Frontiers in Drug, Chemistry and Clinical Research

Review Article ISSN: 2631-5424

The role of non-enzymatic antioxidants on age-related macular degeneration

Rogil Jose de Almeida Torres[1*], Ana Lucia dos Anjos Ferreira[2], Andrea Luchini[3], Rogerio Joao de Almeida Torres[4] and Camila Renata Correa[5]

[1]Sao Paulo State University (UNESP), Medical School, Botucatu, SP, Brazil

[2]Sao Paulo State University (UNESP), Medical School, Botucatu, SP, Brazil

[3]Centro Oftalmologico de Curitiba, Curitiba, PR, Brazil

[4]Hospital Angelina Caron, Campina Grande do Sul, PR, Brazil

[5]Sao Paulo State University (UNESP), Medical School, Botucatu, SP, Brazil

Abstract

Age-related macular degeneration (AMD) is the major cause of irreversible blindness in the elderly and the oxidative stress is considered the main triggering factor of this disease. Several laboratory studies, performed *in vitro* and *in vivo*, have demonstrated the beneficial effects of non-enzymatic antioxidants on the reduction of oxidative stress biomarkers, as well as the increase in the expression of antioxidant enzymes. Such effects have been expected to culminate in the stabilization or cure of AMD. Nevertheless, the results obtained from large populational studies are not so consistent and positive as observed in laboratory experiments. Consequently, this review aims to approach the role and effects of the main non-enzymatic antioxidants in AMD. The adverse effects regarding the abusive use of antioxidants are also discussed.

Introduction

Age-related macular degeneration (AMD) is the main cause of blindness in the elderly. A comprehensive meta-analysis of 39 population-based studies reported the estimate global number of people affected with AMD in 2020 to be 196 million, a number predicted to increase to 288 million by 2040 [1]. Although AMD mechanism is multifactorial, a common denominator is the redox state imbalance. It is known that the retina is a tissue exposed to oxidative stress due to its high metabolism, large concentrations of polyunsaturated fatty acid content, exposure to visible light (between 400 - 700 nm) and the presence of photosensitive molecules such as rhodopsin and lipofuscin [2]. These conditions favor the permanent peroxidation of polyunsaturated lipids in the membrane system of retina photoreceptor cells [3]. Conversely, the retina contains various detoxification systems, involving endogenous antioxidant compounds and enzymes that counteract the oxidative stress. In retinal pigment epithelial (RPE) cells, antioxidants are generated to protect the RPE cells, as well as photoreceptors and other retinal cells [4,5]. With aging, an increase in the reactive oxygen species (ROS) and a reduction in the production of antioxidant enzymes are observed, leading to the susceptibility of the macula to oxidative alterations [5]. Additionally, numerous studies have shown that AMD patients have decreased antioxidant concentrations in their plasma, a condition that may also contribute to the triggering of the degenerative macular disease [6,7]. The excess of ROS causes oxidative damage to the deoxyribonucleic acid (DNA), proteins, and lipids, inducing a mitochondrial dysfunction and ultimately resulting in lipofuscin accumulation in the RPE cells, triggering AMD [8-11]. Despite the evident participation of the redox state imbalance on AMD pathogenesis [2], the clinical trials that used antioxidants in the prevention or treatment of the disease did not present the same positive results obtained *in vivo* and *in vitro* studies. As an example, we mention the Age-Related Eye Disease Study (AREDS), the clinical trial that comprised approximately 5,000 participants that took vitamin supplements 5 to 13 times the recommended daily allowance (RDA) [12]. Report 8 of this study demonstrated that the use of these vitamins could influence positively the progression of the macular disease and recommended their consumption to monocular advanced or intermediate AMD patients (with bilateral large drusen) [13]. On the other hand, AREDS Report 22 affirms that nutrients such as Vitamin A, α-Tocopherol and Vitamin C were not independently associated with AMD [14]. With the objective to clarify conflicting concepts such as the ones reported above, this review discusses the role and performance of the main non-enzymatic antioxidants in AMD. The adverse effects regarding the abusive use of antioxidants are also discussed.

Methods of literature search

We searched publications in two electronic databases (PUBMED and Web of Science) up to September 2021. We used the following keywords and their synonyms in various combinations: age-related macular degeneration; free radicals; low molecular weight antioxidante; antioxidants; dietary supplements (adverse effects).

Antioxidants and AMD

Antioxidants are made up of small organic molecules and large enzymes that function synergistically to increase cell defense and

Correspondence to: Rogil Jose de Almeida Torres, Rua Emiliano Perneta 390, Conjunto 1407, CEP 80420-080, E-mail: rjat@terra.com.br

Key words: *age-related macular degeneration, free radicals, low molecular weight antioxidante, antioxidants, dietary supplements (adverse effects)*

Received: December 24, 2021; **Accepted:** December 29, 2021; **Published:** January 06, 2022

counteract ROS and reactive nitrogen species (RNS) [15]. Any compound that can inhibit oxidation induced either spontaneously or by means of external oxidants is considered to be an antioxidant. This is a relatively simple definition but, at times, it becomes very difficult to evaluate whether a compound actually has an antioxidant action, particularly *in vivo* [16]. Antioxidants located in both the hydrophilic and lipophilic compartments of plasma are actively involved as a defense system against ROS, which are continuously generated in the body due to both normal metabolism and disease. However, when the production of ROS is not controlled, it leads to cellular lipid, protein, and DNA damage in biological system. There are active interactions among antioxidants located in the hydrophilic (ascorbic acid, uric acid, glutathione system, catalase, superoxide dismutase) and lipophilic (tocopherols, carotenoids, bilirubin) compartments of plasma [17]. The antioxidants may be classified into enzymatic or endogenous, constituted by macromolecules, and non-enzymatic, characterized by organic molecules relatively smaller and with low molecular weight [18-20]. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are enzymatic antioxidants, which constitute the primary defense against oxidative RPE damage [21]. Conversely, the non-enzymatic defense system, is represented by thiol compounds, uric acid, and antioxidant compounds from dietary sources such as the vitamins, minerals, and the phenolic compounds [22-24]. In this review study, we have selected the following antioxidants: thiol compounds, uric acid, ascorbic acid (vitamin C), α-tocopherol, β-carotene, lycopene, xanthophylls (lutein, zeaxanthin and astaxanthin), melatonin, Q10 coenzyme, polyphenols and minerals zinc and selenium. It is probable that the generally well-nourished population maintains optimal ranges of antioxidants through a balanced dietary fruit and vegetable intake. However, high doses of a single or limited mixture of antioxidant supplements may not affect the already saturated *in vivo* antioxidant network, but rather could result in an imbalance in the antioxidant network and could possibly even act as pro-oxidants [16].

Non-enzymatic antioxidants

Thiol

The term thiol or sulfhydryl refers to the functional group "-SH" (H: hydrogen and S: sulphur) that has an atom of sulphur instead of an atom of oxygen [25], an essential element for amino acids, proteins, and other biomolecules [26]. The thiols have a biological relevance for being reactive and ubiquitous [26]. Thiol-type compounds are extraordinarily efficient antioxidants that protect the cells against consequences of damage induced by free radicals, due to their ability to react with them latter. These compounds can act as thiol/disulfide component of redox buffer, free radical scavengers, and chelators of metal ions [27,28]. The intracellular and extracellular states of thiols play a critical role in the determination of the protein structure and function, regulation of the enzymatic activity and control of the activity of transcription factors [29]. The three major thiol/disulfide systems are maintained at different redox potentials in cells: Glutathione/Glutathione disulfide (GSH/GSSG); Cysteine/Cystine (Cys/CySS) and thioredoxin-1 (TRX1). Consequently, a model was proposed for redox signaling with Cys/CySS, GSH/GSSG, and TRX (-SH)2/(-SS-) as three distinct control nodes for redox-dependent pathways [30]. These pairs of thiol/disulfide in the plasma are considered reliable markers of systemic oxidative stress and antioxidant defense [31-33]. Due to this relevance at the systemic level, plasma GSH/GSSG and Cys/CySS redox have been quantified [34,35]. Studies have demonstrated that metabolites of plasma thiol GSH and Cys become more oxidated with age [36,37], oxidative stress [38,39], and age-related diseases [34,36,40].

Reactive oxygen species generated by retinal exposure to acute excessive light are mediated by SOD, GSH, and the thioredoxin (TRX)/thioredoxin reductase (TRXR) defense system [41-43]. Consequently, the connection between thiol levels and AMD have been under analysis. In the AREDS trial, the subjects who received the combined antioxidant supplement did not have significant Cys/CySS oxidation over time, while those who did not take the supplement were oxidized over time. Similar data were obtained for GSH/GSSG. Together with the data on oxidation after chemotherapy and in smokers, the results support the interpretation that the plasma redox states of GSH/GSSG and Cys/CySS provide useful measures of oxidative stress *in vivo* in humans [44]. Another study revealed a significant decrease in the plasma total thiol (TT) of AMD subjects in relation to control group [45]. Similar results were reported in other studies in which AMD patients had significantly lower levels of TT and native thiol (NT) compared to healthy controls [46,47]. One other study showed that NT was significantly lower in patients with AMD than in healthy individuals. However, there was no statistic difference among the groups regarding the TT status [48]. Consequently, it is worth point out the importance of the following thiols in AMD: GSH, cysteine, thioredoxin-1, homocysteine (Hcy) [34,41,44,45], considering that the glutathione system and the TRX system constitute the major thiol reducing systems [49].

Glutathione: The L-y-glutamyl-L-cysteinylglycin (reduced glutathione; GSH) is mainly present in its reduced form and may be converted into the oxidative form (GSSG) by glutathione peroxidase (GPx). Oxidaze glutathione (GSSG) may be reverted into its reduced form (GSH) by the glutathione reductase (GR) [50]. It is known that GSH, the major endogenous antioxidant, acts primarily in the cytoplasm and mitochondria. In healthy cells, GSH protects cells against oxidative injury. GSH is mainly produced in cytosol and is carried from the cytosol to mitochondria using specific glutathione carriers. Consequently, the increase in the GSH levels in the cytosol represents a useful way to increase protection against the oxidative stress for cytosolic and mitochondrial damage [51]. The efficiency of GSH redox system declines with age [52], contributing to the development and/or progression of age-related toxicities and diseases [52-54], including AMD [55-57]. Experimental studies showed that GSH is released from tissues into the plasma [58], and during oxidative stress, GSSG is also released [59]. Release of both GSH and GSSG varies as a function of tissue concentration indicating that plasma measurements could provide an index of tissue oxidative stress [60]. A deficiency of GSH or a major change in the GSH/GSSG ratio renders cells or cellular organelles vulnerable to stress-induced damage. Therefore, its deficiency would result in tissue injury, associated with the triggering and/or progression of several neurodegenerative diseases, autoimmune diseases, and ocular disorders, such as AMD, glaucoma, Leber's Hereditary Optic Neuropathy, and diabetic retinopathy [61-66]. In AMD, studies have shown a significant reduction of plasma GSH compared to the control group [45,67-69]. Another study reported a significantly lower plasma GSH in older individuals affected by AMD, diabetes, and controls (elderly with no diabetes or AMD) than in younger individuals. Estimates of redox potential indicated that the plasma GSH pool is considerably more oxidized in all older groups [34]. It is also important to highlight that data of gene and protein expression identify GSH as one of the most prominent antioxidants in these structures [70-71]. Hence, the presence of the GSH antioxidant system, including the enzymes involved in GSH metabolism and regeneration, has been documented in retinal cells, such as photoreceptor outer segments, Müller glial cells, RPE cells, and retinal astrocytes [66,72-76]. Corroborating these studies, it has been demonstrated that GSH

depletion induced in human RPE (ARPE-19) cells caused ferroptosis, autophagy, and stress-induced premature senescence (SIPS) [77]. RPE from αB crystalline mice (- / -) and ARPE-19 cells transfected with αB crystalline siRNA were subjected to endoplasmic reticulum (ER) stress induced by tunicamycin. The resulting cell death was associated with increased levels of ROS, depletion of glutathione in the mitochondria, decreased of superoxide dismutase activity, increased release of cytochrome c and activation of caspases 3 and 4 [78].

Due to their potential to delay the onset of AMD or slow its evolution, several molecules have been tested to increase GSH expression in RPE cells or in the serum. L-carnitine is synthesized from lysine and methionine and plays a crucial role in fat metabolism by transporting long-chain fatty acids to produce energy via β-oxidation and oxidative phosphorylation [79]. GSH plasma level increase was associated to L-carnitine supplementation (for 3 month) in AMD patients [80]. Experimentally, L-carnitine elevated GSH level in RPE cells (from human donor eyes) treated with hydrogen peroxide (H2O2) [81]. It was shown that GSH depletion-dependent cell death may be prevented by selective inhibitors of ferroptosis, iron chelator deferoxamine (DFO), as well as autophagic inhibitors with lysosomal inhibitor bafilomycin A1 (Baf-A1) (75 nM) and 3-MA (10 mM) (77). The ester derivatives of N-acetylcysteine, N-acetylcysteine methyl ester, N-acetylcysteine ethyl ester (NACET), N-acetylcysteine propyl ester, and N-acetylcysteine butyl ester caused an increase of GSH in ARPE-19 cells treated with hydroquinone (HQ) [51,82]. The pretreatment of ARPE-19 cells with NACET, induced an oxidative stress resistance and increased the intracellular GSH pool available to act as natural antioxidant defense. Moreover, the ability of NACET to increase GSH levels in rats' eyes after oral administration was demonstrated [83] The ARPE-19 cells co-exposed to tauroursodeoxycholic acid (TUDCA), a taurine-conjugated bile acid, had higher cell viability and lower cell death rate compared to cells exposed to H2O2 alone. TUDCA significantly increased antioxidant capacity in H2O2-treated RPE cells by decreasing the generation of ROS and malondialdehyde (MDA), upregulating the expression of antioxidant genes, and increasing the generation of GSH [84]. Madecassoside (MADE), a bioactive triterpenoid saponin isolated from Centella Asiatic, protected ARPE-19 cells against hydrogen peroxide-induced oxidative stress and apoptosis through the activation of nuclear factor-E2-related factor 2/Heme oxygenase 1 (NrF2/HO-1) pathway, inducing an increase of the GSH level [85]. This increase in ARPE- 19 cells undergoing oxidative stress was also observed with exendin-4 (EX4) (a glucagon-like peptide-1 receptor agonist) [86], thymoquinone (TQ) (an active component derived from Nigella sativa) [87], aloperine (a quinolizidine alkaloid) [88], phillyrin (obtained from an extract of the dried fruit of Forsythia suspensa) [89], curcumin [90], astaxanthin [91], ferulic acid (FA) (a natural polyphenol antioxidant) [92], among other antioxidants.

Cysteine: Cysteine (Cys) plays an important role in the GSH two-step biosynthesis. First, L-cysteine and glutamate combine in the presence of γ-glutamylcysteine synthetase to produce a dipeptide and then it is converted into GSH with glycine in the presence of glutathione synthetase [93]. Oxidative stress biomarkers determined in plasma from 77 AMD patients and 75 controls revealed a relationship between a plasma oxidative stress biomarker, Cys and CySS genotype [94]. It is important to emphasize that an L-cysteine has high hydrophilicity, thus justifying a lower accumulation in the RPE cells when administered exogenously. Therefore, with a view to making Cys a preventive and/or therapeutic possibility the use of a lipophilic prodrug approach should be considered. It has been shown that lipophilic small molecule drugs enhance RPE permeability better than hydrophilic small molecule drugs [95-97]. The N-acetyl-L-cysteine (a prodrug of L-cysteine) (NAC) is a potent antioxidant as the bioavailability of the parent drug, L-cysteine [98-99]. NAC controls the redox state in the cells by reducing free radicals directly through its scavenging activity, and it reduces oxidized proteins through its thiol-disulfide exchange activity [100-102]. NAC has proven to be an efficient antioxidant for eye-related diseases in rats and humans [103-105]. NAC protective effect in mice [57], as well as its intravitreal administration have been documented to protect light-induced retinal degeneration [106]. The pre-treatment with NAC protected RPE cells from oxidation-induced mitochondrial dysfunction [107]. NAC has been effective in protecting the retina from oxidative damage when applied topically to the eye, as shown in rd10+/+ mice, a model of retinitis pigmentosa. These results have important implications for AMD because the authors showed that when applied to the cornea, NAC was able to penetrate the posterior segment and protect the retina [108]. In NAC-treated mice with laser-induced lesions, 4-hydroxynonenal (4-HNE)-modified protein induction and nuclear factor-κB (NF-kB) activation in nuclear extracts were markedly suppressed compared to vehicle-treated mice. The recruitment of macrophages and neutrophils was inhibited and the levels of monocyte chemoattractant protein-1 (MCP-1), C-X-C Motif Chemokine Ligand 1 (CXCL1), vascular endothelial growth factor (VEGF) and VEGFR-1 were also lower in NAC-treated mice compared to vehicle-treated ones. Furthermore, the extent of induced choroidal neovascularization (CNV) was significantly less in NAC-treated mice compared to vehicle-treated mice [109]. Both, NAC and N-acetylcysteine amide (NACA) [110-112], have been used with ARPE-19 cells to show protection against oxidative damage. An experiment showed that the four derivates of N-acetylcysteine ester, N-Acetylcysteine methyl ester, NACET, N-Acetylcysteine propyl ester, and N-acetylcysteine butyl ester, provide cell and mitochondrial protection against oxidation damage in ARPE-19 cells in comparison with NAC. This study concluded that the cysteine ester prodrugs are more effective in protecting ARPE-19 cells from oxidative stress than the commercially available antioxidants [51]. Corroborating this study, NACET has shown to increase viability in the RPE cells undergoing oxidative stress more effectively than NAC, due to its direct and faster capacity to act with oxidative agents, increasing the pool of intracellular GSH [83]. Other studies have demonstrated the increase in cellular GSH levels through the conversion to NAC and then to L-cysteine using NACET [98-99]

The Cys/CySS redox couple quantitatively represents the largest pool of low-molecular- weight thiols and disulfides in plasma [31]. It has been reported that Cys / CySS oxidation stimulates the adhesion of monocytes to vascular endothelial cells through up-regulation of various adhesion molecules via an NF-kB-dependent pathway [113]. It has been shown that in more oxidized Cys / CySS environments, cultured ARPE-19 cells are more susceptible to oxidant-induced apoptosis [114].

NAC is available as both an Food and Drug Administration (FDA)-approved prescription drug and an over-the-counter dietary supplement, thereby facilitating its clinical use. It also has a long history of successful use in multiple conditions where elevated ROS induce pathology. Currently, NAC is approved for oral and intravenous administration in the treatment of acetaminophen overdose [100,115-116].

With these positive effects, it is important to determine the substances that induce the increase of plasma cysteine, as for example, the increased oral intake of zinc to modulate the plasma CySS concentration [117]. Corroborating this study, the pilot interventional

study shows that a 5-day course of antioxidant and zinc supplements can modify plasma levels of CySS, suggesting that this oxidative stress biomarker could help predict how likely an individual is to benefit from AREDS supplementation. Further, CySS may be useful for the evaluation of new AMD therapies, particularly those hypothesized to affect redox status [118]. S-allyl L-cysteine (SAC), a bioactive component from aged garlic extracts, can effectively attenuate hydroquinone-induced oxidative damage in ARPE-19 cells, showing that SAC modulates oxidative stress-induced RPE apoptosis, and thereby potentially offers new insights for AMD treatment [119].

It is known that the bioavailability and stability of NAC are limited due to its hydrophobicity and free sulfhydryl group, which can be easily oxidized. The half-life of NACs after oral or intravenous administration is approximately six hours [120]. This short half-life suggests that, to obtain a better effect on AMD, NAC must be used more frequently and in the form of nanoparticles [121-122].

Thioredoxin: The TRX system is a ubiquitous thiol-reducing system that includes proteins, TRX-interacting protein (TXNIP), TRXR, and nicotinamide adenine dinucleotide phosphate (NADPH). TRX is a small protein with two redox-active cysteine residues in an active center (Cys-Gly-Pro-Cys-), which is present in many different prokaryotes and eukaryotes and appears to be present in all living cells [49,123]. Oxidized TRX is reduced by TRXR in the presence of NADPH [124]. TRX expression is induced by a variety of forms of stress, including virus infection, mitogens, phorbol myristate acetate (PMA), X-ray and ultra-violet irradiation, hydrogen peroxide, and ischemic reperfusion [124,125]. Two classical TRX isoforms, including TRX1 in the cytosol/nucleus and TRX2 in mitochondria, are both essential, and inactivating mutations in TRX genes are embryonically lethal [126]. TRX maintains the function of metabolic enzymes whose catalytic activity depends on the presence of disulfide bonds. TRX proteins, TRX1 and TRX2, protect cells by scavenging intracellular ROS [123]. TRX activity and expression are negatively regulated by TXNIP [127]. The inhibition of TRXs by TXNIP leads to an increase in oxidative stress and to an increase in NF-kB expression [128]. In RPE cells, as well as in photoreceptors and other retinal cells, the TRX system combines with antioxidants such as the SOD and GSH for detoxification of ROS [4,41,42,129]. Expression of TRX1 was observed in cytoplasm of ARPE-19 cells, whereas expression of TRX2 was identified in the mitochondria [130]. Overexpression of TRX protects cells from cytotoxicity, elicited by oxidative stress in both *in vitro* and *in vivo* models [131-132]. This protective effect was observed in the mitochondria of RPE cells upon oxidative stress, including acute light-induced injury [42,133]. The multi-functional compounds containing functional groups, which can independently chelate redox metals and quench free radicals, such as 4-(5-hydroxypyrimidin-2-yl)-N,N-dimethyl-3,5-dioxopiperazine-1-sulfonamide (compound 4) and 4-(5-hydroxy-4,6-dimethoxypyrimidin-2-yl)-N,N-dimethyl-3,5-dioxopiperazine-1-sulfonamide (compound 8), reduced the oxidative insult in 2-week dark adapted Wistar rats exposed to 1000 lx of light for 3 hours. In this study, the intense light exposure induced the expression of both TRX and TRXR in the neural retina of untreated rats, confirming the protective effect of the TRX system [133]. Another study showed that TRX2 is more effective in improving the survival of cells exposed to 4-HNE than TRX1, and when TRXs are overexpressed, there is a reduction in the accumulation of perinuclear NF-kB, characterizing TRXs as potent antioxidant proteins of RPE cells [130]. Hence, the substances that can increase TRXs levels in RPE have become relevant. The pretreatment of curcumin on retina-derived cell lines (661W and ARPE-19), protected these cells from H2O2-induced cell death by up-regulating cellular protective enzymes, such as HO-1 and TRX [134]. The geranylgeranylacetone (GGA), an acyclic polyisoprenoid, promoted cytoprotective effects against retinal photooxidative damage by means of induction of TRX and heat shock protein 72 (Hsp72), predominantly in the RPE. Light-induced upregulations of 8-hydroxy-2-deoxyguanosine and 4-HNE-modified protein, markers of oxidative stress, were inhibited by GGA pretreatment. It has been concluded that TRX is a neurotrophic factor released from RPE cells and plays a crucial role in maintaining photoreceptor cell integrity [129]. The 17β-estradiol (βE2) exerted antioxidative effects following light-induced retinal degeneration. βE2 up-regulated NrF2, which triggered phase-2 antioxidant enzyme expression (SOD 1 and 2, catalase, glutaredoxins 1 and 2, and thioredoxins 1 and 2), reduced ROS production, and ameliorated retinal damage [135]. Hydroxytyrosol (the major antioxidant polyphenol in olives) treatment simultaneously protected against acrolein-induced inhibition of NrF2 and peroxisome proliferator-activated receptor coactivator 1 alpha (PPARGC1α) in ARPE-19 cells. The activation of NrF2 led to activation of phase II detoxifying enzymes, including γ-glutamyl-cysteinyl-ligase, NADPH-quinone-oxidoreductase 1 (NQO1), HO-1, SOD, peroxiredoxin and TRX as well as other antioxidant enzymes, while the activation of PPARGC1α led to increased protein expression of mitochondrial transcription factor A, uncoupling protein 2 and mitochondrial complexes [136]. Intraperitoneal and oral administration of sulforaphane (SF), a naturally occurring antioxidant (found as a precursor of glucosinolate in broccoli), has increased the expression of TRX in retinal tissue and upregulated genes with cytoprotective effects against light-induced damage to photoreceptors and RPE in mice [136-138] It was shown that systemic administration of SF could delay photoreceptor degeneration by inducing the activity of extracellular-signal-regulated protein kinases (ERKs) and up-regulating TRX/TRXR/NrF2 system in the retinas of tub/tub mice [139]. SF increased the ability of RPE 19 cell against oxidative stress through up-regulating translation of TRX1 and the nuclear translocation of NrF2, and down-regulating inflammatory mediators and chemokines [140].

Uric acid

Uric acid, a naturally occurring antioxidant, is the end-product of purine metabolism, and it has been proposed to be an important plasma antioxidant [141]. There is growing evidence that urate, a product of uric acid, is beneficial against ONOO- (peroxynitrite) mediated damage and contributes to the total antioxidant defense pool *in vivo* [142-144]. Uric acid eliminates hydroxyl (OH •), and superoxide radicals ($O2 \bullet^-$), as well as inhibits DNA and lipid oxidation in cell membranes. The capacity of the uric acid to prevent oxidation of the biological components by reactive species defines its crucial antioxidant role *in vivo*, delaying the accumulation of tissue lesion markers mediated by reactive species [145]. Several studies report the qualitative and quantitative role of uric acid as an antioxidant substance, which acts by eliminating free radicals and reducer of temporary metal ions, which are converted into little reactive forms [146]. Altered uric acid metabolism could thus possibly play a role in the pathogenesis and damage related to AMD [147]. A possible relationship between serum uric acid and AMD was assessed in 232 participants, divided into 3 groups: Non-Neovascular AMD, and Neovascular AMD (nAMD) and control groups. The mean uric acid levels in those with non-neovascular AMD and the control group was almost identical, making any association between serum uric acid and non-neovascular AMD very unlikely. However, the significantly higher mean serum uric acid level in the nAMD group compared

to the control group when this case group was studied individually indicates a possible association between raised serum uric acid levels and neovascular AMD [148]. More studies should be performed with the objective to certify uric acid as an important marker of AMD.

Vitamin C

Vitamin C is a water-soluble vitamin that can reduce most physiologically relevant ROS/RNS [149]. Additionally, it regenerates the α-Tocopherol and participates in the protective mechanism against lipoperoxidation [150]. It is mainly found in citric fruit such as the orange, lemon, strawberry and dark-green vegetables such as broccoli and spinach, as well in tomatoes, peppers and watermelon. The daily recommended consumption in the United States of America (U.S.A) is 75 mg for women and 90 mg for men [151].

It has experimentally been demonstrated that ascorbate enhibits VEGF expression in RPE cells [152]. The protective factor of vitamin C may be also observed in the retina of rats undergoing luminous radiation [153] and in ARPE-19 cells exposed to H2O2 and ultraviolet B (UVB) [154]. However, a large population study, performed in different countries, does not correlate ascorbic acid supplementation with early or late AMD [14,155-158], whereas in another study, with a limited number of participants, the protective role of vitamin C on AMD was reported [159].

Vitamin E

Vitamin E comprises 8 different forms (α-, β-, γ- e δ-tocopherol e α-, β-, γ- e δ-tocotrienol) [160], being considered a lipo-soluble antioxidant [161]. The main dietary sources of alpha-tocopherol are whole grains, vegetable oil (olive, sunflower, corn and soybean), eggs, and nuts [162]. The α-tocopherol is the most predominant tocopherol in the retina and in the human plasma [163]. The tocopherol antioxidant activity is expressed in the lipid membrane of the cells, where it can act by breaking the chain reaction and preventing the propagation of free radical reactions, as is the case in the peroxidation of the lipoproteins found in the retina and in the macular region [158,164]. The serum concentration of α-tocopherol is regulated by the α-tocopherol transfer protein (α-TTP), involved in its transport from the liver to the other organs [165]. Investigating the protective effect of vitamin E on the oxidative stress in α-TTP knockout rats, which were raised and fed a vitamin E-deficient diet, previous study reported an increase in the lipid peroxidation and degeneration of neurons. The α-TTP deficient rats presented alterations in the retinal function identified by the attenuation in “a" e "b" waves at the electroretinogram (ERG) test, and by the loss of outer and inner segments of photoreceptors. This result shows α-tocopherol to be a powerful antioxidant in the retina that protects against the retinal degeneration related to oxidative stress [166]. Additionally, its effect on Phase II enzymes and its protective role on acrolein-induced toxicity in ARPE-19 cells have been experimentally demonstrated. The pretreatment with α-tocopherol activated the Keap1/NrF2 pathway by increasing NrF2 expression and inducing its translocation to the nucleus. Consequently, the expression and/or activity of the following Phase II enzymes increased: glutamate cysteine ligase (GCL), NQO1, HO-1, glutathione S-transferase (GSTs) and SOD; total antioxidant capacity and glutathione also increased. This antioxidant defense enhancement protected ARPE-19 cells from an acrolein-induced decrease in cell viability, lowered reactive oxygen species and protein oxidation levels, and improved mitochondrial function. These results suggest that α-tocopherol protects ARPE-19 cells from acrolein-induced cellular toxicity, not only as a chain-breaking antioxidant, but also as a Phase II enzyme inducer [167]. Nevertheless, some populational studies report the use of alpha-tocopherol in AMD as very controversial. Some studies suggested a negative association of vitamin E intake with AMD [162,168] and other (AREDS Report 22) concluded that vitamin E intake does not correlate with AMD [14]. Some other studies suggest that there is no significant reduction in AMD risk among vitamin E users [156,169], whereas other report that an increased consumption of foods high in vitamin E was associated with a low incidence of AMD [158,162].

Regarding the serum levels, the results are inconsistent. A study showed a strong relationship between a high alpha-tocopherol plasma level and AMD decreased prevalence [158]. Conversely, another study did not detect such association [170]. Vitamin E deficiency in humans is rare and usually observed in isolated cases of abnormal fat absorption or metabolism in the diet, rather than in diets low in Vitamin E [171]. The daily recommended consumption in the US is 15 mg/d from food (22 IU/d from natural source vitamin E or 33 IU/d of synthetic form) [151].

Carotenoids

Carotenoids are C40-based isoprenoids of the tetraterpene family and are biosynthesized by the linkage of two C20 geranylgeranyl diphosphate molecules [172-173]. Carotenoids contain polyene chain, long conjugated double bonds, which carry out antioxidant activities by quenching singlet oxygen and scavenging radicals to terminate chain reactions. The biological benefits of carotenoids may be due to their antioxidant properties attributed to their physical and chemical interactions with cell membranes [174]. They are responsible for the coloration of some fruits and vegetables [175]. Lycopene, β-carotene, xanthophylls and hydrocarbon are among the 20 important carotenoids found in the human tissue [176]. About 50% of carotenoids may be converted into vitamin A, being nominated provitamin A. They are not considered essential as they are not necessary to mammals receiving an adequate intake of vitamin A, a condition that might change in the absence of such adequate intake. Examples of provitamins include α-carotene, β-carotene, and α-cryptoxanthin [177]. Non-provitamin A carotenoids have been associated with a variety of health benefits [178,179]. This group of carotenoids includes xanthophylls: lutein, zeaxanthin, and astaxanthin, as well as lycopene [180,181]. Epidemiological studies identified the association of high dietary carotenoid intake with reduced risks of breast, cervical, ovarian, colorectal, cardiovascular, and eye diseases [182]. The U.S. Department of Agriculture reported that the average daily intake of lutein by Americans is about 1.7 mg per day, and in Europe, it is 2.3 mg per day. However, these values are far below the recommended dietary intake level of 6 to 14 mg per day to reduce the risk of macular degeneration and cataract [183].

The macula lutea of the eye, also known as the yellow spot, contains high concentrations of macular xanthophylls. The peak concentrations of lutein and zeaxanthin appear at the center of the fovea [184]. These carotenoids concentrate on the plexiform layer of the macula and are responsible for the yellowish color of this region [185]. Carotenoids have been known to be the most effective singlet oxygen quenchers, their activities much higher than those of other retinal antioxidants, tocopherols, and thiols [186-188]. Additionally, through their chain-breaking abilities in lipid membrane peroxidative reactions and free radical extinction, carotenoids have been shown to be important inhibitors of lipofuscin formation [188,189]. Considering the blue light an important inducer of free radicals, and consequently a triggering factor of AMD, it has been shown that the effectiveness of the blue light filter follows the order lutein> zeaxanthin> β-carotene> lycopene. These results indicate that xanthophylls (especially lutein) are the best

blue light filters among all carotenoids available in blood plasma [190]. There is no sufficient strength and consistency studies to support any intake recommendation for all carotenoids. Data on the adverse effects of consuming too much supplementary carotenoids are contradictory. For this reason, there are not setting upper limits for intake of carotenoids. It has been strongly recommended people to use caution before taking these nutrients in high doses and recommend supplementation only to prevent or control a vitamin A deficiency [151].

Beta-carotene: Beta-carotene is one of the 600 carotenoids identified in nature, considered the most known abundant and efficient provitamin A present in food. A study with 156 patients with early and late AMD and a control group of 156 healthy people reported that beta-carotene serum levels weren't significantly associated with AMD [170]. Another randomized, double-blind, placebo-controlled study (n = 22,071 apparently healthy physicians; 40-84 YO), was carried out to test whether beta-carotene supplementation (50 mg/day), would affect the incidence of AMD. After 12 years of treatment, the study reported 162 AMD cases in the beta-carotene supplementation group against 170 cases in the control group, indicating that beta-carotene intake did not interfere in the incidence of AMD [191]. Surprisingly, a study with 494 participants, followed up after 12 months of anti-vascular endothelial growth factor therapy, revealed that a higher intake of dietary β-carotene was associated with an increased risk of intra-retinal fluid (IRF) and pigment epithelial detachment (PED) [192]. Due to its association with the incidence increase in pulmonary cancer, and consequently an increase in the mortality rate of smokers and workers exposed to asbestos [193], beta-carotene used in AREDS was replaced by lutein and zeaxanthin in AREDS 2 [14,194].

Lycopene: Lycopene is a carotenoid with known antioxidant and anti-inflammatory properties. Its long, acyclic polyene chain gives it a singlet oxygen quenching capacity higher than that of β-carotene and α-tocopherol [186,195,196]. This highly unsaturated, non-oxygenated carotenoid is responsible for the red color in fruits and vegetables. Tomatoes and tomato-based products are the most common sources of lycopene in the human diet and account for more than 85% of the dietary intake of this carotenoid in North America [197-199]. Guava, watermelon, papaya, and surinam cherry are additional sources of lycopene [200]. Besides its antioxidant capacity, there are many other potential non-oxidant mechanisms through which lycopene may protect against chronic diseases, including the regulation of the gene expression, antiproliferative capacity, and hormonal and immunological modulation, among others [195,201,202].

A study that exposed ARPE-19 cells to tumor necrosis factor alpha (TNFα) revealed that lycopene can reduce TNF-α-induced monocyte adhesion and H2O2-induced cell damage in RPE cells. Furthermore, lycopene inhibited the expression of intercellular adhesion molecule 1 (ICAM-1) and eliminated NF-kB activation for up to 12h in RPE cells treated with TNFα. Lycopene positively regulated NrF2 levels in nuclear extracts and increased the transactivity of antioxidant response elements. The use of NrF2 small interfering RNA (siRNA) blocked the inhibitory effect of lycopene on TNF-α-induced ICAM-1 expression and NF-kB activation. Lycopene also increased intracellular GSH levels and GCL expression. After treatment with lycopene, TNF-α-induced ROS production was abolished [203]. Tomato extract containing high levels of beta-carotene, lycopene, and traces of lutein exerted protective effects on ARPE-19 cells treated with H2O2. Nitrotyrosine formation was considerably reduced in cells incubated with tomato extract compared with controls after H2O2 treatment. Protein carbonyls were reduced by 30 %, and the formation of thiobarbituric acid-reactive substances was reduced by 140 % in cells incubated with tomato extract. This study provides the experimental evidence for protective effects of dietary tomatoes rich in carotenoids on oxidative stress in the RPE [204]. The analysis of monocytes that were isolated from patients with nAMD, cultured, matured in macrophages and polarized to classic [M1 (stimulated by IFNγ and lipopolysaccharide (LPS))] and alternative [M2 (stimulated with IL-4 and IL -13)] phenotypes, showed that combinations of lutein and carnosic acid with zinc and standardized tomato extract or with beta-carotene yielded an antioxidative, anti-inflammatory, and antiangiogenic effect on M1 and M2 macrophages. These effects were manifested in the upregulation of antioxidative genes (HO-1, SOD1) and the downregulation of pro-angiogenic genes and pro-inflammatory genes [stromal cell-derived factor 1(SDF-1), TNF-alpha, IL-6, MCP-1). Lutein monotherapy or a combination of lutein and zinc had less effect on the expression of these genes [205]. A study revealed that increased serum antioxidant levels were associated with a decreased prevalence of late AMD, but not with early AMD, in an older Japanese population. On the other hand, a combination of alpha-, beta-carotenes and lycopene was protectively associated with late AMD, though alpha-, beta-carotenes and lycopene individually showed no statistically significant relationship with late AMD. The carotenoid family (beta-cryptoxanthin, alpha-, beta-carotenes, lycopene, and lutein and zeaxanthin) was also related to late AMD [206]. Another population study with 263 individuals, aged between 50 and 88 years, reported that higher levels of serum carotenoids, in particular zeaxanthin and lycopene, are associated with a lower likelihood of having exudative AMD [207]. However, the results of nine prospective cohort studies that included 149,203 people, with 1,878 cases of early AMD, indicated that vitamin A, vitamin C, vitamin E, zinc, lutein, zeaxanthin, alpha carotene, beta carotene, beta cryptoxanthin and lycopene have little or no effect on the primary prevention of early AMD. The three randomized controlled trials did not show that antioxidant supplements prevented early AMD [208].

Xanthophylls: Xanthophylls constitute most of the carotenoids in nature, and lutein, zeaxanthin, and astaxanthin are among the varieties that can potentially affect the progression of AMD [209]. The direct and indirect antioxidant actions of xanthophylls involve blue light filtration [210], quenching of singlet oxygen [211-213]. It is known that macular xanthophylls are located transversely in the lipid bi-layer of the retinal membrane, protecting the retina against peroxidation and photo-damage [214-216]. They also prevent blue light exposure to fovea's photoreceptors significantly [217]. Carotenoids also protect the retina against oxidative damage by repairing α-tocopherol and acting synergistically with vitamin C [218].

Lutein and zeaxantine

utein and zeaxanthin, members of the xanthophylls' family, are considered the main carotenoids and have been associated with prevention of cataract and macular degeneration [219-222]. Basil, parsley, kale, leek, pistachio, egg yolk, red pepper represent the main dietary sources of lutein, whereas avocado, corn and wheat (Einkorn, Khorasan, Durum) contain lutein and zeaxanthin [223-225]. They are powerful antioxidants that react against free radicals [226], delaying the lipid peroxidation of the cell membrane and are strongly associated with the AMD risk prevention or decrease [213,227-231]. They are found in the retina and known to be highly effective protective agents against singlet oxygen [232,233]. The highest concentration of macular xanthophylls is found in the outer plexiform layer, which is a layer of neuronal synapses between photoreceptor cells and secondary neurons [234]. Macular xanthophylls are also present in the layer of

rod outer segments and in RPE cells [235-237]. Zeaxanthin is a major carotenoid pigment contained in human retina, predominantly found in the central fovea, whereas lutein is more present in the peripheral region. Zeaxanthin is, therefore, the most efficient antioxidant in the area where the risk of oxidative damage is higher [238,239]. It has been shown that zeaxanthin activates the enzymes of Nrf2-mediated Phase II enzymes, increasing the antioxidant capacity and preventing cell death *in vivo* and *in vitro* [240]. In ARPE-19 cells, lutein treatment significantly increased the transcripts of NQO1 by 1.7 ± 0.1-fold, GLC regulatory subunit (GCLm) by 1.4 ± 0.1-fold, and HO-1 by 1.8 ± 0.3-fold, indicating that lutein not only serves as a direct antioxidant but also activates NrF2 in ARPE-19 cells [241]. It is known that the pigments are responsible for filtering and the absorption of blue light, attenuating the oxidative stress and protecting the retina [235,242]. There is evidence that lutein and zeaxanthin can increase the density of the macular pigment [243,244]. Corroborating the findings about the experimental analyses, the study "Lutein Nutrition Effects Measured by Autofluorescence" (LUNA) showed that supplementation with 12mg lutein and 1 mg of zeaxanthin resulted in a significant increase of the macular pigment density (MPOD) in most participants, including those with macular degeneration [243,244]. Recent study (systematic review and meta-analysis) investigated the minimum concentration of lutein/zeaxanthin (L/Zea) intake (dietary and/or supplement) able to change MPOD in adults (3,189 participants; mean age, 43 YO; 42% male) with healthy eyes. It was found that dose (L/Zea for 3 – 12 months) of 5 mg/d to <20 mg/d and dose of ≥20 mg/d increased 0.04 and 0.11 units in MPOD, respectively. The effects of L/Zea (for 3- 6 months) intake at doses <5 mg/d (dietary sources) is less clear [245]. A study (1,787 women; age, 50-79 YO) showed that the inclusion of bioactive substances such as lutein and zeaxanthin in the diet, can protect against macular degeneration [222]. A meta-analysis revealed that lutein, zeaxanthin, and meso-zeaxanthin supplementation improved macular pigment optical density both in AMD patients and healthy subjects with a dose-response relationship [246]. Evaluating the effects of lutein (5 to 20 mg/d) and zeaxanthin (0 to 20 mg/d) supplementation on visual function from AMD patients (1,176 participants; age, > 65 YO; followed 6 – 36 months), previous systematic review and meta-analysis showed a significant visual acuity improvement in a dose-dependent manner. In fact, each 1-mg/d increase in these carotenoids supplementation was related to a 0.003 reduction in logarithm of the minimum angle of resolution level of visual acuity as compared to baseline [247]. Previous meta-analysis (limited to cohort studies) showed dietary intake of lutein and zeaxanthin is not associated with reduced risk of early AMD, whereas an increase in the intake of these carotenoids may be protective against late AMD. Authors were unable to evaluate the associations in the dose–response meta-analysis due to insufficient dose data [248]. Another epidemiologic study with 380 participants reported that the risk of AMD (early or late) was significantly higher in people with lower plasma concentrations of zeaxanthin. Lower plasma concentration of lutein or lutein plus zeaxanthin was also associated with a higher tendency of AMD, although with no statistical significance [249]. The association between risk of AMD (and cataract) and plasma lutein, zeaxanthin and other carotenoids was studied in 899 people over 60 YO. It was observed that the high plasma level of zeaxanthin was associated with the AMD risk decrease. High plasma levels of lutein and lutein plus zeaxanthin were also related to the decrease in AMD risk. Other analyzed carotenoids such as alpha- and beta-carotene, beta-cryptoxanthin and lycopene were not significantly correlated with AMD. The results suggest an important protective role of xantophyills, mainly of zeaxanthin, in AMD [250]. Other studies have shown that the lutein and zeaxanthin diet was inversely associated with nAMD [14,251]. Conversely, a study with 8,222 people over 40 years of age, did not confirm this inverse relationship of carotenoids in the diet or in the serum of any AMD types. It concluded that the relationship between these carotenoids and AMD may be influenced by age, race and further prospective studies in separate populations must be performed [252]. Corroborating this report, other relevant studies did not present significant associations between the lutein and zeaxanthin diet and prevalence of early or late AMD [253,254]. It is important to mention that serum variations of lutein and zeaxanthin, related to their intake, may be caused by the competition of the serum, tissues, including the retina, as well as by other unknown factors, extrinsic or endogenous, which influence their absorption and/or distribution [255].

Lutein and zeaxanthin benefits remain controversial. The FDA carried out a review study to verify and attest to the scientific evidence of the lutein and zeaxanthin role in macular degeneration and cataract [256]. Based on the reviews, FDA concluded that the agent role of lutein and zeaxanthin to reduce the risk of macular degeneration and/or cataract requires further findings (http://www.cfsan.fda.gov/~dms/ssaguide.html). Finally, AREDS 2, which used 10 mg of lutein and 2 mg of zeaxanthin in the formulation, concluded that compared to the placebo, addition of lutein/zeaxanthin and/or omega-3 fatty acids to the previous AREDS formulation showed no significant effect on AMD progression or visual acuity loss [257].

Astaxanthin

Astaxanthin (3,3'-dihydroxy-β,β-carotene4,4'-dione; molar mass 596.84 g/mol) (AST) is a carotenoid [174], which belongs to the family of xanthophylls. It is typically found in marine environments, especially in microalgae and seafood such as salmonids, shrimps, crabs and lobsters [258,259], contributing to the pinkish-red color of these crustaceans [260]. Astaxanthin provides protection against oxidative stress by scavenging ROS in both the inner and outer layers of the cellular membrane [261-263]. Astaxanthin scavenges mostly peroxyl radicals, protecting fatty acids and biological membranes against lipid peroxidation [264,265]. Due to its unique molecular structure, AST displays some important biological properties, mainly represented by strong antioxidant, anti-inflammatory and antiapoptotic activities [266-268]. It has been shown AST has ten times higher antioxidant activity than lutein, canthaxanthin, β-carotene and hundred times higher than α-tocopherol [265,269,270]. Its antioxidant efficacy has been confirmed by several studies, which report a significant reduction in the levels of oxidative markers, such as malondialdehyde (MDA) and isoprostanes, and increased levels of antioxidant agents such as SOD [271-273]. Astaxanthin also inhibits the production of inflammatory mediators (iNOS, COX-2, TNF-α, and IL-1β) by blocking NF-kB activation and as a consequent suppression of IKK activity and I(kappa)B-alpha degradation [267]. Additionally, AST reduced H2O2-induced cell viability loss, cell apoptosis, and intracellular generation of ROS. Furthermore, treatment with AST activated the Nrf2-ARE pathway. Consequently, Phase II enzymes NQO1, HO-1, GCLM, and GCLC mRNA and proteins were increased. AST inhibited expression of H2O2-induced cleaved caspase-3 protein. Activation of the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway was involved in the protective effect of AST on the ARPE-19 cells [269]. Corroborating this study, other findings show that AST promotes a significant increase in the mRNA expression of NrF-2 as well as its downstream genes, including SOD, GCL regulatory subunit, and GPx [274]. Another study revealed that AST can limit caspase synthesis, Ca2+ release and excessive ROS production with anti-apoptotic effect

on ARPE-19 cells treated with hydroquinone [91]. The protective effect of AST against light-induced retinal damage was experimentally demonstrated [275]. Similarly, this study also demonstrated the protective role of AST against oxidative stress model in mice model [276]. In an AMD experimental model, AST promoted a significant suppression of choroidal neovascularization (CNV) induced by laser photocoagulation due to the downregulation of various inflammatory mediators including ICAM-1, and MCP-1, macrophage-derived VEGF, and IL-6, and endothelial derived-VEGFR [277].

The FDA has approved the use of AST as food colorant in animal and fish feed [278]. The structure of astaxanthin is very similar to that of lutein and zeaxanthin. However, the antioxidant activity and ultraviolet light protection effect of astaxanthin is stronger than that of lutein and zeaxanthin. Consequently, it can be inferred that the deposition of astaxanthin in the eye could provide superior protection against UV light and oxidation of retinal tissues pointing to the potential of astaxanthin for eye health maintenance [279]. Due to the antioxidant effect, AST has been tested as nutritional supplement in patients with AMD [279]. In a randomized clinical trial carried out in patients with non-advanced AMD, an improvement in the evolution of the disease by supplementation with antioxidants and carotenoids, including AST (4 mg), was observed [280]. The multicenter, prospective open-label randomized study, 145 patients were randomly assigned to 2 different treatment groups: interventions were lutein (10 mg), zeaxanthin (1 mg), astaxanthin (4 mg), and antioxidants/vitamins supplementation formula or no dietary supplementation for 2 years. This study reported that patients treated with lutein/zeaxanthin and AST together with other nutrients were more likely to present clinically meaningful stabilization/improvements in visual acuity (VA), contrast sensitivity (CS), and visual function for 24 months when compared with nontreated subjects [281]. Astaxanthin is safe, with no side effects when it is consumed with food. It is lipid soluble, accumulates in animal tissues after feeding of AST to rats and no toxic effects were found [282-284]. It is recommended to administer AST with omega-3 rich seed oils such as chia, flaxseed, fish, nutella, walnuts and almonds. A study reported that no adverse effects were found with the administration of AST (6 mg/day) in adult human subjects [174,285].

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) (MT) is a hormone that possesses an efficient antioxidant capacity [286]. It is produced by the pineal gland, within the retina, as well as in other tissues [287-292]. Light information from the eye is passed on through the retinohypothalamic tract (RHT), reaching the suprachiasmatic nuclei (SCN), where MT secretion takes place. The secretion of MT by the pineal body is circadian, with high levels at night and very low secretion during daylight [286,293]. It partly regulates our day–night rhythmicity including various physiologic functions, such as the cardiovascular system, immune system, the aging process, among others [289, 294-296]. In older patients, MT levels have been shown to be reduced significantly at night, suggesting a physiological role in the ageing process [286]. Additionally, the light that reaches the retina of older people is markedly reduced because of senile miosis and presence of cataract [297-298]. A decrease in MT production in aged persons may cause a reduction of the antioxidant activity, which would directly affect the scavenging of many reactive oxygen species and the stabilization of the cellular membrane and thus induce the oxidative damage, which is thought to be one of the causes of AMD [299,300]. Melatonin receptors have been detected in the RPE, photoreceptors, retinal ganglion cells, horizontal cells and amacrine cells [301]; however, its production is almost exclusively carried out by the photoreceptor cells [302,303]. Once produced, MT is not stored but freely diffuses out of the cells. The amount of MT produced by the retina is small compared to that in the pineal gland. In a few instances, retinal MT may contribute to the levels of the hormone in the blood [304]. Both the pineal and the retinal MT reach the RPE cells [305]. Locally produced MT may lead to a relatively high MT concentration surrounding photoreceptors, thus protecting these cells by inducing the expression of various antioxidant enzymes by activation of MT receptors [289,292,306]. In this regard, MT has been reported to protect the RPE and photoreceptors against oxidative processes [286,307], playing a partial protective role on RPE cells against H2O2 damage [308], and a neuroprotective and antiapoptotic role by reducing the oxidative stress damage in retinal ganglion cells (RGCs) [309]. It has been reported daily administration of MT (3mg) may protect the retina and delay the progression of AMD [309].

A prospective, cross-sectional and observational study was carried out to examine whether MT daytime levels differ between pseudophakic patients with AMD and pseudophakic subjects without any ocular pathology of the same age. The serum analysis of 69 pseudophakic patients, 50 of those with exudative and non-exudative AMD and 19 control patients, revealed that pseudophakic patients with AMD produce more MT during the day compared to pseudophakic subjects without AMD. This study suggested that such a finding may be caused by the reduced visual acuity in patients with AMD, whereby less light reaches the photoreceptors, allowing MT secretion to continue during the day. Because MT also acts as an antioxidant, and daytime levels are higher in patients with AMD, these results might be interpreted as a rescue factor [299]. Besides the serum analysis, the non-invasive diagnosis of MT expression may also be performed by oral epithelium analysis and determination of the main metabolite of the urinary 6-hydroxymelatonin-sulfate (6-HMS) [299,310-311]. The 6-sulfatoxymelatonin levels (aMT6s), the major metabolite of melatonin in urine was determined in 43 AMD patients and 12 controls who did not have AMD. It was observed that the urinary aMT6s level in AMD patients was 40% lower than in age- and gender-matched controls [311]. By analyzing AMD physiopathogenesis and MT action mechanism, it is possible to infer that this hormone is likely to prevent and treat AMD [312].

Coenzyme q10

Coenzyme Q10 (CoQ10) is considered a lipo-soluble endogenously synthesized provitamin. Its oxidized form is called ubiquinone and the reduced one ubiquinol [313]. CoQ10 may be found in all cell membranes of the human body, although larger concentrations are present in heart, liver, brain, and skeletal muscle. It is located in the inner mitochondrial membrane, where it interacts with specific enzymes, and acting as an essential coenzyme in the mitochondrial respiratory chain [314]. Although CoQ10 can be synthesized by human cells, it can also be provided by diets. Small amounts are found in eggs, cereals, milk products, dried fruits such as nuts, and in vegetables (mainly spinach and broccoli). Beef, fish, and poultry are rich sources of CoQ10. In its reduced form, it is a powerful antioxidant that has the capacity to protect the proteins of the mitochondrial membrane, the phospholipids and the DNA from oxidative damage, and regenerate other antioxidants such as the ascorbic acid and a-tocopherol [315,316]. In circulation, CoQ10 is carried bound to lipoproteins, in particular low-density lipoprotein (LDL) in its reduced form (CoQ10H2), which can be easily oxidized to CoQ10. When LDL is exposed to oxidative stress *in vitro*, CoQ10 is the first antioxidant to be depleted. As CoQ10H2 is considered to inhibit lipid peroxidation in LDL and has a low threshold for oxidation, the

CoQ10H2/CoQ10 ratio can be used as a potential marker to determine the oxidative stress LDLs have been subjected to *in vivo* [317]. The normal range of CoQ10 concentration in human plasma is 0.8–1.2 mg/L [318]. Deficiency of CoQ10 may be defined as the presence of reduced levels of CoQ10 in tissues or cells, relating it to the increase in the oxidative stress and associating it with ageing [313]. To estimate its concentration, its plasma level is measured [318-319]. Specifically, CoQ10 levels in the retina can decline by approximately 40% with age and may be linked to the progression of AMD [320-321]. A study that analyzed coenzyme CoQ10 levels in the plasma and platelets from 19 exudative AMD patients and 19 age-matched controls showed that most patients had lower plasma CoQ10 content than most controls. Additionally, plasma from controls showed greater capacity to oppose the oxidative damage, suggesting that free radicals play a pathogenic role in AMD and that CoQ10 may have a protective effect [322]. The use of idebenone, a coenzyme Q10 synthetic quinone analog, revealed potential antiapoptotic and cytoprotective effects on cultured ARPE-19 cells under conditions of oxidative stress [323].

Polyphenols: Polyphenols or dietary phenolic compounds are known to be the largest group of phytochemicals and a group of natural compounds sharing common structural features [324]. More than 8,000 phenolic structures are currently known, and among them over 4,000 flavonoids have been identified. Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols [324]. For years, the beneficial health effects of polyphenols were associated to their antioxidant capacity, largely demonstrated *in vitro*. They are known to be efficient scavengers of most oxidative species, by H-atom transfer or single electron transfer to a stabilizing radical [325]. Moreover, the capacity of many polyphenols to form stable complexes of metallic ions, such as iron and copper, which can act as catalyzers in the production of oxidative species [for example, OH •, O2 • ⁻, H2O2] has also been accepted [325,326]. Polyphenols are said to present pleiotropic effects, impacting multiple molecular targets and intracellular signaling cascades, most of them interconnected [327], acting as modulators of the genetic expression and signaling pathways related to the cell function and protection, activating the antioxidant response element (ARE), regulating the cell oxidative state, and modulating the activity of the oxidative enzymes, such as xanthine oxidase, protein kinase C and nitric oxide synthases [328,329]. Besides the antioxidant effects, polyphenols present anti-inflammatory [330], cardiovascular [331], tumor suppressor [332], and neuroprotective properties, among others [333-337]. From the ocular perspective, several studies have suggested that the consumption of different polyphenols from natural sources such as fruit and vegetables can contribute to preserve vision and even reverse visual impairment in certain visual disorders [338,339]. Consequently, polyphenolic compounds can help prevent photoreceptor cell damage caused by ROS, and thus can have beneficial effects on the visual function in retinal degenerative diseases.

Chemically, dietary polyphenols can be divided into the following groups: phenolic acids, flavonoids, and polyphenolic amides [324]. In each group, several studies *in vitro*, *in vivo* and/or populational have been performed and have shown the potential benefits of polyphenols in AMD. Phenolic acids, for example, found in fruits and vegetables, in grains and seeds, particularly in the bran or hull [340], inhibited oxidized LDL effects on exacerbating choroidal neovascularization by downregulating cylindromatosis (CYLD) (a tumor suppressor) [341]. On the other hand, the flavonoids can inhibit inflammatory reactions by suppressing the expression of pro-inflammatory genes and molecules involved in retinal degeneration [342]. A population-based cohort study with 2,856 adults aged ≥49 years at baseline, and 2,037 followed up 15 years later, demonstrated an independent and protective association between the dietary intake of flavonoids and the likelihood of having AMD [343]. Studies *in vitro* have demonstrated the great capacity of flavonoids to inhibit the oxidation of LDL-c better than the classic antioxidants, such as alpha-tocopherol [344]. Intravitreally injected chrysin may inhibit induced CNV in brown Norway rats with a diode laser and downregulated hypoxia-inducible factor 1-alpha (HIF-1α) and VEGF expression [345]. Proanthocyanidins, a class of polyphenols, found in berries and fruits such as lingonberry, cranberry, black elderberry, black chokeberry, blackcurrant, blueberry [346], induced the elevation of *in vivo* antioxidant activity and the suppression of retinal lipid oxidation, as well as suppressed apoptotic cell death of the retinal tissue exposed to oxidative stress caused by visible light [347]. Epigallocatechin gallate (EGCG), the main flavonoid present in green tea [348], alleviated mouse laser-induced CNV leakage and reduced CNV area by down-regulating HIF-1α/VEGF/VEGFR2 pathway; M1-type macrophage/microglia polarization, as well as endothelial cell viability, proliferation, migration, and tube formation [349]. It has been demonstrated that other polyphenols also have antioxidant, anti-inflammatory and antiproliferative effects, with a potential to integrate the group of molecules to be used in AMD prevention [350-352].

Zinc

Zinc (Zn), the second most common metal in the human body, is an oligo-element that reaches a total concentration of 2.5 grams [353-357]. Shellfish, oysters, beef, liver, chicken giblets and eggs are considered the best Zn sources. Nuts and vegetables are relatively good Zn sources as well [358]. Usually, Zn is stored in the cell, and distributed to muscles, bones, skin, and liver [354]. The daily Zn recommended dosage is 15 mg [359]. Zinc is involved in the stabilization of the structural membranes and cells by two mechanisms: protection sulfhydryl groups against oxidation and inhibition of the production of oxygen reactive species by transition metals such as iron and copper [356,360]. The retina, mainly the macular region, retains a large concentration of Zn due to the role that it plays in the enzymatic reactions, and consequently, in the prevention of AMD [361]. It is known that Zn is one of the main constituents of superoxide dismutase (SOD), which also defends the organism against the oxygen reactive species [362]. Zinc is found at high levels in the RPE/choroid, photoreceptor inner segments (RIS)/ outer limiting membrane (OLM) layer, outer plexiform layer (OPL), and inner nuclear layer (INL) [363]. The evidence that Zn is essential for the retina is presented in several studies that reveal the depletion of Zn in the body results in a bad adaptation to the dark and reduced amplitudes of the photopic and scotopic electroretinogram (ERG) evaluations [364-365]. Deficiencies of selected trace elements, such as zinc, have also been implicated in the vision loss caused by AMD [361,366,367]. A decrease of 24% in Zn level in the RPE complex and choroid was observed in AMD patients when compared with individuals without AMD [368]. Previous studies in ARPE-19 cells suggest that zinc could activate the transcription factor NrF2 and increase the expression of phase II detoxification genes controlled by the ARE [369]. By activating the ARE/NrF2 pathway, Zn supplementation may upregulate the cystine/glutamate exchanger. Such effect will lead to increased tissue and cellular uptake of CySS and, consequently, decreased plasma CySS concentration [117]. It has been reported that a daily intake of 81 mg of Zn can reduce then visual loss resultant from AMD [370]. The AREDS 8 multicentric study divided participants into 4 groups: 1st group received high daily dosages of antioxidants such as vitamin C (500 mg),

vitamin E (400 IU), and beta-carotene (15 mg); 2nd group received Zn (80 mg) and cupric oxide (2mg); 3rd group received antioxidants plus Zn and cupric oxide, and 4th group received placebo. After 5 years, a decrease in early AMD risk was observed (category 2) for a more advanced stage (category 4 – geographic atrophy or subretinal neovascular membrane) of approximately 17 % for those that consumed only antioxidants, 21% for those treated with Zn and of 25% for those who received antioxidants plus Zn [13]. Another study conducted in 494 patients followed up after 12 months of anti-vascular endothelial growth factor therapy confirmed that a higher intake of dietary Zn was associated with a reduced likelihood of sub-retinal fluid (SRF) at 1 year [192]. A cross-sectional study carried out in 547 participants reported that a low dietary Zn intake was associated with a greater likelihood of SRF presence, particularly in those treated for at least 6 months, and increased macular thickness in treated eyes with nAMD [371]. Nevertheless, conflicting results were found in the literature regarding Zn supplementation and AMD. The Beaver Dam Eye Study observed a discreet association between Zn intake and reduction in early AMD risk [253]. Another randomized study of a control case showed that Zn supplementation does not exert therapeutic effect on AMD patients at advanced stages [372]. A study that assessed the levels of oligoelements in human plasma, including zinc, with 236 patients with nAMD compared to 236 same age controls without AMD, identified higher Zn plasma levels in patients fed supplements. However, there was no significant difference in Zn levels between patients with nAMD and controls. This might be due to the fact that only 36% of the nAMD patients reported supplement usage [373].

Zn plasma levels is not probably a sensitive indicator of an individual's Zn status, as it represents only ~ 0.1% of Zn concentration in the entire body [374]. Hence, to measure Zn plasma levels may not provide a precise measurement of Zn local levels in the macula of AMD patients [373].

Selenium

Selenium (Se) is considered an essential oligoelement for the human body due to its participation in relevant metabolic functions [375], immunological system [376], thyroid hormonal metabolism [377], male infertility [378], neoplasia [379], cardiovascular diseases [380] as well as an antioxidant [381]. Currently, the Recommended Daily Allowance (RDA) is 55 µg/day for healthy adult men or women [382,383]. Selenium-rich foods include Brazil nuts, whole grain rice, and sunflower seeds [384]. This oligoelement is an active component of the Gpx [385], which plays an antioxidant role and protects the body cells, reducing the toxic substances caused by the oxidative stress [386]. GPx, which contains 4 atoms of selenium [386], is responsible for about 30% of the plasma levels of this mineral [385,387]. Se-deficient animals have markedly decreased GPx activity [388]. Selenium is present in very small amounts in the retina [363]. A recent case control study found a borderline association between AMD and low serum selenium concentrations [389]. Comparing 10 patients with nAMD (61.2-76.1 years) and 9 with healthy eyes (66.9-75.1 years), previous study revealed that blood selenium concentration was significantly lower in the AMD group than in the control one [390].

Complications related to use of antioxidants

With the objective to prevent or treat systemic disease, antioxidants have been largely used for decades. However, the indiscriminate use of antioxidants is not risk free. Experimental studies have shown that common antioxidants may accelerate cancer growth and metastasis [391-392]. Moreover, several populational studies and meta-analysis have condemned the abusive use of antioxidants for considering them harmful to health [393-399]. There are several possible explanations for the potential negative effect of antioxidant supplements. Oxygen reactive species, in moderate concentrations, are essential mediators of reactions through which the body eliminates undesirable cells. Antioxidant supplements are scavengers of free radicals and may interfere in the essential defense mechanisms that would help eliminate defective cells, including the precancerous or cancerous ones [396]. The human diet usually contains safe levels of antioxidants; however, high levels of antioxidant supplements are likely to upset a relevant physiological balance [393-397,400]. It is important to remember that antioxidant supplements are synthetic and paradoxically may have pro-oxidant properties [398]. These factors may account for a possible increase in cancer risk [397,400] and cardiovascular diseases [393]. Systematic reviews and meta-analysis of randomized clinical trials have not demonstrated that supplementation of beta-carotene, vitamin A and vitamin E, contribute to a decline in mortality rate. Some analyses have even reported a mortality increase [393,400]. A large interventionist study (n = 18,314), the Carotene and Retinol Efficacy Trial (CARET) [193], administered supplements of beta-carotene (30 mg/day) and retinyl ester (25,000 IU/day) to smokers and workers exposed to asbestos. The study was ended 21 months early due to the evidence of no benefit and substantial evidence of harm in the group that received β-carotene and retinol palmitate, especially women. In fact, the incidence of lung cancer increased 28%, and total mortality was 17% higher in the supplementation group in comparison with the placebo group (n = 8,894). Previous systematic review and meta-analyses showed beta-carotene supplementation has not been shown to have any beneficial effect on cancer prevention. Conversely, it was associated with increased risk not only of lung cancer but also of gastric cancer at doses of 20-30 mg/day, in smokers and asbestos workers [401]. A prospective observational study analyzed the association between prostate cancer risk and the use of antioxidants in 295,344 men registered at the National Institutes of Health (NIH)AARP Diet and Health Study. The participants were cancer free at the registration time. The authors observed that the use of multivitamins 7 times a week was associated with a double risk for prostate cancer. The study was observational, hence eventual doubts may not be excluded; however, it was a well-conducted study with a large sample, which reduces possible error factors [399]. A meta-analysis with 232,600 patients assessed the antioxidant effect of vitamin supplements on the mortality rate per disease. It concluded that the use of beta-carotene, vitamin A and vitamin E in excess may increase mortality rate. This study also observed that vitamin C and selenium are not related to longevity and that further clinical trials should be performed to confirm the effects of these substances [395]. High dose of xanthophylls was associated to increased risk of skin cancer and gastric adenocarcinoma [209]. Excessive consumption of zinc, in turn, may also cause disorders in the immune response [402], high-density lipoprotein decreases [403], and anemia [404]. Selenium supplementation does not seem to prevent type 2 diabetes, and it may increase the risk for the disease [405]. The association between cancer prevention and selenium supplementation is not supported by epidemiological studies [406]. It is important to highlight that this discussion is about the pharmacological supplementation of antioxidants and not about the modulation of the redox balance offered by a diet.

Conclusion and future directions

Antioxidants act in a complementary way. The water-soluble ones can remove free radicals in an aqueous medium, whereas the lipo-soluble exert their role in a lipid one. There are vitamins

that perform better in high oxygen concentrations, whereas others perform better in low oxygen concentrations. This reveals that the association of antioxidants is important to improve its effects and that, when analyzed separately, they do not demonstrate their real impact on AMD. Regarding thiols, besides their essential antioxidant role in the preservation of the vision, they are also important AMD biomarkers. Several molecules can increase the concentration of thiols in the plasma and in the RPE, with special focus on NAC, zinc, and polyphenols. N-acetyl-L-cysteine displays many properties that make it a potential drug for AMD patients, with additional benefits that include its oral administration or topic application to the cornea. Regard to carotenoids, a major attention should be given to xanthophyll. Many studies have revealed that lutein and zeaxanthin supplementation improved macular pigment optical density, demonstrating objectively the importance of lutein and zeaxanthin intake in AMD prevention. Regarding astaxanthin, it is a carotenoid that has been tested in labs and presents promising results in the prevention and/or treatment of AMD. Its antioxidant and anti-inflammatory activities have been observed in experimental studies with a performance higher than that of other carotenoids such as lutein, canthaxanthin and beta-carotene. As to the uric acid, melatonin and CoQ10, important non-enzymatic antioxidants, studies have confirmed their role on the preservation and homeostasis of RPE and sensory retina. However, more studies are necessary to assess the beneficial effects of their supplementation on AMD. Polyphenols, in turn, present antioxidant, anti-inflammatory, and antiangiogenic properties, and may serve as basis for the prevention and future treatments in dry and exudative AMD. As to complications induced by the indiscriminate consumption of antioxidants, it is important to highlight that this discussion only refers to the pharmacological supplementation and not to the modulation of the redox balance offered by a diet, moderate physical exercise, and healthy lifestyles. For these, there is substantial evidence of their protective effect. The use of antioxidants in AMD requires further clarification. Knowledge about the bioavailability, biotransformation, and accurate action of antioxidant supplements in AMD, quantities of vegetables and fruits that may be consumed to obtain an adequate supply of these nutrients, is of vital importance to prevent and treat AMD.

Conflicts of interest

None.

References

1. Wong WL, Su X, Li X, Cheung CM, Klein R, et al. (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* 2: e106-e116.
2. Beatty S, Koh H, Phil M, Henson D, Boulton M (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 45:115-134.
3. Berman ER, Rothman MS (1980) Lipofuscin of human retinal pigment epithelium. *J Ophthalmol* 90: 783-791
4. Lu L, Oveson BC, Jo YJ, Lauer TW, Usui S (2009) Increased expression of glutathione peroxidase 4 strongly protects retina from oxidative damage. *Antioxid Redox Signal* 11: 715-724.
5. Frank RN, Amin RH, Puklin JE (1999) Antioxidant enzymes in the macular retinal pigment epithelium of eyes with neovascular age-related macular degeneration. *Am J Ophthalmol* 127: 694-709.
6. Evereklioglu C, Er H, Doganay S, Cekmen M, Turkoz Y (2003) Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration. *Doc Ophthalmol* 106: 129-136.
7. Nowak M, Swietochowska E, Wielkoszyński T, Marek B, Karpe J (2003) Changes in blood antioxidants and several lipid peroxidation products in women with age-related macular degeneration. *Eur J Ophthalmol* 13: 281-286.
8. Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 94: 909-950.
9. Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde, and related aldehydes. *Free Radic Biol Med* 11: 81-128.
10. Shimokawa I, Higami Y, Horiuchi S, Iwasaki M, Ikeda T (1998) Advanced glycation end products in adrenal lipofuscin. *J Gerontol A Biol Sci Med Sci* 53: B49-B51.
11. Crabb JW, Miyagi M, Gu X, Shadrach K, West KA (2020) Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. *Proc Natl Acad Sci U S A* 12: 14682-14687.
12. Age-Related Eye Disease Study Research Group (1999) The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Control Clin Trials* 20: 573-600.
13. Age-Related Eye Disease Study Research Group (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol* 119: 1417-1436.
14. Age-Related Eye Disease Study Research Group, SanGiovanni JP, Chew EY, Clemons TE, Ferris FL 3rd, et al. (2007) The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Arch Ophthalmol* 125: 1225-1232
15. Young IS, Woodside JV (2001) Antioxidants in health and disease. *J Clin Pathol* 54: 176-186.
16. Aldini G, Yeum KJ, Niki E, Russell RM (2011) Biomarkers for Antioxidant Defense and Oxidative Damage: Principles and Practical Applications; John Wiley & Sons: Hoboken, NJ, USA.
17. Yeum KJ, Russell RM, Krinsky NI, Aldini G (2004) Biomarkers of antioxidant capacity in the hydrophilic and lipophilic compartments of human plasma. *Arch Biochem Biophys* 430: 97-103.
18. Chelikani P, Fita I, Loewen PC (2004) Diversity of structures and properties among catalases. *Cell Mol Life Sci* 61: 192-208.
19. Mirończuk-Chodakowska I, Witkowska AM, Zujko ME (2018) Endogenous non-enzymatic antioxidants in the human body. *Adv Med Sci* 63: 68-78.
20. Nimse SB, Palb D (2015) Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances* 5: 27986-8006.
21. Cai J, Nelson KC, Wu M, Sternberg P Jr, Jones DP (2000) Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 19: 205-221.
22. Bianchi MLP, Antunes LMG (1999) Radicais livres e os principais antioxidantes da dieta. *Rev Nutr* 12: 123-130.
23. Prasad AS, Beck FWJ, Bao B, Fitzgerald JT, Snell DC, et al. (2007) Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr* 85: 837-844.
24. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4: 118-126.
25. Lee PT, Lowinsohn D, Compton RG (2014) The use of screen-printed electrodes in a proof of concept electrochemical estimation of homocysteine and glutathione in the presence of cysteine using catechol. *Sensors* 14: 10395-10411.
26. Atmaca G (2004) Antioxidant effects of sulfur-containing amino acids. *Yonsei Med J* 45: 776-788.
27. Burgoyne JR, Eaton P (2011) Contemporary techniques for detecting and identifying proteins susceptible to reversible thiol oxidation. *Biochem Soc Trans* 39: 1260-1267.
28. Kemp M, Go YM, Jones DP (2008) Non equilibrium thermodynamics of thiol/disulfide redox system: a prespective on redoxsystem biology. *Free Radic Biol Med* 44: 921-937.
29. Wlodek L (2002) Beneficial and harmful effects of thiols. *Pol J Pharmacol* 54: 215-223.
30. Jones DP, Go YM, Anderson CL, Ziegler TR, Kinkade JM Jr, et al. (2004) Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control. *FASEB J* 18: 1246-1248.
31. Jones DP (2006) Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Res* 9: 169-181.

32. Pastore A, Federici G, Bertini E, Piemonte F (2003) Analysis of glutathione: implication in redox and detoxification. *Clinica Chimica Acta* 333: 19-39.

33. Jones DP, Carlson JL, Samiec PS, Sternberg P Jr, Mody VC Jr, et al. (1998) Glutathione measurement in human plasma. Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. *Clin Chim Acta* 275: 175-184.

34. Samiec PS, Drews-Botsch C, Flagg EW, Kurtz JC, Sternberg P Jr, et al. (1998) Glutathione in human plasma: decline in association with aging, age- related macular degeneration, and diabetes. *Free Radic Biol Med* 24: 699-704.

35. Ashfaq SBSC, Abramson JL, Rhodes SD, Jurkovitz C, Vaccarino V, et al. (2006) Plasma glutathione redox state, a novel marker of oxidative stress, correlates with early atherosclerosis in humans. *Am Coll Card* 47: 1005-1011.

36. Jones DP, Carlson JL, Mody VC Jr, Cai J, Lynn MJ, et al. (2000) Redox state of glutathione in human plasma. *Free Radic Biol Med* 28: 625-635.

37. Jones DP, Mody VC Jr, Carlson JL, Lynn MJ, Sternberg P Jr (2002) Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. *Free Radic Biol Med* 33: 1290-1300.

38. Moriarty SE, Shah JH, Lynn M, Jiang S, Openo K, et al. (2003) Oxidation of glutathione and cysteine in human plasma associated with smoking. *Free Radic Biol Med* 35: 1582-1588.

39. Jonas CR, Puckett AB, Jones DP, Griffith DP, Szeszycki EE, et al. (2000) Plasma antioxidant status after high-dose chemotherapy: a randomized trial of parenteral nutrition in bone marrow transplantation patients. *Am J Clin Nutr* 72: 181-189.

40. Ashfaq S, Abramson JL, Jones DP, Rhodes SD, Weintraub WS, et al. (2006) The relationship between plasma levels of oxidized and reduced thiols and early atherosclerosis in healthy adults. *J Am Coll Cardiol* 47: 1005-1011.

41. Ohira A, Honda O, Gauntt CD, Yamamoto M, Hori K, et al. (1994) Oxidative stress induces adult T cell leukemia derived factor/thioredoxin in the rat retina. *Lab Invest* 70: 279-285.

42. Gauntt CD, Ohira A, Honda O, Kigasawa K, Fujimoto T, et al. (1994) Mitochondrial induction of adult T cell leukemia derived factor (ADF/hTx) after oxidative stresses in retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 35: 2916-2923.

43. Yamamoto M, Sato N, Tajima H, Furuke K, Ohira A, et al. (1997) Induction of human thioredoxin in cultured human retinal pigment epithelial cells through cyclic AMP-dependent pathway; involvement in the cytoprotective activity of prostaglandin E1. *Exp Eye Res* 65: 645-652.

44. Moriarty-Craige SE, Carlson JL, Lynn M, Gensler G, Bressler S, et al. (2005) Antioxidant supplements prevent oxidation of cysteine/cystine redox in AMD patients. *Amer J Ophthalmol* 140: 1020-1026.

45. Javadzadeh A, Ghorbanihaghjo A, Bahreini E, Rashtchizadeh N, Argani H, et al. (2010) Plasma oxidized LDL and thiol-containing molecules in patients with exudative age-related macular degeneration. *Mol Vis* 16: 2578-2584.

46. Arıkan Yorgun M, Toklu Y, Altınkaynak H, Tanrıverdi B, Ergin M (2016) A Novel Tool for the Assessment Oxidative Stress in Age-Related Macular Degeneration: Thiol/Disulfide Homeostasis Revisited. *Curr Eye Res* 41: 1584-1589.

47. Elbay A, Ozer OF, Akkan JCU, Celik U, Kutlutürk I, Koytak A (2017) Comparison of serum thiol-disulphide homeostasis and total antioxidant-oxidant levels between exudative age-related macular degeneration patients and healthy subjects. *Int Ophthalmol* 37: 1095-1101.

48. Aktaş S, Sağdık HM, Tetikoğlu M, Aktaş H, Özcura F, et al. (2017) Dynamic thiol/disulfide homeostasis in patients with age-related macular degeneration. *Arq Bras Oftalmol* 80: 234-237.

49. Holmgren A (1989) Thioredoxin and glutaredoxin systems. *J Biol Chem* 264: 13963-1396.

50. Meister A, Anderson ME (1983) Glutathione. *Ann Rev Biochem* 52: 711-760.

51. Kularatne RN, Bulumulla C, Catchpole T (2020) Protection of human retinal pigment epithelial cells from oxidative damage using cysteine prodrugs. *Free Radic Biol Med* 152: 386-394.

52. Kretzscharm M, Muller D (1993) Aging, training and exercise. A review of effects on plasma glutathione and lipid peroxides. *Sports Med* 10: 196-209.

53. Flagg EW, Coates RJ, Jones DP, Eley JW, Gunter EW (1993) Plasma total glutathione in humans and its association with demographic and health-related factors. *Br J Nutr* 70: 797-808.

54. Lang CA, Naryshkin S, Schneider DL, Mills BJ (1992) Low blood glutathione levels in healthy aging adults. *J Lab Clin Med* 120: 720-725.

55. Tate DJ Jr, Newsome DA, Oliver PD (1993) Metallothionein shows an age-related decrease in human macular retinal pigment epithelium. Invest. *Ophthalmol Vis Sci* 34: 2348-2351.

56. Liles MR, Newsome DA, Oliver PD (1991) Antioxidant enzymes in the aging human retinal pigment epithelium. *Arch Ophthalmol* 109: 1285-1288.

57. Tanito M, Nishiyama A, Tanaka T, Masutani H, Nakamura H, et al. (2002) Change of redox status and modulation by thiol replenishment in retinal photooxidative damage. *Invest Ophthalmol Vis Sci* 43: 2392-2400.

58. Sies H, Graf P (1985) Hepatic thiol and glutathione efflux under the influence of vasopressin, phenylephrine and adrenaline. *Biochem J* 226: 545-549.

59. Adams JD Jr, Lauterburg BH, Mitchell JR (1983) Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. *J Pharmacol Exp Ther* 227: 749-754.

60. Ookhtens M, Kaplowitz N (1998) Role of the liver in interrogans homeostasis of glutathione and cyst(e)ine. *Semin Liver Dis* 18: 313-329.

61. Ferrington DA, Ebeling MC, Kapphahn RJ, Terluk MR, Fisher CR, et al. (2017) Altered bioenergetics and enhanced resistance to oxidative stress in human retinal pigment epithelial cells from donors with age-related macular degeneration. *Redox Biol* 13: 255-265.

62. Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL (2005) Systemic reduction in glutathione levels occurs in patients with primary open-angle glaucoma. *Invest Ophthalmol Vis Sci* 46: 877-883.

63. Perricone C, De Carolis C, Perricone R (2009) Glutathione: a key player in autoimmunity. *Autoimmun Rev* 8: 697-701.

64. Qin L, Mroczkowska SA, Ekart A, Patel SR, Gibson JM (2014) Patients with early age-related macular degeneration exhibit signs of macro- and micro-vascular disease and abnormal blood glutathione levels. *Graefes Arch Clin Exp Ophthalmol* 252: 23-30.

65. Lutchmansingh FK, Hsu JW, Bennett FI, Badaloo AV, McFarlane-Anderson N, et al. (2018) Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PLoS One* 13: e0198626.

66. Sreekumar PG, Ferrington DA, Kannan R (2021) Glutathione metabolism and the novel role of mitochondrial GSH in retinal degeneration. *Antioxidants (Basel)* 10: 661.

67. Coral K, Raman R, Rathi S, Rajesh M, Sulochana KN, et al. (2006) Plasma homocysteine and total thiol content in patients with exudative age-related macular degeneration. *Eye (Lond)* 20: 203-207.

68. Bharathselvi M, Biswas S, Raman R, Selvi R, Coral K, et al. (2016) Homocysteine & its metabolite homocysteine-thiolactone & deficiency of copper in patients with age related macular degeneration - A pilot study. *Indian J Med Res* 143: 756-762.

69. Zafrilla P, Losada M, Perez A, Caravaca G, Mulero J (2013) Biomarkers of oxidative stress in patients with wet age-related macular degeneration. *J Nutr Health Aging* 17: 219-222.

70. Marc RE, Jones BW, Watt CB, Vazquez-Chona F, Vaughan DK (2008) Extreme retinal remodeling triggered by light damage: implications for age related macular degeneration. *Mol Vis* 14: 782-806.

71. Huster D, Hjelle OP, Haug FM, Nagelhus EA, Reichelt W (1998) Subcellular compartmentation of glutathione and glutathione precursors. A high resolution immunogold analysis of the outer retina of guinea pig. *Anat Embryol (Berl)* 198: 277-287.

72. Sreekumar PG, Spee C, Ryan SJ, Cole SP, Kannan R (2012) Mechanism of RPE cell death in α-crystallin deficient mice: a novel and critical role for MRP1-mediated GSH efflux. *PLoS One* 7: e33420.

73. Hsu SC, Molday RS (1994) Glucose metabolism in photoreceptor outer segments. Its role in phototransduction and in NADPH-requiring reactions. *J Biol Chem* 8: 17954-17959.

74. Lu SC, Bao Y, Huang ZZ, Sarthy VP, Kannan R (1999) Regulation of gamma-glutamylcysteine synthetase subunit gene expression in retinal Müller cells by oxidative stress. *Invest Ophthalmol Vis Sci* 40: 1776-1782.

75. Jiang S, Moriarty SE, Grossniklaus H, Nelson KC, Jones DP (2002) Increased oxidant-induced apoptosis in cultured nondividing human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 43: 2546-2553.

76. Guo X, Jiang Q, Tuccitto A, Chan D, Alqawlaq S, et al. (2018) The AMPK-PGC-1α signaling axis regulates the astrocyte glutathione system to protect against oxidative and metabolic injury. *Neurobiol Dis* 113: 59-69.

77. Sun Y, Zheng Y, Wang C, Liu Y (2018) Glutathione depletion induces ferroptosis, autophagy, and premature cell senescence in retinal pigment epithelial cells. *Cell Death Dis* 9: 753.

78. Dou G, Sreekumar PG, Spee C, He S, Ryan SJ, et al. (2012) Deficiency of αB crystallin augments ER stress-induced apoptosis by enhancing mitochondrial dysfunction. *Free Radic Biol Med* 53: 1111-1122.

79. Bertelli A, Conte A, Ronca G (1994) L-propionyl carnitine protects erythrocytes and low density lipoproteins against peroxidation. *Drugs Exp Clin Res* 20: 191-197.

80. Ates O, Alp HH, Mumcu U, Azizi S, Cinici E, et al. (2008) The effect of L-carnitine treatment on levels of malondialdehyde and glutathione in patients with age related macular degeneration. *Eurasian J Med* 40: 1-5.

81. Shamsi FA, Chaudhry IA, Boulton ME, Al-Rajhi AA (2007) L-carnitine protects human retinal pigment epithelial cells from oxidative damage. *Curr Eye Res* 32: 575-584.

82. Kularatne RN, Bulumulla C, Catchpole T, Takacs A, Christie A, et al. (2020) Protection of human retinal pigment epithelial cells from oxidative damage using cysteine prodrugs. *Free Radic Biol Med* 152: 386-394

83. Tosi GM, Giustarini D, Franci L, Minetti A, Imperatore F, et al. (2021) Superior Properties of N-Acetylcysteine Ethyl Ester over N-Acetyl cysteine to prevent retinal pigment epithelial cells oxidative damage. *Int J Mol Sci* 9: 600.

84. Alhasani RH, Almarhoun M, Zhou X, Reilly J, Patterson S, et al. (2020) Tauroursodeoxycholic acid protects retinal pigment epithelial cells from oxidative injury and endoplasmic reticulum stress In Vitro. *Biomedicines* 21: 367.

85. Zhou J, Chen F, Yan A, Xia X (2020) Madecassoside protects retinal pigment epithelial cells against hydrogen peroxide-induced oxidative stress and apoptosis through the activation of Nrf2/HO-1 pathway. *Biosci Rep* 40: BSR20194347.

86. Cui R, Tian L, Lu D, Li H, Cui J (2020) Exendin-4 protects human retinal pigment epithelial cells from H2O2-Induced oxidative damage via activation of NRF2 signaling. *Ophthalmic Res* 63: 404-412.

87. Hu X, Liang Y, Zhao B, Wang Y (2019) Thymoquinone protects human retinal pigment epithelial cells against hydrogen peroxide induced oxidative stress and apoptosis. *J Cell Biochem* 120: 4514-4522.

88. Zhang J, Zhou H, Chen J, Lv X, Liu H (2020) Aloperine protects human retinal pigment epithelial cells against hydrogen peroxide-induced oxidative stress and apoptosis through activation of Nrf2/HO-1 pathway. *J Recept Signal Transduct Res* 30: 1-7.

89. Du Y, You L, Ni B, Sai N, Wang W, et al. (2020) Phillyrin Mitigates Apoptosis and Oxidative Stress in Hydrogen Peroxide-Treated RPE Cells through Activation of the Nrf2 Signaling Pathway. *Oxid Med Cell Longev* 12: 2684672.

90. Zhu W, Wu Y, Meng YF, Wang JY, Xu M, et al. (2015) Effect of curcumin on aging retinal pigment epithelial cells. *Drug Des Devel Ther* 9: 5337-5344.

91. Yiğit M, Güneş A, Uğuz C, Yalçın TÖ, Tök L, et al. (2019) Effects of astaxanthin on antioxidant parameters in ARPE-19 cells on oxidative stress model. *Int J Ophthalmol* 18: 930-935.

92. Xie K, Jin B, Zhu H, Zhou P, Du L (2020) Ferulic acid (FA) protects human retinal pigment epithelial cells from H2 O2 -induced oxidative injuries. *J Cell Mol Med* 24: 13454-13462.

93. Lu SC (2013) Glutathione synthesis. *Biochim Biophys Acta* 1830: 3143-3153.

94. Brantley MA Jr, Osborn MP, Sanders BJ, Rezaei KA, Lu P, et al. (2012) Plasma biomarkers of oxidative stress and genetic variants in age-related macular degeneration. *Am J Ophthalmol* 153: 460-467.

95. Pitkänen L, Ranta VP, Moilanen H, Urtti A (2005) Permeability of retinal pigment epithelium: effects of permeant molecular weight and lipophilicity. *Invest Ophthalmol Vis Sci* 46: 641-646.

96. Mannermaa E, Vellonen KS, Urtti A (2006) Drug transport in corneal epithelium and blood-retina barrier: emerging role of transporters in ocular pharmacokinetics. *Adv Drug Deliv Rev* 58: 1136-1163.

97. Del Amo EM, Rimpelä AK, Heikkinen E, Kari OK, Ramsay E, et al. (2017) Pharmacokinetic aspects of retinal drug delivery. *Prog Retin Eye Res* 57: 134-185.

98. Giustarini D, Milzani A, Dalle-Donne I, Tsikas D, Rossi R (2012) N-Acetylcysteine ethyl ester (NACET): a novel lipophilic cell-permeable cysteine derivative with an unusual pharmacokinetic feature and remarkable antioxidant potential. *Biochem Pharmacol* 84: 1522-1533.

99. Giustarini D, Galvagni F, Dalle Donne I, Milzani A, Severi FM, et al. (2018) N-acetylcysteine ethyl ester as GSH enhancer in human primary endothelial cells: A comparative study with other drugs. *Free Radic Biol Med* 126: 202-209.

100. Dilger RN, Baker DH (2007) Oral N-acetyl-L-cysteine is a safe and effective precursor of cysteine. *J Anim Sci* 85: 1712-1718.

101. Zafarullah M, Li WQ, Sylvester J, Ahmad M (2003) Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 60: 6-20.

102. Laragione T, Bonetto V, Casoni F, Massignan T, Bianchi G, et al. (2003) Redox regulation of surface protein thiols: identification of integrin alpha-4 as a molecular target by using redox proteomics. *Proc Natl Acad Sci U S A* 100: 14737-14741.

103. Osada H, Okamoto T, Kawashima H, Toda E, Miyake S, et al. (2017) Neuroprotective effect of bilberry extract in a murine model of photo-stressed retina. *PLoS One* 12: e0178627.

104. Zheng Q, Ren Y, Reinach PS, She Y, Xiao B, et al. (2014) Reactive oxygen species activated NLRP3 inflammasomes prime environment-induced murine dry eye. *Exp Eye Res* 125: 1-8.

105. Schimel AM, Abraham L, Cox D, Sene A, Kraus C, et al. (2011) N-acetylcysteine amide (NACA) prevents retinal degeneration by up-regulating reduced glutathione production and reversing lipid peroxidation. *Am J Pathol* 178: 2032-2043.

106. Saito Y, Tsuruma K, Shimazawa M, Nishimura Y, Tanaka T (2016) Establishment of a drug evaluation model against light-induced retinal degeneration using adult pigmented zebrafish. *J Pharmacol Sci* 131: 215-218.

107. Terluk MR, Ebeling MC, Fisher CR, Kapphahn RJ, Yuan C, et al. (2019) N-Acetyl-L-cysteine Protects Human Retinal Pigment Epithelial Cells from Oxidative Damage: Implications for Age-Related Macular Degeneration. *Oxid Med Cell Longev* 14: 5174957.

108. Lee SY, Usui S, Zafar AB, Oveson BC, Jo YJ, et al. (2011) N-Acetylcysteine promotes long-term survival of cones in a model of retinitis pigmentosa. *J Cell Physiol* 226: 1843-1849.

109. Hara R, Inomata Y, Kawaji T, Sagara N, Inatani M (2010) Suppression of choroidal neovascularization by N-acetyl-cysteine in mice. *Curr Eye Res* 35: 1012-1020.

110. Bertram KM, Baglole CJ, Phipps RP, Libby RT (2009) Molecular regulation of cigarette smoke induced-oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. *Am J Physiol Cell Physiol* 297: C1200-C1210.

111. Kagan DB, Liu H, Hutnik CM (2012) Efficacy of various antioxidants in the protection of the retinal pigment epithelium from oxidative stress. *Clin Ophthalmol* 6: 1471-1476.

112. Carver KA, Yang D (2016) N-Acetylcysteine amide protects against oxidative stress-induced microparticle release from human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 57: 360-371.

113. Go YM, Jones DP (2005) Intracellular proatherogenic events and cell adhesion modulated by extracellular thiol/disulfide redox state. *Circulation* 111: 2973-2980.

114. Jiang S, Moriarty-Craige SE, Orr M, Cai J, Sternberg P Jr (2005) Oxidant-induced apoptosis in human retinal pigment epithelial cells: dependence on extracellular redox state. *Invest Ophthalmol Vis Sci* 46: 1054-1061.

115. Kanter MZ (2006) Comparison of oral and i.v. acetylcysteine in the treatment of acetaminophen poisoning. *Am J Health Syst Pharm* 63: 1821-1827.

116. Whyte AJ, Kehrl T, Brooks DE, Katz KD, Sokolowski D (2010) Safety and effectiveness of acetadote for acetaminophen toxicity. *J Emerg Med* 39: 607-611.

117. Moriarty-Craige SE, Ha KN, Sternberg P Jr, Lynn M, Bressler S, et al. (2007) Effects of long-term zinc supplementation on plasma thiol metabolites and redox status in patients with age-related macular degeneration. *Am J Ophthalmol* 143: 206-211.

118. Brantley MA Jr, Osborn MP, Sanders BJ, Rezaei KA, Lu P, et al. (2012) The short-term effects of antioxidant and zinc supplements on oxidative stress biomarker levels in plasma: a pilot investigation. *Am J Ophthalmol* 153: 1104-1109.

119. Sun ZW, Chen C, Wang L, Li YD, Hu ZL (2020) S-allyl cysteine protects retinal pigment epithelium cells from hydroquinone-induced apoptosis through mitigating cellular response to oxidative stress. *Eur Rev Med Pharmacol Sci* 24: 2120-2128.

120. Bavarsad Shahripour R, Harrigan MR, Alexandrov AV (2014) N-acetylcysteine (NAC) in neurological disorders: mechanisms of action and therapeutic opportunities. *Brain Behav* 4: 108-122.

121. Markoutsa E, Xu P (2017) Redox Potential-Sensitive N-Acetyl Cysteine-Prodrug Nanoparticles Inhibit the Activation of Microglia and Improve Neuronal Survival. *Mol Pharm* 14: 1591-1600.

122. Ebeling MC, Polanco JR, Qu J, Tu C, Montezuma SR (2020) Improving retinal mitochondrial function as a treatment for age-related macular degeneration. *Redox Biol* 34: 101552.

123. Holmgren A (1985) Thioredoxin. *Annu Rev Biochem* 54: 237-271.

124. Sachi Y, Hirota K, Masutani H, Toda K, Okamoto T, et al. (1995) Induction of ADF/TRX by oxidative stress in keratinocytes and lymphoid cells. *Immunol Lett* 44: 189-193.

125. Moon S, Fernando MR, Lou MF (2005) Induction of thioltransferase and thioredoxin/thioredoxin reductase systems in cultured porcine lenses under oxidative stress. *Invest Ophthalmol Vis Sci* 46: 3783-3789.

126. Lillig CH, Holmgren A (2007) Thioredoxin and related molecules - from biology to health and disease. *Antioxid Redox Signal* 9: 25-47.

127. Perrone L, Devi TS, Hosoya K, Terasaki T, Singh LP (2009) Thioredoxin interacting protein (TXNIP) induces inflammation through chromatin modification in retinal capillary endothelial cells under diabetic conditions. *J Cell Physiol* 221: 262-272.

128. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H, et al. (1999) Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. *J Biol Chem* 274: 21645-21650.

129. Tanito M, Kwon YW, Kondo N, Bai J, Masutani H, et al. (2005) Cytoprotective effects of geranylgeranylacetone against retinal photooxidative damage. *J Neurosci* 25: 2396-2404.

130. Sugano E, Isago H, Murayama N, Tamai M, Tomita H (2013) Different anti-oxidant effects of thioredoxin 1 and thioredoxin 2 in retinal epithelial cells. *Cell Struct Funct* 38: 81-88.

131. Nishinaka Y, Masutani H, Nakamura H, Yodoi J (2001) Regulatory roles of thioredoxin in oxidative stress-induced cellular responses. *Redox Rep* 6: 289-295.

132. Nakamura H, Nakamura K, Yodoi J (1997) Redox regulation of cellular activation. *Annu Rev Immunol* 15: 351-369.

133. Randazzo J, Zhang Z, Hoff M, Kawada H, Sachs A, et al. (2011) Orally active multi-functional antioxidants are neuroprotective in a rat model of light-induced retinal damage. *PLoS One* 6: e21926.

134. Mandal MN, Patlolla JM, Zheng L, Agbaga MP, Tran JT, et al. (2009) Curcumin protects retinal cells from light-and oxidant stress-induced cell death. *Free Radic Biol Med* 46: 672-679.

135. Zhu C, Wang S, Wang B, Du F, Hu C, et al. (2015) 17β-Estradiol up-regulates Nrf2 via PI3K/AKT and estrogen receptor signaling pathways to suppress light-induced degeneration in rat retina. *Neuroscience* 24: 328-339.

136. Zhu L, Liu Z, Feng Z, Hao J, Shen W, et al. (2010) Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *J Nutr Biochem* 21: 1089-1098.

137. Gao X, Talalay P (2004) Induction of phase 2 genes by sulforaphane protects retinal pigment epithelial cells against photooxidative damage. *Proc Natl Acad Sci U S A* 101: 10446-10451.

138. Tanito M, Masutani H, Kim YC, Nishikawa M, Ohira A (2005) Sulforaphane induces thioredoxin through the antioxidant-responsive element and attenuates retinal light damage in mice. *Invest Ophthalmol Vis Sci* 46: 979-987.

139. Kong L, Tanito M, Huang Z, Li F, Zhou X, et al. (2007) Delay of photoreceptor degeneration in tubby mouse by sulforaphane. *J Neurochem* 101: 1041-1052.

140. Ye L, Yu T, Li Y, Chen B, Zhang J, et al. (2013) Sulforaphane enhances the ability of human retinal pigment epithelial cell against oxidative stress, and its effect on gene expression profile evaluated by microarray analysis. *Oxid Med Cell Longev* 2013: 413024.

141. Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* 78: 6858-6862.

142. Kean RB, Spitsin SV, Mikheeva T, Scott GS, Hooper DC (2000) The peroxynitrite scavenger uric acid prevents inflammatory cell invasion into the central nervous system in experimental allergic encephalomyelitis through maintenance of blood-central nervous system barrier integrity. *J Immunol* 165: 6511-6518.

143. Spitsin SV, Scott GS, Mikheeva T, Zborek A, Kean RB, et al. (2002) Comparison of uric acid and ascorbic acid in protection against EAE. *Free Radic Biol Med* 33: 1363-1371.

144. Wittenstein B, Klein M, Finckh B, Ullrich K, Kohlschutter A (2002) Plasma antioxidants in pediatric patients with glycogen storage disease, diabetes mellitus, and hypercholesterolemia. *Free Radic Biol Med* 33: 103-110.

145. Stinefelt B, Leonard SS, Blemings KP, Shi X, Klandorf H (2005) Free radical scavenging, DNA protection, and inhibition of lipid peroxidation mediated by uric acid. *Ann Clin Lab Sci* 35: 37-45.

146. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA (2005) Uric acid and oxidative stress. *Curr Pharm Des* 11: 4145-4151

147. Mehryar M, Farvardin M, Hosseini H, Aslani M (2006) Potential role of uric acid in the molecular pathogenesis of age-related macular degeneration. *Med Hypotheses* 66: 793-795.

148. Subramani S, Khor SE, Livingstone BI, Kulkarni UV (2010) Serum uric acid levels and its association with age-related macular degeneration (ARMD). *Med J Malaysia* 65: 36-40.

149. Halliwell B (1999) Vitamin C: poison, prophylactic or panacea? *Trends Biochem Sci* 24: 255-259.

150. Ames BN (2001) DNA damage from micronutrient deficiencies is likely to be a major cause of cancer. *Mutat Res* 475: 7-20.

151. Monsen ER (2000) Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. *J Am Diet Assoc* 100: 637-640.

152. Sant DW, Camarena V, Mustafi S, Li Y, Wilkes Z, et al. (2018) Ascorbate Suppresses VEGF Expression in Retinal Pigment Epithelial Cells. *Invest Ophthalmol Vis Sci* 59: 3608-3618.

153. Organisciak DT, Wang HM, Li ZY, Tso MO (1985) The protective effect of ascorbate in retinal light damage of rats. *Invest Ophthalmol Vis Sci* 26: 1580-1588.

154. Oh S, Kim YJ, Lee EK, Park SW, Yu HG (2020) Antioxidative effects of ascorbic acid and astaxanthin on ARPE-19 Cells in an Oxidative Stress Model. *Antioxidants (Basel)* 9: 833.

155. Smith W, Mitchell P, Webb K, Leeder SR (1999) Dietary antioxidants and age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology* 106: 761-767.

156. Christen WG, Ajani UA, Glynn RJ, Manson JE, Schaumberg DA, et al. (1999) Prospective cohort study of antioxidant vitamin supplement use and the risk of age-related maculopathy. *Am J Epidemiol* 149: 476-484..

157. Evans JR, Lawrenson JG (2017) Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. *Cochrane Database Syst. Rev* 7: CD000253.

158. Delcourt C, Cristol JP, Tessier F, Léger CL, Descomps B (1999) Age-related macular degeneration and antioxidant status in the POLA study. POLA Study Group. Pathologies Oculaires Liées à l'Age. *Arch Ophthalmol* 117: 1384-1390.

159. Zor RK, Erşan S, Küçük E, Yıldırım G, Sarı İ (2020) Serum malondialdehyde, monocyte chemoattractant protein-1, and vitamin C levels in wet type age-related macular degeneration patients. *Ther Adv Ophthalmol* 12: 2515841420951682.

160. Brigelius-Flohé R, Kelly FJ, Salonen JT, Neuzil J, Zingg JM (2002) The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr* 76: 703-716.

161. Thomas SR, Stocker R (2000) Molecular action of vitamin E in lipoprotein oxidation: implications for atherosclerosis. *Free Radic Biol Med* 28: 1795-1805.

162. van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, et al. (2005) Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA* 294: 3101-3107.

163. Sies H, Stahl W, Sundquist AR (1992) Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. *Ann N Y Acad Sci* 669: 7-20.

164. Winkler BS, Boulton ME, Gottsch JD, Sternberg P (1999) Oxidative damage and age-related macular degeneration. *Mol Vis* 5: 32.

165. Kono N, Ohto U, Hiramatsu T, Urabe M, Uchida Y, et al. (2013) Impaired α-TTP-PIPs interaction underlies familial vitamin E deficiency. *Science* 31: 1106-1110.

166. Yokota T, Igarashi K, Uchihara T, Jishage K, Tomita H, et al. (2001) Delayed-onset ataxia in mice lacking alpha -tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. *Proc Natl Acad Sci U S A* 18: 15185-15190.

167. Feng Z, Liu Z, Li X, Jia H, Sun L, et al. (2010) α-Tocopherol is an effective Phase II enzyme inducer: protective effects on acrolein-induced oxidative stress and mitochondrial dysfunction in human retinal pigment epithelial cells. *J Nutr Biochem* 21: 1222-1231.

168. West S, Vitale S, Hallfrisch J, Muñoz B, Muller D (1994) Are antioxidants or supplements protective for age-related macular degeneration? *Arch Ophthalmol* 112: 222-227.

169. Christen WG, Glynn RJ, Chew EY, Buring JE (2010) Vitamin E and age-related macular degeneration in a randomized trial of women. *Ophthalmology* 117: 1163-1168.

170. Smith W, Mitchell P, Rochester C (1997) Serum beta carotene, alpha tocopherol, and age-related maculopathy: the Blue Mountains Eye Study. *Am J Ophthalmol* 124: 838-840.

171. Manor D, Morley S (2007) The α-tocopherol transfer protein. *Vitamins and Hormones* 76: 45-65.

172. Tapiero H, Townsend DM, Tew KD (2004) The role of carotenoids in the prevention of human pathologies. *Biomed Pharmacother* 58: 100-110.

173. Namitha KK, Negi PS (2010) Chemistry and biotechnology of carotenoids. *Crit Rev Food Sci Nutr* 50: 728-760.

174. Ambati RR, Phang SM, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications-a review. *Mar Drugs* 7: 128-152.

175. Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 43: 228-265.

176. El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, et al. (2004) Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Arch Biochem Biophys* 430: 37-48.

177. Scott KJ, Rodriquez-Amaya D (2000) Pro-vitamin A carotenoid conversion factors: Retinol equivalents—Fact or fiction? *Food Chem* 69: 125-127.

178. Bohn T (2008) Bioavailability of non-provitamin a carotenoids. *Curr Nutr Food Sci* 4: 240-258.

179. Murillo AG, Fernandez ML (2016) Potential of dietary non-provitamin a carotenoids in the prevention and treatment of diabetic microvascular complications. *Adv Nutr* 15: 14-24.

180. Brazionis L, Rowley K, Itsiopoulos C, O'Dea K (2009) Plasma carotenoids and diabetic retinopathy. *Br J Nutr* 101: 270-277.

181. Rühl R (2013) Non-pro-vitamin A and pro-vitamin A carotenoids in atopy development. *Int Arch Allergy Immunol* 161: 99-115.

182. Milani A, Basirnejad M, Shahbazi S, Bolhassani A (2017) Carotenoids: biochemistry, pharmacology and treatment. *Br J Pharmacol* 174: 1290-1324.

183. Harikumar KB, Nimita CV, Preethi KC, Kuttan R, Shankaranarayana ML (2008) Toxicity profile of lutein and lutein ester isolated from marigold flowers (Tagetes erecta). *Int J Toxicol* 27: 1-9.

184. Hammond BR Jr, Wooten BR, Snodderly DM (1997) Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A Opt Image Sci Vis* 14: 1187-1196.

185. Handelman GJ, Dratz EA, Reay CC, van Kuijk JG (1988) Carotenoids in the human macula and whole retina. *Invest Ophthalmol Vis Sci* 29: 850-855.

186. Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 274: 532-538.

187. Di Mascio P, Murphy ME, Sies H (1991) Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. *Am J Clin Nutr* 53: 194S-200S.

188. Sundelin SP, Nilsson SE (2001) Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants. *Free Radic Biol Med* 31: 217-225.

189. Nilsson SE, Sundelin SP, Wihlmark U, Brunk UT (2003) Aging of cultured retinal pigment epithelial cells: oxidative reactions, lipofuscin formation and blue light damage. *Doc Ophthalmol* 106: 13-16.

190. Junghans A, Sies H, Stahl W (2001) Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch Biochem Biophys* 15: 160-164.

191. Christen WG, Manson JE, Glynn RJ, Gaziano JM, Chew EY, et al. (2007) Beta carotene supplementation and age-related maculopathy in a randomized trial of US phy sicians. *Arch Ophthalmol* 125: 333-339.

192. Dharamdasani Detaram H, Liew G, Russell J, Vu KV, Burlutsky G, et al. (2020) Dietary antioxidants are associated with presence of intra- and sub-retinal fluid in neovascular age-related macular degeneration after 1 year. *Acta Ophthalmol* 98: e814-e819.

193. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, et al. (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 334: 1150-1155. [Crossref]

194. Age-Related Eye Disease Study 2 Research Group, Chew EY, Clemons TE, Sangiovanni JP, Danis RP, et al. (2014) Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA Ophthalmol* 132: 142-149.

195. Heber D, Lu QY (2002) Overview of mechanisms of action of lycopene. *Exp Biol Med (Maywood)* 227: 920-923.

196. Ferron AJT, Aldini G, Francisqueti-Ferron FV, Silva CCVA, Bazan SGZ (2019) Protective effect of tomato-oleoresin supplementation on oxidative injury recoveries cardiac function by improving β-adrenergic response in a diet-obesity induced model. *Antioxidants (Basel)* 8: 368.

197. Takeoka GR, Dao L, Flessa S, Gillespie DM, Jewell WT, et al. (2001) Processing effects on lycopene content and antioxidant activity of tomatoes. *J Agric Food Chem* 49: 3713-3717.

198. Rao AV, Agarwal S (1999) Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *Nutr Res* 19: 305-323.

199. Agarwal S, Rao AV (2000) Tomato lycopene and its role in human health and chronic diseases. *CMAJ* 19: 739-744.

200. Arab L, Steck S (2000) Lycopene and cardiovascular disease. *Am J Clin Nutr* 71: 1691-1695.

201. Rao AV, Rao LG (2007) Carotenoids and human health. *Pharmacol Res* 55: 207-216.

202. Stahl W, Ale-Agha N, Polidori MC (2002) Non-antioxidant properties of carotenoids. *Biol Chem* 383: 553-558.

203. Yang PM, Wu ZZ, Zhang YQ, Wung BS (2016) Lycopene inhibits ICAM-1 expression and NF-kB activation by Nrf2-regulated cell redox state in human retinal pigment epithelial cells. *Life Sci* 155: 94-101.

204. Chichili GR, Nohr D, Frank J, Flaccus A, Fraser PD, et al. (2006) Protective effects of tomato extract with elevated beta-carotene levels on oxidative stress in ARPE-19 cells. *Br J Nutr* 96: 643-649.

205. Rinsky B, Hagbi-Levi S, Elbaz-Hayoun S, Grunin M, Chowers I (2017) Characterizing the effect of supplements on the phenotype of cultured macrophages from patients with age-related macular degeneration. *Mol Vis* 6: 889-899.

206. Michikawa T, Ishida S, Nishiwaki Y, Kikuchi Y, Tsuboi T, et al. (2009) Serum antioxidants and age-related macular degeneration among older Japanese. *Asia Pac J Clin Nutr* 18: 1-7.

207. Zhou H, Zhao X, Johnson EJ, Lim A, Sun E, et al. (2011) Serum carotenoids and risk of age-related macular degeneration in a chinese population sample. *Invest Ophthalmol Vis Sci* 17: 4338-4344.

208. Chong EW, Wong TY, Kreis AJ, Simpson JA, Guymer RH (2007) Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 335: 755.

209. Aziz E, Batool R, Akhtar W, Rehman S (2020) Xanthophyll: Health benefits and therapeutic insights. *Life Sci* 240: 117104.

210. Kirschfeld K (1982) Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Proc R Soc Lond B Biol Sci* 216: 71-85.

211. Cantrell A, McGarvey DJ, Truscott TG, Rancan F, Böhm F (2003) Singlet oxygen quenching by dietary carotenoids in a model membrane environment. *Arch Biochem Biophys* 412: 47-54.

212. Trevithick-Sutton CC, Foote CS, Collins M, Trevithick JR (2006) The retinal carotenoids zeaxanthin and lutein scavenge superoxide and hydroxyl radicals: a chemiluminescence and ESR study. *Mol Vis* 12: 1127-1135.

213. Lim BP, Nagao A, Terao J, Tanaka K, Suzuki T (1992) Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. *Biochim Biophys Acta* 1126: 178-184.

214. Subczynski WK, Wisniewska A, Widomska J (2010) Location of macular xanthophylls in the most vulnerable regions of photoreceptor outer-segment membranes. *Arch Biochem Biophys* 1: 61-66.

215. Conn PF, Schalch W, Truscott TG (1991) The singlet oxygen and carotenoid interaction. *J Photochem Photobiol B* 11: 41-47.

216. Woodall AA, Britton G, Jackson MJ (1997) Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxyl radicals: relationship between carotenoid structure and protective ability. *Biochim Biophys Acta* 20: 575-586.

217. Ahuja S, Crocker E, Eilers M, Hornak V, Hirshfeld A, et al. (284) Location of the retinal chromophore in the activated state of rhodopsin. *J Biol Chem* 10: 10190-10201.

218. Parodi MB, Mollo MR, Brunoro A, Arrigo A, Romano F (2018) Micronutrients and benefits of supplementation for reducing the risk of progression of age-related macular degeneration-an update. *Eur Ophthalmic Rev* 12: 39-44.

219. Alves-Rodrigues A, Shao A (2004) The science behind lutein. *Toxicol Lett* 150: 57-83.

220. Hammond BR Jr, Wooten BR, Snodderly DM (1996) Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. *Vision Res* 36: 3003-3009.

221. Malinow MR, Feeney-Burns L, Peterson LH, Klein ML (1980) Diet-related macular anomalies in monkeys. *Invest Ophthalmol Vis Sci* 19: 857-863.

222. Moeller SM, Parekh N, Tinker L, Ritenbaugh C, Blodi B (2006) Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-Related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. *Arch Ophthalmol* 124: 1151-1162

223. Li LH, Lee JC, Leung HH, Lam WC (2020) Lutein Supplementation for Eye Diseases. *Nutrients* 12: 1721.

224. Tabeshpour J, Razavi BM, Hosseinzadeh H (2017) Effects of Avocado (Persea americana) on Metabolic Syndrome: A Comprehensive Systematic Review. *Phytother Res* 31: 819-837.

225. Scott TM, Rasmussen HM, Chen O, Johnson EJ (2017) Avocado consumption increases macular pigment density in older adults: A randomized, controlled trial. *Nutrients* 23: 919.

226. Jorgensen K, Skibsted LH (1993) Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z Lebensm Unters Forsch* 196: 423-429.

227. Krinsky NI, Landrum JT, Bone RA (2003) Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu Rev Nutr* 23: 171-201.

228. Yu B, Wang J, Suter PM, Russell RM, Grusak MA (2012) Spirulina is an effective dietary source of zeaxanthin to humans. *Br J Nutr* 108: 611-619.

229. Nolan JM, Power R, Stringham J, Dennison J, Stack J (2016) Enrichment of macular pigment enhances contrast sensitivity in subjects free of retinal disease: Central Retinal Enrichment Supplementation Trials-Report 1. *Invest Ophthalmol Vis Sci* 57: 3429-3439.

230. Gammone MA, Riccioni G, D'Orazio N (20150 Marine Carotenoids against Oxidative Stress: Effects on Human Health. *Mar Drugs* 30: 6226-6246.

231. Wu J, Cho E, Willett WC, Sastry SM, Schaumberg DA (2015) Intakes of Lutein, Zeaxanthin, and Other Carotenoids and Age-Related Macular Degeneration During 2 Decades of Prospective Follow-up. *JAMA Ophthalmol* 133: 1415-1424.

232. Handelman GJ, Dratz EA, Reay CC, van Kuijk FJGM (1988) Carotenoids in the human macular and whole retina. *Invest Ophthalmol Vis Sci* 29: 850-855.

233. Krinsky NI (1989) Antioxidant functions of carotenoids. *Free Radic Biol Med* 7: 617-635.

234. Bone RA, Landrum JT, Tarsis SL (1985) Preliminary identification of the human macular pigment. *Vision Res* 25: 1531-1535.

235. Snodderly DM, Auran JD, Delori FC (1984) The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci* 25: 674-685.

236. Rapp LM, Maple SS, Choi JH (2000) Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol Vis Sci* 41: 1200-1209.

237. Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY (2001) Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Exp Eye Res* 72: 215-223.

238. Snodderly DM (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr* 62: 1448S-1461S.

239. Khachik F, Bersnstein PS, Garland DL (1997) Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 38: 1802-1811.

240. Zou X, Gao J, Zheng Y, Wang X, Chen C, et al. (2014) Zeaxanthin induces Nrf2-mediated phase II enzymes in protection of cell death. *Cell Death Dis* 5: e1218.

241. Frede K, Ebert F, Kipp AP, Schwerdtle T, Baldermann S (2017) Lutein Activates the Transcript ion Factor Nrf2 in Human Retinal Pigment Epithelial Cells. *J Agric Food Chem* 65: 5944-5952.

242. Krinsky NI (1979) Carotenoid protection against oxidation. *Pure Appl Chem* 51: 649-660.

243. Ciulla TA, Hammond BR Jr (2004) Macular pigment density and aging, assessed in the normal elderly and those with cataracts and age-related macular degeneration. *Am J Ophthalmol* 138: 582-587.

244. Trieschmann M, Beatty S, Nolan JM, Hense HW, Heimes B, et al. (2007) Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res* 84: 718-728.

245. Wilson LM, Tharmarajah S, Jia Y, Semba RD, Schaumberg DA (2021) The effect of lutein/zeaxanthin intake on human macular pigment optical density: A systematic review and meta-analysis. *Adv Nutr* 12: 2244-2254.

246. Ma L, Liu R, Du JH, Liu T (2016) Lutein, Zeaxanthin and Meso-zeaxanthin Supplementation Associated with Macular Pigment Optical Density. *Nutrients* 12: 426.

247. Liu R, Wang T, Zhang B, Qin L, Wu C (2014) Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 16: 252-258.

248. Ma L, Dou HL, Wu YQ, Huang YM, Huang YB (2012) Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. *Br J Nutr* 107: 350-359.

249. Gale CR, Hall NF, Phillips DI, Martyn CN (2003) Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci* 44: 2461-2465.

250. Delcourt C, Carrière I, Delage M, Barberger-Gateau P, Schalch W (2006) POLA Study Group. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: the POLA Study. *Invest Ophthalmol Vis Sci* 47: 2329-2335.

251. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 272: 1413-1420.

252. Mares-Perlman JA, Fisher AI, Klein R, Palta M, Block G (2001) Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol* 153: 424-432.

253. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein BE, Brady WE (1998) Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol* 148: 204-214.

254. Flood V, Smith W, Wang JJ, Manzi F, Webb K (2002) Dietary antioxidant intake and incidence of early age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology0* 109: 2272-2278.

255. Gruber M, Chappell R, Millen A, LaRowe T (2004) Correlates of serum lutein + zeaxanthin: findings from the Third National Health and Nutrition Examination Survey. *J Nutr* 134: 2387-2394.

256. Trumbo PR, Ellwood KC (2006) Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Administration's evidence-based review system for health claims. *Am J Clin Nutr* 84: 971-974.

257. Age-Related Eye Disease Study 2 Research Group (2013) Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 309: 2005-2015.

258. Bjerkeng B, Peisker S, Ytrestøyl Å (2007) Digestibility and muscle retention of astaxanthin in Atlantic salmon, Salmo salar, fed diets with the red yeast Phaffia rhodozyma in comparison with synthetic formulated astaxanthin. *Aquaculture* 269: 476-489.

259. EFSA (European Food Safety Authority) (2005) Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the European commission on the safety of use of colouring agents in animal human nutrition. *EFSA J* 291: 1-40.

260. Yuan JP, Peng J, Yin K, Wang JH (2011) Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. *Mol Nutr Food Res* 55: 150-165.

261. McNulty HP, Byun J, Lockwood SF (2007) Differential effects of carotenoids on lipid peroxidation due to membrane interactions: X-ray diffraction analysis. *Biochim Biophys Acta* 1768: 167-174.

262. Kidd P (2011) Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. *Altern Med Rev* 16: 355-364.

263. Fakhri S, Abbaszadeh F, Dargahi L, Jorjani M (2018) Astaxanthin: A mechanistic review on its biological activities and health benefits. *Pharmacol Res* 136: 1-20.

264. Yang Y, Pham TX, Wegner CJ, Kim B (2014) Astaxanthin lowers plasma TAG concentrations and increases hepatic antioxidant gene expression in diet-induced obesity mice. *Br J Nutr* 112: 1797-1804.

265. Naguib YM (2000) Antioxidant activities of astaxanthin and related carotenoids. *J Agric Food Chem*. 48: 1150-1154.

266. Giannaccare G, Pellegrini M, Senni C (2020) Clinical Applications of Astaxanthin in the Treatment of Ocular Diseases: Emerging Insights. *Mar Drugs* 18: 239.

267. Lee SJ, Bai SK, Lee KS, Namkoong S, Na HJ (2003) Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation. *Mol Cells* 31: 97-105.

268. Dong LY, Jin J, Lu G, Kang XL (2013) Astaxanthin attenuates the apoptosis of retinal ganglion cells in db/db mice by inhibition of oxidative stress. *Mar Drugs* 11: 960-974.

269. Li Z, Dong X, Liu H, Chen X, Shi H (2013) Astaxanthin protects ARPE-19 cells from oxidative stress via upregulation of Nrf2-regulated phase II enzymes through activation of PI3K/Akt. *Mol Vis* 19: 1656-1666.

270. Miki W (1991) Biological functions and activities of animal carotenoids. *Pure Appl Chem* 63: 141-146.

271. Choi HD, Kim JH (2011) Effects of astaxanthin on oxidative stress in overweight and obese adults. *Phytother Res* 25: 1813-1818.

272. Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G (2008) Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. *J Nutr Biochem* 19: 491-504.

273. Kim JH, Chang MJ, Choi HD, Youn YK (2011) Protective effects of Haematococcus astaxanthin on oxidative stress in healthy smokers. *J Med Food* 14: 1469-1475.

274. Yang Y, Kim B, Lee J (2013) Astaxanthin structure, metabolism, and health benefits. *J Hum Nutr Food Sci* 1: 1-11.

275. Otsuka T, Shimazawa M, Nakanishi T (2013) Protective effects of a dietary carotenoid, astaxanthin, against light-induced retinal damage. *J Pharmacol Sci* 123: 209-218.

276. Nakajima Y, Inokuchi Y (2008) Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress in-vitro and in mice in-vivo. *J Pharm Pharmacol* 60: 1365-1374.

277. Izumi-Nagai K, Nagai N, Ohgami K, Satofuka S (2008) Inhibition of choroidal neovascularization with an anti-inflammatory carotenoid astaxanthin. *Invest Ophthalmol Vis Sci* 49: 1679-1685.

278. Pashkow FJ, Watumull DG, Campbell CL (2008) Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *Am J Cardiol* 22: 58D-68D.

279. Guerin M, Huntley ME, Olaizola M (2003) Haematococcus astaxanthin: applications for human health and nutrition. *Trends Biotechnol* 21: 210-216.

280. Parisi V, Tedeschi M, Gallinaro G (2008) Carotenoids and antioxidants in age-related maculopathy italian study: multifocal electroretinogram modifications after 1 year. *Ophthalmology* 115: 324-333.

281. Piermarocchi S, Saviano S, Parisi V, Tedeschi M, Panozzo G, et al. (2012) Carotenoids in Age-related Maculopathy Italian Study (CARMIS): two-year results of a randomized study. *Eur J Ophthalmol* 22: 216-225.

282. Ranga Rao A, Baskaran V, Sarada R, Ravishankar GA (2013) In vivo bioavailability and antioxidant activity of carotenoids from micro algal biomass—A repeated dose study. *Food Res Int* 54: 711-717.

283. Ranga Rao A, Raghunath Reddy RL, Baskaran V, Sarada R (2010) Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties elucidated in rat model. *J Agric Food Chem* 11: 8553-8559.

284. Stewart JS, Lignell A, Pettersson A, Elfving E, Soni MG (2008) Safety assessment of astaxanthin-rich microalgae biomass: Acute and subchronic toxicity studies in rats. *Food Chem Toxicol* 46: 3030-3036.

285. Spiller GA, Dewell A (2003) Safety of an astaxanthin-rich Haematococcus pluvialis algal extract: a randomized clinical trial. *J Med Food* 6: 51-56.

286. Liang FQ, Green L, Wang C, Alssadi R, Godley BF (2004) Melatonin protects human retinal pigment epithelial (RPE) cells against oxidative stress. *Exp Eye Res* 78: 1069-1075.

287. Ianăş O, Manda D, Câmpean D, Ionescu M, Soare G (1999) Effects of melatonin and its relation to the hypothalamic-hypophyseal-gonadal axis. *Adv Exp Med Biol* 460: 321-328.

288. Alarma-Estrany P, Pintor J (2007) Melatonin receptors in the eye: location, second messengers and role in ocular physiology. *Pharmacol Ther* 113: 507-522.

289. Tosini G, Fukuhara C (2003) Photic and circadian regulation of retinal melatonin in mammals. *J Neuroendocrinol* 15: 364-369.

290. Vanecek J (1998) Cellular mechanisms of melatonin action. *Physiol Rev* 78: 687-721.

291. Chiquet C, Claustrat B, Thuret G, Brun J, Cooper HM (2006) Melatonin concentrations in aqueous humor of glaucoma patients. *Am J Ophthalmol* 142: 325-327.

292. Chanut E, Nguyen-Legros J, Versaux-Botteri C, Trouvin JH, Launay JM (1998) Determination of melatonin in rat pineal, plasma and retina by high-performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl* 709: 11-18.

293. Iuvone PM, Tosini G, Pozdeyev N, Haque R, Klein DC (2005) Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. *Prog Retin Eye Res* 24: 433-456.

294. Witt-Enderby PA, Radio NM, Doctor JS, Davis VL (2006) Therapeutic treatments potentially mediated by melatonin receptors: potential clinical uses in the prevention of osteoporosis, cancer and as an adjuvant therapy. *J Pineal Res* 41: 297-305.

295. Ekmekcioglu C (2006) Melatonin receptors in humans: biological role and clinical relevance. *Biomed Pharmacother* 60: 97-108.

296. Pandi-Perumal SR, Srinivasan V, Maestroni GJ (2006) Melatonin: Nature's most versatile biological signal? *FEBS J* 273: 2813-2838.

297. Weale RA (1988) Age and the transmittance of the human crystalline lens. *J Physiol* 395: 577-587.

298. Weale RA (1982) The age variation of "senile' cataract in various parts of the world. *Br J Ophthalmol* 66: 31-34.

299. Schmid-Kubista KE, Glittenberg CG, Cezanne M, Holzmann K, Neumaier-Ammerer B (2009) Daytime levels of melatonin in patients with age-related macular degeneration. *Acta Ophthalmol* 87: 89-93.

300. Liang FQ, Godley BF (2003) Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. *Exp Eye Res* 76: 397-403.

301. Alarma-Estrany P, Pintor J (2007) Melatonin receptors in the eye: location, second messengers and role in ocular physiology. *Pharmacol Ther* 113: 507-522.

302. Cahill GM, Besharse JC (1993) Circadian clock functions localized in xenopus retinal photoreceptors. *Neuron* 10: 573-577.

303. Liu C, Fukuhara C, Wessel JH, Iuvone PM, Tosini G (2004) Localization of Aa-nat mRNA in the rat retina by fluorescence in situ hybridization and laser capture microdissection. *Cell Tissue Res* 315: 197-201.

304. Underwood H, Binkley S, Siopes T, Mosher K (1984) Melatonin rhythms in the eyes, pineal bodies, and blood of Japanese quail (Coturnix coturnix japonica). *Gen Comp Endocrinol* 56: 70-81.

305. Wiechmann AF, Campbell LD, Defoe DM (1999) Melatonin receptor RNA expression in Xenopus retina. *Brain Res Mol Brain Res* 8: 297-303.

306. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F (2004) Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 36: 1-9.

307. Marchiafava PL, Longoni B (1999) Melatonin as an antioxidant in retinal photoreceptors. *J Pineal Res* 26: 184-189.

308. Rosen RB, Hu DN, Chen M, McCormick SA, Walsh J (2012) Effects of melatonin and its receptor antagonist on retinal pigment epithelial cells against hydrogen peroxide damage. *Mol Vis* 18: 1640-1648.

309. Del Valle Bessone C, Fajreldines HD, de Barboza GED, Tolosa de Talamoni NG, Allemandi DA, et al. (2019) Protective role of melatonin on retinal ganglionar cell: In vitro an in vivo evidences. *Life Sci* 218: 233-240.

310. Konovalov SS, Polyakova VO (2017) Melatonin: the possibility to analyse the marker of age-related pathology in the buccal epithelium and urine. *Klin Med (Mosk)* 95: 136-139.

311. Rosen R (2009) Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol Vis* 21: 1673-1679.

312. Mehrzadi S, Hemati K, Reiter RJ, Hosseinzadeh A (2020) Mitochondrial dysfunction in age-related macular degeneration: melatonin as a potential treatment. *Expert Opin Ther Targets* 24: 359-378.

313. Littarru GP, Tiano L (2007) Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol Biotechnol* 37: 31-37.

314. Spindler M, Beal MF, Henchcliffe C (2009) Coenzyme Q10 effects in neurodegenerative disease. *Neuropsychiatr Dis Treat* 5: 597-610.

315. Migliore L, Molinu S, Naccarati A, Mancuso M (2004) Evaluation of cytogenetic and DNA damage in mitochondrial disease patients: effects of coenzyme Q10 therapy. *Mutagenesis* 19: 43-49.

316. Acosta MJ, Vazquez Fonseca L, Desbats MA, Cerqua C, Zordan R, et al. (2016) Coenzyme Q biosynthesis in health and disease. *Biochim Biophys Acta* 1857: 1079-1085.

317. Lagendijk J, Ubbink JB, Vermaak WJ (1996) Measurement of the ratio between the reduced and oxidized forms of coenzyme Q10 in human plasma as a possible marker of oxidative stress. *J Lipid Res* 37: 67-75.

318. Mancini A, Festa R, Raimondo S, Pontecorvi A (2011) Hormonal influence on coenzyme Q10 levels in blood plasma. *Int J Mol Sci* 12: 9216-9225.

319. Bhagavan HN, Chopra RK (2006) Coenzyme Q10: absorption, tissue uptake, metabolism and pharmacokinetics. *Free Radic Res* 40: 445-453.

320. Qu J, Kaufman Y, Washington I (2009) Coenzyme Q10 in the human retina. *Invest Ophthalmol Vis Sci* 50: 1814-1818.

321. Desbats MA, Morbidoni V, Silic-Benussi M, Doimo M, Ciminale V (2016) The COQ2 genotype predicts the severity of coenzyme Q10 deficiency. *Hum Mol Genet* 25: 4256-4265.

322. Blasi MA, Bovina C, Carella G, Genova ML, Jansen AM, et al. (2011) Does coenzyme Q10 play a role in opposing oxidative stress in patients with age-related macular degeneration? *Ophthalmologica* 215: 51-54.

323. Arend N, Wertheimer C, Laubichler P, Wolf A, Kampik A (2015) Idebenone Prevents Oxidative Stress, Cell Death and Senescence of Retinal Pigment Epithelium Cells by Stabilizing BAX/Bcl-2 Ratio. *Ophthalmologica* 234: 73-82.

324. Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2: 1231-1246.

325. Procházková D, Boušová I, Wilhelmová N (2011) Antioxidant and prooxidant properties of flavonoids. *Fitoterapia* 82: 513-523.

326. Leopoldini M, Russo N (2011) The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* 125: 288-306.

327. Barrajón-Catalán E, Herranz-López M, Joven J, Segura-Carretero A, Alonso-Villaverde C, Menéndez JA, et al. (2014) Molecular promiscuity of plant polyphenols in the management of age-related diseases: far beyond their antioxidant properties. *Adv Exp Med Biol* 824: 141-159.

328. Santos-Buelga C, González-Paramás AM, Oludemi T, Ayuda-Durán B, González-Manzano S (2019) Plant phenolics as functional food ingredients. *Adv Food Nutr Res* 90: 183-257.

329. Du Y, Guo H, Lou H (2007) Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. *J Agric Food Chem* 55: 1695-1701.

330. El-Mekkawy S, Shahat AA, Alqahtani AS, Alsaid MS, Abdelfattah MAO, et al. (2020) A Polyphenols-Rich Extract from Moricandia sinaica Boiss. Exhibits Analgesic, Anti-Inflammatory and Antipyretic Activities In Vivo. *Molecules* 30: 5049.

331. Rasouli H, Farzaei MH, Khodarahmi R (2017) Polyphenols and their benefits: A review. *Int J Food Prop* 20: 1700-1741.

332. Focaccetti C, Izzi V, Benvenuto M, Fazi S, Ciuffa S, et al. (2019) Polyphenols as Immunomodulatory Compounds in the Tumor Microenvironment: Friends or Foes? *Int J Mol Sci* 20: 1714.

333. Carballeda-Sangiao N, Chamorro S, de Pascual-Teresa S (2021) A Red-Berry Mixture as a Nutraceutical: Detailed Composition and Neuronal Protective Effect. *Molecules* 27: 3210.

334. Tronina T, Popłoński J, Bartmańska A (2020) Flavonoids as Phytoestrogenic Components of Hops and Beer. *Molecules* 14: 4201.

335. Dudnik A, Gaspar P, Neves AR, Forster J (2018) Engineering of microbial cell factories for the production of plant polyphenols with health-beneficial properties. *Curr Pharm Des* 24: 2208-2225.

336. Kwon EB, Yang HJ, Choi JG, Li W (2020) Protective Effect of Flavonoids from Ohwia caudata against Influenza a Virus Infection. *Molecules* 24: 4387.

337. Tomás-Barberán FA, Selma MV, Espín JC (2016) Interactions of gut microbiota with dietary polyphenols and consequences to human health. *Curr Opin Clin Nutr Metab Care* 19: 471-476.

338. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE (2004) Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch Ophthalmol* 122: 883-892.

339. Fernandez-Gonzalez P, Mas-Sanchez A, Garriga P (2021) Polyphenols and Visual Health: Potential Effects on Degenerative Retinal Diseases. *Molecules* 26: 3407.

340. Kim K-H, Tsao R, Yang R, Cui SW (2006) Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem* 95: 466-473.

341. Mao K, Shu W, Liu L, Gu Q, Qiu Q (2017) Salvianolic Acid A Inhibits OX-LDL Effects on Exacerbating Choroidal Neovascularization via Downregulating CYLD. *Oxid Med Cell Longev* 2017: 6210694.

342. Ortega JT, Parmar T, Golczak M, Jastrzebska B (2021) Protective effects of flavonoids in acute models of light-induced retinal degeneration. *Mol Pharmacol* 99: 60-77.

343. Gopinath B, Liew G, Kifley A, Flood VM, Joachim N, et al. (2018) Dietary flavonoids and the prevalence and 15-y incidence of age-related macular degeneration. *Am J Clin Nutr* 108: 381-387.

344. Vinson JA, Jang J, Dabbagh YA, Serry M, Cai S (1995) Plant polyphenols exhibit lipoprotein-boud antioxidant activity using an vitro oxidatona model for heart disease. *J Agric Food Chem* 43: 2798-2799.

345. Song JH, Moon KY, Lee SC, Kim SS (2020) Inhibition of Hypoxia-Inducible Factor-1α and Vascular Endothelial Growth Factor by Chrysin in a Rat Model of Choroidal Neovascularization. *Int J Mol Sci* 18: 2842.

346. Krenn L, Steitz M, Schlicht C, Kurth H, Gaedcke F (2007) Anthocyanin- and proanthocyanidin-rich extracts of berries in food supplements-analysis with problems. *Pharmazie* 62: 803-812.

347. Ishihara T, Kaidzu S, Kimura H, Koyama Y, Matsuoka Y (2018) Protective Effect of Highly Polymeric A-Type Proanthocyanidins from Seed Shells of Japanese Horse Chestnut (Aesculus turbinata BLUME) against Light-Induced Oxidative Damage in Rat Retina. *Nutrients* 10: 593.

348. Oliveira MR, Nabavi SF, Daglia M, Rastrelli L, Nabavi SM (2016) Epigallocatechin gallate and mitochondria-A story of life and death. *Pharmacol Res* 104: 70-85.

349. Xu J, Tu Y, Wang Y, Xu X, Sun X, et al. (2020) Prodrug of epigallocatechin-3-gallate alleviates choroidal neovascularization via down-regulating HIF-1α/VEGF/VEGFR2 pathway and M1 type macrophage/microglia polarization. *Biomed Pharmacother* 121: 109606.

350. Nagineni CN, Raju R, Nagineni KK, Kommineni VK, Cherukuri A, et al. (2014) Resveratrol suppresses expression of VEGF by human retinal pigment epithelial cells: Potential nutraceutical for age-related macular degeneration. *Aging Dis* 5: 88-100.

351. Chou WW, Wang YS, Chen KC, Wu JM, Liang CL (2021) Tannic acid suppresses ultraviolet B-induced inflammatory signaling and complement factor B on human retinal pigment epithelial cells. *Cell Immunol* 273: 79-84.

352. Allegrini D, Raimondi R, Angi M, Ricciardelli G, Montericcio A, et al. (2021) Curcuma-Based Nutritional Supplement in Patients with Neovascular Age-Related Macular Degeneration. *J Med Food* 19.

353. Prasad AS (1995) Zinc: an overview. *Nutrition* 11: 93-99.

354. Aggett PJ, Comerford JG (1995) Zinc and human health. *Nutr Rev* 53: S16-S22.

355. Andreini C, Banci L, Bertini I, Rosato A (2006) Zinc through the three domains of life. *J Proteome Res* 5: 3173-3178.

356. Bray TM, Bettger WJ (1990) The physiological role of zinc as an antioxidant. *Free Radic Biol Med* 8: 281e91.

357. Wastney ME, Aamodt RL, Rumble WF, Henkin RI (1986) Kinetic analysis of zinc metabolism and its regulation in normal humans. *Am J Physiol* 251: R398-R408.

358. Sandström B (1997) Bioavailability of zinc. *Eur J Clin Nutr* 51: S17-S19.

359. Mezzetti A, Pierdomenico SD, Constantini F, Romano F, De Cesare D, et al. (1998) Copper/zinc ratio and systemic oxidant load: effect of aging and aging-related degenerative diseases. *Free Radic Biol Med* 25: 676-681.

360. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. *Physiol Rev* 73: 79-118.

361. Newsome DA, Swartz M, Leone NC, Elston RC, Miller E (1988) Oral zinc in macular degeneration. *Arch Ophthalmol* 106: 192-198.

362. Grahn BH, Paterson PG, Gottschall-Pass KT, Zhang Z (2001) Zinc and the eye. *J Am Coll Nutr* 20: 106-118.

363. Ugarte M, Grime GW, Lord G, Geraki K, Collingwood JF, et al. (2012) Concentration of various trace elements in the rat retina and their distribution in different structures. *Metallomics* 4: 1245-1254.

364. Mochizuki K, Murase H, Imose M, Kawakami H, Sawada A (2006) Improvement of scotopic electroretinograms and night blindness with recovery of serum zinc levels. *Jpn J Ophthalmol* 50: 532-536.

365. Morrison SA, Russell RM, Carney EA, Oaks EV (1978) Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. *Am J Clin Nutr* 31: 276-281.

366. Weiter J (1988) Macular degeneration is there a nutritional component? *Arch Ophthalmol* 106: 183-184.

367. Silverstone BZ, Landau L, Berson D, Sternbuch J (1985) Zinc and copper metabolism in patients with senile macular degeneration. *Ann Ophthalmo* 17: 419-422.

368. Erie JC, Good JA, Butz JA, Pulido JS (2009) Reduced zinc and copper in the retinal pigment epithelium and choroid in age-related macular degeneration. *Am J Ophthalmol* 147: 276-282.

369. Ha KN, Chen Y, Cai J, Sternberg P Jr (2006) Increased glutathione synthesis through an ARE-Nrf2 dependent pathway by zinc in the RPE. *Invest Ophthalmol Vis Sci* 47: 2709-2715.

370. Mares-Perlman JA, Klein R, Klein BE, Greger JL, Brady WE, et al. (1996) Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch Ophthalmol* 114: 991-997.

371. Dharamdasani Detaram H, Mitchell P, Russell J, Burlutsky G, Liew G (2020) Dietary zinc intake is associated with macular fluid in neovascular age-related macular degeneration. *Clin Exp Ophthalmol* 48: 61-68.

372. Stur M, Tittl M, Reitner A, Meisinger V (1996) Oral zinc and the second eye in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 37: 1225-1235.

373. Heesterbeek TJ, Rouhi-Parkouhi M, Church SJ, Lechanteur YT, Lorés-Motta L, et al. (2020) Association of plasma trace element levels with neovascular age-related macular degeneration. *Exp Eye Res* 201: 108324.

374. Mills CF (1988) Springer-Verlag; London ; New York: Zinc in Human Biology.

375. Diplock AT (1981) Metabolic and functional defects in selenium deficiency. *Philos Trans R Soc Lond B Biol Sci* 14: 105-117.

376. Murr C, Talasz H, Artner-Dworzak E, Schroecksnadel K, Fiegl M, et al. (2007) Inverse association between serum selenium concentrations and parameters of immune activation in patients with cardiac disorders. *Clin Chem Lab Med* 45: 1224-1228.

377. Kaprara A, Krassas GE (2006) Selenium and thyroidal function; the role of immunoassays. *Hell J Nucl Med* 9: 195-203.

378. Mintziori G, Mousiolis A, Duntas LH, Goulis DG (2020) Evidence for a manifold role of selenium in infertility. *Hormones (Athens)* 19: 55-59.

379. Ujiie S, Itoh Y, Kikuchi H (1988) Serum selenium contents and the risk of cancer. *Gan To Kagaku Ryoho* 25: 1891-1897.

380. Oster O, Prellwitz W (1990) Selenium and cardiovascular disease. *Biol Trace Elem Res* 24: 91-103.

381. Hasani M, Djalalinia S, Khazdooz M, Asayesh H, Zarei M, et al. (2019) Effect of selenium supplementation on antioxidant markers: a systematic review and meta-analysis of randomized controlled trials. *Hormones (Athens)* 18: 451-462.

382. Navarro-Alarcon, M. e López-Martínez (2000) Essentiality of selenium in the human body: relationship with different diseases. *Sci Total Environ* 249: 347-371.

383. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds (2000) Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington (DC): National Academies Press (US).

384. Souza ML, Menezes HC (2004) Processing of Brazil nut and meal and cassava flour: quality parameters. *Ciênc Tecnol Aliment* 24: 120-128.

385. Flohe L (1985) The glutathione peroxidase reaction: molecular basis of the antioxidant function of selenium in mammals. *Curr Top Cell Regul* 27: 473-478.

386. Mills GC (1957) Glutathione peroxidase an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 229: 189-197.

387. Harrison I, Littlejohn D, Fell GS (1996) Distribution of selenium in human blood plasma and serum. *Analyst* 121: 189-194.

388. Zakowski JJ, Forstrom JW, Condell RA, Tappel AL (1978) Attachment of selenocysteine in the catalytic site of glutathione peroxidase. *Biochem Biophys Res Commun* 84: 248-253.

389. Tsang NCK, Penfold PL, Snitch PJ, Billson F (1992) Serum levels of antioxidants and age-related macular degeneration. *Doc Ophthalmol* 81: 387-400.

390. Mayer MJ, van Kuijk FJ, Ward B, Glucs A (1998) Whole blood selenium in exudative age-related maculopathy. *Acta Ophthalmol Scand* 76: 62-67.

391. Le Gal K, Ibrahim MX, Wiel C, Sayin VI, Akula MK, et al. (2015) Antioxidants can increase melanoma metastasis in mice. *Sci Transl Med* 7: 308re8.

392. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P (2014) Antioxidants accelerate lung cancer progression in mice. *Sci Transl Med* 6: 221ra15.

393. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ (2003) Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 361: 2017-2023.

394. Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 142: 37-46.

395. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 297: 84257.

396. Salganik RI (2001) The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *J Am Coll Nutr* 20: 464S472S.

397. Caraballoso M, Sacristan M, Serra C, Bonfill X (2003) Drugs for preventing lung cancer in healthy people. *Cochrane Database Syst Rev* 2: CD002141.

398. Vertuani S, Angusti A, Manfredini S (2004) The antioxidants and pro-antioxidants network: an overview. *Curr Pharm Des* 10: 167794.

399. Lawson KA, Wright ME, Subar A, Mouw T, Hollenbeck A (2007) Multivitamin use and risk of prostate cancer in the National Institutes of HealthAARP Diet and Health Study. *J Natl Cancer Inst* 99: 75464.

400. Bjelakovic G, Nikolova D, Simonetti RG, Gluud C (2004) Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet* 364: 1219-1228.

401. Druesne-Pecollo N, Latino-Martel P, Norat T, Barrandon E, Bertrais S (2010) Beta-carotene supplementation and cancer risk: a systematic review and metaanalysis of randomized controlled trials. *Int J Cancer* 127: 172-184.

402. Chandra RK (1984) Excessive intake of zinc impairs immune responses. *JAMA* 252: 1443-1446.

403. Hooper PL, Visconti L, Gary PJ, Johnson GE (1980) Zinc lowers high-density lipoprotein-cholesterol levels. *JAMA* 244: 1960-1961.

404. Patterson WP, Winkelmann M, Perry MC (1985) Zinc-induced copper deficiency: megamineral sideroblastic anemia. *Ann Intern Med* 103: 385-356.

405. Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, et al. (2007) Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann Intern Med* 147: 217-223.

406. Vinceti M, Filippini T, Del Giovane C, Dennert G, Zwahlen M, et al. (2018) Selenium for preventing cancer. *Cochrane Database Syst Rev* 29: CD005195.