to reach the lens and the increased exposure of the lens to the oxygen would cause a nuclear cataract. The main sites of photochemical damage are DNA of epithelial cells and tryptophan of cortical water-soluble proteins (crystallins); *i.e.* the main chromophores of the crystalline lens. Oxidative damage that occurs to proteins and DNA is the leading cause of nuclear cataract. DNA damage, however, is able to affect also differentiation and cell death, besides protein synthesis. Also photo-oxidation of lenticular chromophores is able to develop macromolecular aggregates, through cross-linking proteins, leading to clouding onset [11]. In addition, an oxidative damage to the cell membranes of both epithelial cells and external cortical fibers, which absorb all of the UVB radiation, results in lipid peroxidation, photopolymerization of intrinsic membrane protein and alteration of transporting systems. The cortical cataract forms when there is a damage to fiber cell membranes that, in turn,

leads to altered sodium, potassium, calcium, and water concentration. The development of membrane phospholipids peroxidation products such as MDA is able to inactivate some essential antioxidant enzymes, for example, catalase and glutathione reductase, inducing protein polymerization and cross-linking[8]. Therefore, even if during the young adult age an antioxidant system protects the lens against oxidative damage, this system is not a 100% effective, and leads to accumulation of damage over a lifetime.

Enzyme activity-related changes With aging the levels of different enzymes decrease and among these there are: catalase, superoxide dismutase, glutathione peroxidase, glutathione synthetase, glutamilcisteina synthetase and glutathione reductase [28]. The superoxide dismutase and glutathione peroxidase have a reduced activity by approximately 70%, especially at nuclear level. The activity of superoxide dismutase is completely absent in advanced cataract. This enzyme is involved in lipid and hydrogen peroxides degradation, and its amount increases from the period between birth until 15 years of age, while slowly decreasing after them [9]. The decrease of glutathione observed with the aging in human lenses was associated with a decreased uptake of L-cyst (e)ine, a decreased glutathione synthesis and possibly with an increase in protein-free oxidized glutathione [59]. The ubiquitin-dependent proteolytic pathway is a primary proteolytic system which is involved in the selective degradation of oxidatively damaged proteins in the old lens, especially in the nucleus of old lens, and it undergoes dramatic changes with aging, including a decreased level of ubiquitin conjugates; ubiquitin conjugation activity decreases with age and in response to oxidative stress[60-61].

PREVENTION AND DISCUSSION

Unfortunately, up to date, there are no effective strategies in preventing age-related cataract progression. Several studies, without promising results, focused on the potential role of antioxidant vitamins and other micronutrients for the delaying of lens clouding [28,62]. To confirm this, in 2012 Mathew *et al* [63] analyzed data from nine trials involving 117 272 subjects ranging from 35 years of age or older, but found no evidence to prove that supplementation with antioxidant, beta-carotene, vitamin C or vitamin E would prevent or slow the progression of age-related cataract. In addition, in 2006 Gritz *et al* [64] studied whether antioxidant supplements could decrease the progression of cataract in rural South India. Antioxidant supplementation with β-carotene, vitamin C and vitamin E did not affect cataract progression in a population with a high prevalence of cataract whose diet is generally deficient in antioxidants. Only few studies highlighted a significant reduction of cataractogenesis due to dietary antioxidants intake, among these studies there are ascorbic acids against lenticular, nuclear as well as cortical opacities in females and carotenoids against posterior subcapsular opacities in non-smoking women[65-66].

More recently, there is an increasing and renewed interest about L-carnitine (LC) and curcumin, due to their role in ending or slowing the progression of age related eye diseases, as well as secondary and systemic disease, dry eye syndrome and genetic eye disorders [67-70]. LC is an amine synthesized from lysine and methionine in a vitamin C-dependent process and it is involved in aerobic glucose metabolism, in oxidative phosphorylation and in fatty acids oxidation into mitochondrial matrix. In addition, in the eye, it seems that LC plays a role in protecting lenticular transparency due to its osmotic properties [70-73]. A study of Kocer *et al* [73] determined the antioxidant role of LC against ionizing radiation-induced cataracts in the lenses of rats. Furthermore, the lenticular content of an indicator of lipid peroxidation, MDA, was measured. Irradiation significantly increased the MDA level as an end product of lipid peroxidation, while LC administration toghether with irradiation significantly decreased the MDA level. LC may protect against the damage produced by gamma radiation by increasing the activity of the superoxide dismutase enzyme (SOD) and by scavenging free radicals generated by ionizing radiation. Acetyl-LC has also shown to have antioxidant and protective properties against selenite-induced cataract both *in vitro* and *in vivo*, due to an increased activity of the antioxidant catalase and glutathione peroxidase (GPX-Px) enzymes [74]. Acetyl-LC, but not LC, could further be able to inhibit the glycosylation of lenticular proteins as well as affect the generation of AGEs [75]. In recent years there has been an increasing interest in studying methionine sulfoxide reductase A (MSRA) and its properties. Methionine sulfoxide, a methionine oxidation product, is one of the oxidative stress products, which reaches high levels during progression of lenticular opacity, whereas it is almost absent in normal crystalline. Methionine sulfoxide production is closely related to the inactivation of several proteins, whose reversibility depends on MSRA. In 2004 Kantorow *et al* [76] demonstrated that overexpression of MSRA protected lens cells against oxidative stress damage, whereas silencing of the MSRA gene makes lens cells more sensitive to oxidative stress damage. In fact, the silencing of the MSRA gene results in the loss of the mitochondrial membrane potential and in an increased reactive oxygen species (ROS) production in human cell lens, also in the absence of oxidative stress [77]. The authors also established that MSRA can restore the *in vitro* activity of cytochrome c (cyt c) through its repair of protein methionine sulfoxide. These results support the hypothesis that MSRA is important for the maintenance of lens transparency and it provides evidence that repair of mitochondrial cyt c by MSRA could play an

important role in the defense of the lens against cataract formation [78-79]. In addition, both MSRA and α-crystallins have also been implicated in apoptotic control to cell survival and are translocated to the mitochondria in oxidative stress conditions. It has been demonstrated that oxidation of methionine leads to loss of chaperone function that can be restored by MSRA repair. These results provide insight into the mechanism of cataract development and it is possible that MSRA overexpression may provide a basis for the developmet of intelligently designed intervention for this condition[78-79].

Since aging increases methionine oxidation, MSRA alone or combined with other therapies could represent a long term protection. Recent publications also refer MSRA an antiapoptotic effect against high-glucose-induced damage[80]. However, the most promising results in counteracting lenticular opacity have been obtained by reducing UV light-induced oxidative stress. This aim was reached by Andley *et al* [7] by using UV- absorbing contact lenses. In this study, UVB exposure of human lens induces damages in actin and cytoskeletal microtubules found in cultured human epithelial cells, and this decrease was partially prevented by UV-blocking contact lenses. UVB-irradiated normal human lenses showed marked reductions in αA-crystallin, αB-crystallin, aldehyde dehydrogenase 1, βS-crystallin, βB2-crystallin, and glyceraldehydes 3-phosphate dehydrogenase (G3PDH), and UV-absorbing contact lenses significantly prevented these alterations [7]. By the same token, Hains *et al* [81] proposed that polymerization products resulting from 3-OH-KYN oxidation may be utilized in connection with any lens systems or similar devices such as ophthalmic devices including plastic or glass sunglasses, protective eyewear such as welders or skiers masks or goggles, and hard (hydrophobic) or soft (hydrophilic) contact or IOLs; glass or plastic windows such as automobile, building or airplane windows; glass or plastic packaging material such as beverage and food containers; thin plastic sheets; umbrellas; canopies; and other similar devices or substances suitable for the protection of humans or radiation sensitive substances from radiation.

As well known, the crystallins become increasingly coloured (from yellow to brown) with age, and cataract develops. These events lead to a reduced visual acuity and altered color perception, in addition to contrast sensitivity and depth perception. The clinical procedure is represented by phacoemulsification of cataract and lens replacement with the appropriate IOL. If on the one hand lens opacity leads to impairment of visual acuity, on the other hand it represents an advantage for retina, due to the UV-filter properties of caratact against retinal damages [81-82]. With regards to UV-filter properties, polymerization products resulting from 3-OH-KYN oxidation have been identified as the leading cause for crystalline lens clouding, and moreover it is possible to obtain *in vitro* a synthetic water-soluble version of the yellow pigment of the lens. Color shade can be modified by changing the precursors concentrations and polymerization conditions (pH, temperature and incubation time). This solution, with a light absorption spectrum similar to the normal human lens one, may be embedded in the lenses of glasses and in several other devices. Further studies, both *in vivo* and *in vitro*, have shown the possible role of different antioxidants in counteracting UV radiation [82-84]. Among these, pyruvate, caffeine and epigallocatechin gallate (EGCG) were the most effective substances. The oral or topical administration of pyruvate (5 mmol/L) has been shown to inhibit the formation of cataracts diabetes- and galactosemia-induced in animal models [82]. Caffeine eye drops (added with hydroxypropyl-methyl cellulose 0.9% in saline containing 72 mol/L caffeine, 5 times/d) has been shown to be both effective in protecting the lens against UVA and UVB induced damage as well as to inhibit the sodium selenite- and galactose-induced cataract [85-86]. The EGCG is the most effective and most abundant catechin in green tea [87]. In recent years it has been shown its possible antioxidant role in ophthalmology. In particular, concerning the lens, it seems that local administration of EGCG in saline is able to reach the capsule of crystalline lens, and further carry out its antioxidant role. Already at doses of 5 μg/mL it is able to reduce UVA induced inactivation of catalase from 10% to 50%[82-84]. In addition, EGCG seems to block UVB-induced activation of matrix metalloproteinase-2 (MMP-2) and -9 and nuclear factor-κB (NF-κB): all of these proteins are involved both in lenticular epithelial cells migration and cataractogenesis[88].

In 2005 Demir *et al* [89] showed that octreotide may play a protective role for cornea and conjunctiva *in vivo* against UV radiation-induced damage. It is a somatostatin synthetic analogue but with a prolonged duration, and besides its strong inhibitory effect against growth hormone release it may also play a role of oxygen free radicals scavenger. It is hoped that the synthesis of a topical formulation of this substance, as it could prevent both UV radiation-induced cataractogenesis and UV radiation combined with oral ingestion of psoralens (PUVA)-induced cataract, may be a widely accepted treatment for many skin diseases. Demir *et al* [89] received results in a study conducted in order to evaluate α-lipoic acid protective effect. It is a cofactor for the α-ketodehydrogenase complexes and it is the principal coenzyme involved in acyl-group transfer reactions. Due to the α-lipoic acid protective effect, Demir *et al* [89] observed that a reduced production of MDA and increased levels of SOD and GSH-Px. The 30 mg/d of α-lipoic acid *per os* were able to inhibit naphthalene-induced lenticular opacity in two different murine models by affecting post-translational

modifications and protecting α-crystalline chaperone properties, as well as lens opacification due to streptozocin administration (a molecule able to induce diabetes mellitus)[90-92]. In 2010 Cagini *et al* [35] demonstrated the presence of α-lipoic acid in the aqueous and proved that its concentration increased after it was administered in the form of eye drops, reaching maximum values after around 93min.

In conclusion, it is possible that due to the renewed interests in several other anti-oxidants, such as 1-3 dimethylthiourea, already known for its protective effect against visible light in the retina, the number of molecules that could be effective in clinical practice continues to grow [88-94]. As mentioned above and because of the last findings, there is a real possibility for the usage of antioxidant agents against cataract, but results within the general phenomenon of lens clouding still need to be verified, as its UV radiation-induced damage is only one of the many causes.

CONCLUSION

Lens aging is closely related with its loss of transparency. Lenticular changes that occur with aging represent an index of general aging of the body. In this case, however, lens aging affects both the loss of accommodation, presbyopia, and decreased visual acuity. The knowledge of functions and molecular mechanisms that regulate the transparency of the lens, allows a better understanding of cataract. The most important changes are lenticular morphological-functional, clinically evident, biochemical-metabolic and ultrastructural not evident changes. Neverthless, biochemical changes are most promising in preventing lens clouding.

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