Zinc Nutrition and Inflammation in the Aging Retina

Gilbert, R., Peto, T., Lengyel, I., & Emri, E. (2019). Zinc Nutrition and Inflammation in the Aging Retina. Molecular Nutrition & Food Research. https://doi.org/10.1002/mnfr.201801049

Published in:
Molecular Nutrition & Food Research

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2019 Wiley. This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Zinc Nutrition and Inflammation in the Aging Retina

| Journal: | Molecular Nutrition and Food Research |
| --- | --- |
| Manuscript ID | mnfr.201801049.R1 |
| Wiley - Manuscript type: | Review |
| Date Submitted by the Author: | n/a |
| Complete List of Authors: | Gilbert, Rosie; Moorfields Eye Hospital; UCL, Institute of Ophthalmology Peto, Tunde; Queen's University Belfast Faculty of Medicine Health and Life Sciences Lengyel, Imre; Queen's University Belfast Faculty of Medicine Health and Life Sciences; UCL, Institute of Ophthalmology Emri, Eszter ; Queen's University Belfast Faculty of Medicine Health and Life Sciences |
| Keywords: | zinc, retina, AMD, inflammation, complement, supplements, T-cells, uveitis |
Zinc Nutrition and Inflammation in the Aging Retina
Rosie Gilbert*1,2, Tunde Peto3, Imre Lengyel 2,3 and Eszter Emri3

Author affiliations:

1. Moorfields Eye Hospital NHS Foundation Trust, City Road, London, EC1V 2PD
2. UCL Institute of Ophthalmology, Bath Street, London, EC1V 2EL
3. School of Medicine, Dentistry and Biomedical Science, Queen’s University Belfast, 97 Lisburn Road, Belfast, BT9 7BL

*Corresponding author: rose.gilbert@gmail.com

Acknowledgements:

The work was supported by the Eye-Risk project funded from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 634479.

Financial and competing interest disclosure:

The other authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.
Zinc is an essential nutrient for human health. It plays key roles in maintaining protein structure and stability, serves as catalytic factor for many enzymes and regulates diverse fundamental cellular processes. Zinc is important in affecting signal transduction and, in particular, in the development and integrity of the immune system, where it affects both innate and adaptive immune responses. The eye, especially the retina/choroid complex, has an unusually high concentration of zinc compared to other tissues. The highest amount of zinc is concentrated in the retinal pigment epithelium (RPE) (RPE/choroid, 292 ± 98.5 µg/g dry tissue), followed by the retina (123 ± 62.2 µg/g dry tissue). The interplay between zinc and inflammation has been explored in other parts of the body but, so far, has not been extensively researched in the eye. Several lines of evidence suggest that ocular zinc concentration decreases with age, especially in the context of age-related disease. Thus, a hypothesis that retinal function could be modulated by zinc nutrition was proposed, and subsequently trialled clinically. In this review, we outline the distribution and the potential role of zinc in the retina-choroid complex, especially in relation to inflammation and immunity, and summarize the clinical studies to date.
**Introduction**

Zinc is an essential micronutrient for all organisms, critically required for normal cellular processes as well as for normal metabolism [1]. The adult human contains 2-3 g of zinc, making it the second most abundant trace element in the human body [2]. Zinc is absorbed in the small intestine and is excreted via the skin, in sweat, via the kidneys, in urine, and via the large intestine/colon, in faeces. Only ~0.1% of total body zinc content is in the blood plasma [3]. Daily intake of zinc is needed to maintain adequate body levels: in the UK the recommended daily zinc intake (reference nutrient intake) is 9.5 mg for an adult man and 7 mg for an adult woman [4]. The Food Standards Agency and the Department of Health in the UK advise that intake of zinc should not exceed 25 mg per day, but often people take supplements >80 mg/day [5, 6].

Approximately 60% of the total body zinc content is found in skeletal muscle and 30% in bone mass [3]. Of the remaining organs and tissues, the eye has an unusually high zinc content, with the highest amount of zinc concentrated in the retinal pigment epithelium (RPE/choroid, 292 ± 98.5 µg/g dry tissue), followed by the retina (123 ± 62.2 µg/g dry tissue) [7-20]. Zinc exists in the other ocular tissues, in the following (descending) order of content: the ciliary body, iris, optic nerve, sclera, cornea and the lens [21, 22].

The neurosensory retina is a multiple layered tissue, which lines the back of the eye and connects with the brain via the optic nerve [23]. Light has to transduce the entire thickness of the retina until it reaches the photosensitive ‘rod and cone’ photoreceptor cell layer. Blood supply to the neurosensory retina occurs through retinal blood vessels, which originate from the central retinal artery. Transport of small molecules, including proteins and lipids, across retinal blood vessels is controlled by endothelial tight junctions, which constitute the inner blood–retinal barrier [24, 25]. The optic nerve is composed of ganglion cell axons, which are the ‘output’ neurons of the retina, constituting its innermost layer [23]. The retina itself is built up of three layers of cellular bodies and two layers of synapses. In the inner nuclear layer, there are the cell bodies of bipolar, amacrine and horizontal cells, while the outer nuclear layer consists of photoreceptor cell bodies. All these cells contribute to the visual cycle, which may be summarised as the conversion of a photochemical ‘message’ from visible light into a neural signal, which can be interpreted by the brain.

Adherent junctions between rods, cones and the photoreceptor inner segments creates a barrier, called the outer limiting membrane, which separates the retina region from the subretinal space. In the subretinal space, the retinal pigment epithelium (RPE) creates a single monolayer. Its main function is support the maintenance of the retina, through reducing the backscattering light via its high pigment content, and removing by-products of the visual cycle. It also prevents new vessel growth into the retinal layers from the choroidal vasculature underneath. Bruch’s membrane, consisting of extracellular matrix proteins, proteoglycans, glucosaminoglycans, separates the RPE from the choroid. Together, the RPE and the Bruch membrane form the outer blood–retinal barrier, which prevents the
entrance of macromolecules and immune cells from the underlying choroid into the photoreceptor layer. Thus, the integrity of the RPE and Bruch’s membrane are essential for homeostasis in the retina. The choroid, located below Bruch’s membrane, contains a dense network of blood vessels (the choriocapillaris), which supplies oxygen and nutrients to the RPE, outer retina and optic nerve. Unlike the endothelial tight junctions of the retinal vessels, the choroid endothelium is fenestrated, which enables the transport of molecules to the metabolically demanding RPE. A simplified diagram of the retina-choroid complex is shown in Figure 1. The choroid contains tissue-resident melanocytes, fibroblasts, macrophages, mast cells and dendritic cells [26]. Muller cells, a specialised form of local retinal glial cell, are, in contrast, found throughout the retina. As part of the normal aging process, waste material accumulates in the retina-RPE and RPE-choroid interface. Its advanced accumulation can lead to disease, such as age-related macular degeneration (AMD). In this review, we summarize the distribution and known role of zinc in retina-choroid complex, in addition to its contribution to waste material accumulation.

Zinc is the only essential transition metal ion that lacks biological redox activity. It is a Lewis acid, meaning that it acts as a proton donor [27]. This feature makes zinc the ideal enzymatic cofactor [28]. Zinc can either participate, directly, in chemical catalysis or, indirectly, by maintaining protein structure or stability. For this reason, it has an important regulatory role in a wide variety of biological processes, acting as a catalyst for more than 300 enzymes and contained within thousands of proteins, as a zinc finger domain [29-36]. [37-41]. Zinc plays a key role in fundamental cellular processes such as DNA synthesis, RNA transcription, cell division and activation [42], as well as in prevention of cell apoptosis [43]. It also has a significant role in affecting signal transduction for cellular function [44], in particular for the development and integrity of the immune system, affecting both innate and adaptive immune responses, which represent non-specific or specific responses, respectively, to foreign macromolecules (antigens) [37-41]. Innate immunity is characterised by physical barriers, fixed receptors based on pathogen molecular patterns, limited immunological memory and the fact that it does not require immunisation (priming) [41]. Acquired immunity is characterised by clonally variable receptors based on gene rearrangement, development of immunological memory, B-cell and/or T-cell activation, cytotoxic T-cells and antibody production [41].

The European Food Safety Authority (EFSA) Panel 2009 report states that a cause and effect relationship has been satisfactorily established between the dietary intake of zinc and normal function of the immune system, normal DNA synthesis and cell division, protection of DNA, proteins and lipids from oxidative damage, maintenance of normal bone, normal cognitive function, normal fertility and reproduction, normal metabolism of fatty acids, normal acid-base metabolism, normal vitamin A metabolism and maintenance of normal vision [45]. This report, however, did not conclude that that inadequate intake of zinc, leading to impaired function of the above-mentioned health relationships, occurs in the general EU population, based on the evidence provided to the panel [45]. In this review, which focuses on the role
of zinc in inflammation in the aging retina, we will summarize the physiological role of zinc in the retina (Table 1) and then discuss how zinc nutrition might affect retinal inflammation and function, with reference to the available literature about zinc and immunity.
| Cell type          | Zinc study focus/ main finding                                                                 | Reference |
|-------------------|-----------------------------------------------------------------------------------------------|-----------|
| Developing retina | Role of Zic family of zinc finger transcription factors in early retinal progenitor cells in mouse retina | [46]      |
|                   | relative axial myopia in Egr-1 (ZENK) -/- KO mice                                               | [47]      |
|                   | role of Zic-3 in intra-retinal axon projection in chicken                                       | [48]      |
|                   | Zic-2 regulates retinal ganglion cell axon repulsion in mice                                   | [49]      |
|                   | mutation in ZNF644 associates with high myopia                                                  | [50]      |
|                   | ZIC408 in retinal vasculogenesis in zebrafish                                                   | [51]      |
|                   | Spalt in cone photoreceptor and retinal horizontal cell development in mice                    | [52]      |
|                   | ZIP4 knockout mice, exencephalia, severe growth retardation, and hydrocephaly, was accompanied by unilateral or bilateral anophthalmia. | [53]      |
|                   | Zip13 knockout caused sunken eyes which were also associated with down slanting palpebral fissures | [54]      |
| Adult retina      | zinc deficiency in retinitis pigmentosa                                                         | [55]      |
|                   | improved dark adaptation in cirrhotic upon zinc supplementation                                | [56]      |
|                   | adequate dietary zinc is protective against AMD                                                 | [57-62]   |
|                   | inhibition of complement activation in AMD upon zinc supplementation                            | [63]      |
|                   | reduced zinc level in retina with aging and in AMD in vivo                                      | [18, 20]  |
|                   | stimulatory effect of Zn on ERG b wave amplitude in vertebrate retina                           | [64-68]   |
|                   | retinal gene expressional changes upon zinc-deficient/sufficient diet in rat retina             | [69, 70]  |
|                   | horizontal and bipolar cells role of extracellular zinc in regulation of AMPA mediates postsynaptic signals in skate horizontal cells and bipolar cells. | [70-72]   |
|                   | inhibition of extracellular zinc on hemi-gap-junction channel currents on retinal horizontal cells in bass | [73]      |
|                   | zinc release by depolarisation of retinal cells in rat                                          | [74]      |
| Acronym | Description | Reference |
|---------|-------------|-----------|
| amacrine cells | modulation of A-type potassium currents by zinc in retinal horizontal cells | [75] |
| amacrine cells | differential modulation of signal from cones to horizontal cells by extracellular zinc | [76] |
| Zn(2+) | modulates light responses of bipolar and amacrine cells in carp retina | [77] |
| amacrine cells | modulatory role of endogenous zinc upon glycinergic response in amacrine cells in rat retina | [78] |
| amacrine cells | Suppression by zinc of transient OFF responses of carp amacrine cells on GABA receptors | [79] |
| photoreceptors | Synaptically released zinc from rod photoreceptors in zebrafish | [80] |
| photoreceptors | modulatory role of extracellular zinc upon calcium activity the photoreceptor terminal | [81] |
| | role of Yin Yang 1 in melatonergic function of retinal photoreceptors. | [82] |
| | zinc co-release with glutamate by photoreceptors; cytoprotective role in skate retina | [81, 83] |
| | translocation of zinc within photoreceptors in light stimulation | [17] |
| | stabilization of disc membranes in bovine and mice retina and in vitro model modelling | [84-88] |
| | role of KLF15 in repression of photoreceptor-specific gene expression in non-photoreceptor cells in vitro molecular model system | [89] |
| RPE | zinc decrease in RPE layer in disease in vitro | [9] |
| RPE | regulatory role of melanin synthesis and melanosome formation in RPE cells in vitro and in vivo in rats | [90-92] |
| RPE | protective role of zinc against oxidative stress in vitro RPE | [32, 93, 94] |
| RPE | role of zinc in phagocytic and lysosomal activity in vitro RPE | [95-98] |
| RPE | role of zinc in apoptosis in in vitro RPE | [99] |
| RPE | protective role of zinc against UV-induced DNA damage in vitro RPE | [100] |
| Tissue          | Effect                                                                 | Reference |
|-----------------|------------------------------------------------------------------------|-----------|
| enhanced pigmentation upon zinc supplementation in vitro | [101]       |
| role of snail zinc finger transcription factor in epithelial mesenchymal transition of human ARPE-19 retinal pigment epithelial cells | [102]       |
| sub-RPE space   | high content of zinc in sub-RPE waste material                          | [103]       |
| choroid         | blockade of choroidal endothelial cell migration upon AREDS zinc        | [104]       |

Table 1: Summary of studies providing evidence for the physiological role of zinc in the retina
Zinc in the normal retina

The retina-choroid complex contains the highest concentration of zinc in the eye, as measured using a wide variety of techniques in different species, including humans [7-20].

Figure 1: Simplified diagram ([105]; reproduced with permission) depicting the cellular organization of the retina/RPE/choroid complex. Total cellular zinc concentration (left shaded bar) represents the total zinc content of the different layers of retina-choroid complex and the grayscale bar shows the total content relative to the each layers. The majority of total zinc is tightly bound to proteins so the level of exchangeable zinc, available for biochemical processes, is maintained within a narrow concentration range (right shaded bar), and the grayscale bar shows the amount of exchangeable zinc content relative to the each layers. Cho, choroid; BM, Bruch's membrane; RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer and GC, ganglion cell layer. In the grayscale colour bar, white represents the lowest concentration of zinc and black represents the highest concentration of zinc.

Total cellular zinc concentration is estimated to be approximately one hundred micromoles [106]. The majority of intracellular zinc is tightly bound to proteins, compartmentalized and sequestered with high binding affinities (in the picomolar to femtomolar range), which allows increased cellular zinc utilization [74, 78, 103, 107-115]. As a consequence of this high protein binding affinity, the level of available zinc for biochemical processes is maintained within a narrow concentration range [106] and is referred to as “free”, “labile”, “readily releasable” or “exchangeable” zinc [116, 117]. These biochemical findings rule out that proteins with low affinity zinc binding sites might be the physiologically relevant proteins for regulatory role of zinc [118]. In physiological circumstances, other essential metal ions, such as calcium,
magnesium, iron and copper can interfere with each other, and share regulation of biological processes, hence these interactions should always be considered when biological activity of zinc is being assessed [118].

The majority of the changes in cellular zinc levels are mediated through 24 zinc transmembrane proteins (ten zinc efflux transporters, called ZnT, and fourteen influx transporters, called ZIP) encoded for by two solute-linked carrier (SLC) gene families, SLC30 and SLC39, respectively [119]. These transporters are regulated transcriptionally, translationally, and at the protein level through heterodimer formation, ubiquitination, phosphorylation, and proteolysis [120-123]. Under steady state conditions, a primary function of cytosolic zinc-binding proteins is to buffer the relatively large zinc content found in most cells to a cytosolic zinc(II) ion concentration in the picomolar range [117]. Under non-steady state conditions, cytosolic zinc-binding proteins act together to modulate transient changes in cytosolic zinc ion concentration in a process called ‘zinc muffling’ [117]. If intracellular zinc influx is increased, muffling reactions will dampen the resulting rise in cytosolic zinc ion concentration and, eventually, restore the cytosolic zinc ion concentration to its original value by shuttling zinc ions into subcellular stores or by removing zinc ions from the cell [117]. In addition, muffling reactions provide a potential means to control changes in cytosolic zinc ion concentrations for purposes of cell signalling in what would otherwise be considered a buffered environment not conducive for signalling. The potential downstream effects of these processes are summarised in Figure 2.

Metallothioneins (MTs) are zinc-ion-binding proteins [124] and their gene expression is tightly controlled mainly by MTF-1 transcription factor. Several isoforms of MTs exist and they show cell-type specificity [124]. Zinc is bound exclusively to the sulphur donors of cysteines in zinc/thiolate clusters [125]. One MT molecule can bind seven zinc ions, through different binding affinities to metals [117, 125-127]. With lower zinc binding affinity, MTs react as redox proteins. They sequester or release zinc, depending on the local redox state, thereby trapping, not only any zinc for storage, but also serve as zinc acceptors and donors for other proteins in a dynamic way (zinc buffering in non-steady state conditions) [118]. Therefore, the redox inert zinc can influence the function of numerous proteins, transcription factors and enzymes ([116, 117, 125]. However, there are also redox modulation-independent mechanisms, which seem to modulate phosphorylation signalling [128] and zinc has long-term effect on these gene expression levels. MTF-1 seems to be a primary zinc sensor and induces gene expressional changes thereby, directly or indirectly, inducing other transcriptional regulators [129].
Figure 2: Schematic diagram depicting how zinc buffering/muffling and involvement of high zinc binding affinity proteins can maintain steady state conditions, with a variety of downstream effects, which may ultimately influence immune function. Intracellular zinc level is tightly controlled. Cytosolic zinc-binding proteins are buffering the relatively large zinc content found in most cells to a cytosolic zinc(II) ion concentration in the picomolar range, and also act together to modulate transient changes in cytosolic zinc ion concentration in a process called ‘zinc muffling’. If intracellular zinc influx is increased, muffling reactions will dampen the resulting rise in cytosolic zinc ion concentration and restore the cytosolic zinc ion concentration to its original value by shuttling zinc ions into subcellular stores or by removing zinc ions from the cell. The potential downstream events can be immediate/short term (change in enzyme activation) and long-term effects (gene/protein/secretional change). All of these can lead to changes in immune function, however this is yet to be elucidated in the retina-choroid complex.

In order for zinc to have an effect, the bioavailable zinc level needs to change. There are two ways that this can happen. The first is characterised by the transport of zinc from and to the extracellular space. The second is by releasing zinc from molecules or intracellular stores. One of the most well studied effects of the releasing zinc into the extracellular space is the effect on glutamate receptors in neurons in the brain. The released extracellular zinc binds and inhibits the postsynaptic N-methyl-D-aspartate receptor [125, 130] and modulate synaptic transmission. A similar effect is observed in the neurosensory retina [78, 108].

In retinal tissue, zinc is usually stored in intracellular compartments in ganglion cells, the horizontal and amacrine cells [78, 108]. Zinc localization in retinal Muller cells has also been demonstrated in previous studies. Depolarization of the retinal neurons can induce zinc release at the plexiform layers [74], which provided the first evidence for the hypothesized neuro modulator role for zinc in the retina [68, 131, 132]. Endogenous zinc is co-released with glutamate from synaptic terminals of photoreceptors and, by negative feedback, reduces calcium entry and concomitant
vesicular release of glutamate [81, 83]. This is, therefore, likely to protect the retina from glutamate excitotoxicity [83].

Zinc can be released from the cellular compartments or stores by molecules such as MTs, by external stimuli. Amongst the many roles of zinc in intracellular processes, zinc release can lead to the activation and phosphorylation of the MAPK/ERK pathway [133] and/or zinc transporters [134]. Intracellular increase in free zinc can generate effects in seconds, called “zinc sparks”, or in minutes, called “zinc waves” [117]. The downstream molecular events are not yet well characterized [135]. Therefore, the direct molecular events determining zinc stimuli and acceptance of zinc, as a second messenger, need further investigation [118].

In addition to its presence in organelles, intracellular zinc is present in the outer segments of photoreceptors [103, 110-112], where the availability of zinc is dependent on light-dark adaptation. In fact, zinc is required for the stabilization of disc membranes in the outer segments [136], probably through the stabilization of rhodopsin [17]. However, additional low affinity zinc binding to specific histidine sites can also lead to de-stabilization of rhodopsin [86], and decreased thermal stability [87], suggesting that rhodopsin might be less stable in light than in dark. This zinc-induced de-stabilization of rhodopsin might be especially relevant in inherited genetic retinal diseases, such as retinitis pigmentosa, which is associated with the Pro23His mutation [86], further supporting zinc-induced instability of rhodopsin [86]. It is of note that retinitis pigmentosa is associated with systemic zinc deficiency [55]. Outer segment zinc content can also affect the conversion of vitamin A (retinol) to retinal [137] by the zinc-dependent alcohol dehydrogenase [138, 139] and this can be clinically manipulated by zinc supplementation [140]. Zinc may also be involved in dark adaptation by affecting the synthesis or release of retinol-binding proteins in the liver [141]. Where and how this reported increase in total zinc in the outer segment [17] originates from is an important factor, which is still to be determined.

Zinc is abundantly enriched in the sub-retinal space [142], especially in sub-RPE deposits [143]. The increased levels of zinc found in the sub-RPE space could be explained by the dysfunction of zinc-rich RPE, occurring in disease states. Indeed, elemental distribution of Zn- showed preferential decrease of zinc in the RPE layer in disease [9]. Zinc content and retention is mediated, at least partly, by pigmentation of the RPE [9], as melanin synthesis and melanosome formation appears to be regulated by zinc [92]. The RPE is also rich in exchangeable zinc [144] localized to the Golgi apparatus [108], melanosomes and lysosomes [14, 91, 108-110, 112, 145-149]. The macula RPE may contain less zinc than the peripheral retinal RPE, and it is thought that macula zinc levels may be further affected by aging [150]. Bruch’s membrane contains a substantial amount of zinc, especially in eyes with AMD (which is discussed in more detail in a later section) [103]. As some of this zinc is in the exchangeable form [103], it is possible that there is an extracellular zinc milieu that requires active control for the regulation of extracellular proteins, involved in the immune response, [151] or for the remodeling of the extracellular matrix by metalloproteinases [152].
The highest concentration of zinc in the eye has been localized to the choroid [18, 19]. As the choroid is a highly pigmented layer, it is likely that pigmentary zinc contributes to this enrichment. It is worth noting that the immunohistological features of two well recognized uveitis syndromes with similar phenotypes: Vogt-Koganagi Harada syndrome and sympathetic ophthalmia, characterized by choroidal inflammation and serous retinal detachments, support a delayed type of hypersensitivity (T-cell-mediated) mechanism directed towards the uveal melanocytes of the choroid, which is inducing and/or perpetuating an autoimmune-type chorioretinal inflammation [153, 154]. In combination with the evidence linking zinc and choroidal pigment, this hints at a possible relationship between zinc, choroidal pigment and chorioretinal inflammation, where accumulation of zinc and exposure of antigens derived from choroidal pigment, as a result of RPE dysfunction in AMD, could be contributing to retinal inflammation. However, at the present time, relatively little is known about the role and function of zinc in the choroid, and its relationship to inflammation, other than that zinc appears to accumulate with increasing age [18] and that it may contribute to the developing zinc deficiency in the inner layers of an aged eye [20]. What we do know about the relationships between zinc, inflammation and immunity, which may be of relevance to retinal inflammation, is summarized in later sections.
Suboptimal dietary zinc status and deficiency: clinical manifestations

An adequate daily intake of zinc is necessary to achieve a steady state for proper immune function, because there is no specialized zinc storage system in the human body. Therefore, zinc deficiency often occurs because of malnutrition, especially in the elderly [155, 156]. Physiologic effects of systemic zinc deficiency are associated with a number of diverse biochemical and immunological changes in the human body [157]. Notably, a clinical syndrome suspected to be secondary to zinc deficiency was first reported from Iran in 1961, consisting of wide range of deficits including growth retardation, male hypogonadism, skin changes, mental lethargy, hepatosplenomegaly, iron deficiency anaemia, and geophagia [158]. Subsequent interest concerning the effect of zinc deficiency on visual function came from the observation that abnormal visual dark adaptation in patients with alcoholic liver disease (cirrhosis) was improved by zinc supplementation [140]. The mechanism for this could be the effect of zinc on the synthesis or release of retinol-binding proteins in the liver [141]. Similar clinical observations regarding zinc and dark adaption were made in a case series of sickle cell anaemia patients [159], and also healthy human subjects [160], which suggested that these effects on visual function may be related to dietary zinc deficiency. Since then, an increasing amount of research has shown that diet influences local retinal zinc homeostasis. Zinc supplementation results in a higher amount of intracellular retinal zinc [161], whilst a zinc deficient diet induces retinal gene expressional changes [69]. Light induced retinal degeneration and visual cell loss in rats resulted in gene expressional changes (attributed to inflammation, apoptosis, cytokines, the innate immune response and related receptors) and this type of induced retinal degenerative change was shown to decrease in response to the addition of a zinc oxide, plus rosemary, antioxidant mixture [70]. As described earlier, zinc-induced de-stabilization of rhodopsin might be relevant in inherited genetic retinal diseases such as retinitis pigmentosa [88], which is also associated with systemic zinc deficiency [55]. Also, the conversion of vitamin A (retinol) to retinal [137] by the zinc-dependent alcohol dehydrogenase [138, 139] may be clinically manipulated by zinc supplementation [140].

Worldwide, zinc deficiency in human subjects has since been reported to occur in physiological conditions where there is an increased requirement for zinc, for example, during childhood and pregnancy [162], and it was originally observed that these deficiencies occurred in countries where primarily cereal proteins are consumed by the population [1]. Although, in both industrialized and developing countries, there may be inadequate dietary intake of zinc, inhibitors of zinc absorption are most likely the most common causative factor in zinc deficiency [163]. Phytate, which is present in staple carbohydrate-based foods, such as cereals, corn and rice, is now known to have a strong negative effect on zinc absorption from composite meals [163]. Thus, zinc deficiency is believed to occur on a spectrum, in any given population, ranging from severe cases to marginally deficiency and suboptimal status [1, 163] and may be secondary to both dietary intake and absorption.
The aging retina: age-related macular degeneration

In developed countries, up to 30% of elderly people are zinc deficient and it has been recognised that zinc may have an important role in degenerative disorders of aging, as a regulator of the antioxidant pathway and as an anti-inflammatory agent [70, 164]. Aging of the retina, specifically, the central retinal macula area of the eye, is considered one of the most important clinical problems affecting vision. In particular, age-related macular degeneration (AMD) affects about one quarter of people over the age of 65 years [165] and late stage disease accounts for approximately 50% of legal blindness in Europe and North America [166, 167].

A key feature of AMD is the presence of extra-cellular deposits between the choroid and the RPE [168]. These deposits vary in size and have been classified in a number of different ways [169-172]. Clinically, the term drusen is used to monitor progression of visual loss, as the other types of deposits are not readily visualized. Small drusen may not always be associated with risk of visual loss. However, multiple large drusen, located in the macular region, increase the risk of AMD (Figure 3). Drusen accumulation occurs naturally with age and an individual druse has the capacity to distort and rupture through the RPE and pushing into the neural retina [172]. Hence, development and progression of sub-RPE deposit formation are likely to be a key factor in AMD pathogenesis. Understanding how such deposits are formed is key to elucidating the mechanistic basis of this disabling eye condition [151].

The composition of sub-RPE deposits, the so-called drusen, is complex [173, 174]. In addition to proteins and lipids, drusen contain anomalous deposits of zinc, some of which is in the exchangeable (ionic or loosely protein bound) form [103]. However, the origin of the millimolar zinc in the Bruch’s membrane, which is the extracellular matrix in which the sub-RPE deposits are formed, is still not clear.
Figure 3: Clinical retinal fundus photograph of the right eye macula showing the soft, large, confluent yellow drusen (sub-RPE deposits), which are characteristic of high risk AMD.

The ‘dry’ form of AMD can be classified into early, intermediate and advanced stages [175-177]. In the early stages, extracellular deposits start to accumulate at the apical and basal side of the RPE. As the disease progresses, the size of the drusen increases, to more than 125 µm in diameter, and RPE pigmentary abnormalities appear. The aetiology of dry AMD is not well understood but in geographic atrophy, the end stage of dry AMD, it is known that the RPE cells slowly degenerate and may atrophy completely - a progression that takes many years before advanced vision loss develops. In addition to the geographic atrophy of dry AMD, the other common clinical variant of late stage AMD, is an aggressive ‘wet’ form, in which the integrity of Bruch’s membrane is broken, and rapidly progressive choroidal neovascularization (CNV) and vision loss develops. Neovascular wet AMD can be treated with some success with intravitreal anti-VEGF injections [178] but there is currently no treatment for dry AMD.

In vitro studies of RPE cells have validated that there is a decrease in endogenous zinc levels with increasing age, and that the basolaterally localized ZIP2 and ZIP4 is reduced as a function of RPE age [179]. As is the case with other cells and tissues, the RPE can be damaged by too much or too little zinc [99, 180]. Newsome et al. demonstrated that levels of zinc are reduced in human eyes with signs of AMD [181]. This was proposed to lead to increased oxidative stress [32, 93, 94], deficits in phagocytic and lysosomal functions [95, 97, 98], macromolecule synthesis- and caspase-dependent apoptosis [99], increased photic injury [165] and UV-induced DNA damage [100] in the RPE. As described earlier, light-induced retinal degeneration and visual cell loss in rats results in gene expressional changes related to inflammation, apoptosis, cytokine production and innate immune responses; and
these pathways can be suppressed by zinc supplementation, in combination with Age-Related Eye Disease Study (AREDS) antioxidant supplement formula and other antioxidants [70]. Furthermore, the effect of AREDS formula plus zinc has been investigated on mouse choroidal endothelial cells, demonstrating a blockage of endothelial cell migration and a decrease in the number of macrophages bound to endothelial cells [104].

This short and, no doubt, incomplete list shows that changes that the zinc in RPE may potentially play a multitude of roles in the development of AMD. However, it does not provide an answer to the question of from where the millimolar zinc levels of zinc in the Bruch’s membrane are derived. However, it is known that the RPE has very high concentrations of intracellular total zinc (see Figure 1). In addition, one of the RPE’s functions is the phagocytosis and processing of the zinc-rich photoreceptor outer segments, potentially enriching the RPE zinc content further. As RPE damage is thought to be the precursor for the development and progression of AMD [165], abnormal zinc release from RPE may occur as the consequence the damage highlighted above. The choroid is also rich in zinc and changes associated with AMD here [182] may also contribute to the accumulation of zinc in the Bruch’s membrane. These suggest that buffering zinc in the Bruch’s membrane could be important in mediating sub-RPE deposit formation and hence the development of AMD.

**Clinical studies of zinc and AMD**

A single centre study of a randomized controlled trial of daily dose of zinc sulphate (100mg) versus placebo, showed a significantly positive treatment effect on visual acuity change compared to baseline, with a decreased likelihood of final visual acuity deterioration [57]. Thus, the idea that restoring zinc balance through diet or supplementation may protect against AMD provides an interesting and potentially inexpensive intervention strategy and subsequent clinical trials have attempted refine this concept [58]. Evidence from the large randomized, placebo-controlled AREDS clinical trials, which initially evaluated high-dose supplementation with vitamins C and E, beta carotene, with or without zinc (zinc oxide 80mg) and copper [59], and later added xanthophyll carotenoids, lutein and zeaxanthin with or without omega-3 fatty acids supplementation in AREDS 2, suggested that these components may help protect against the progression to AMD and related vision loss [35, 36][183, 184]. In particular, it has been shown that retinal degenerative pathways are suppressed by zinc supplementation, in combination with the AREDS formula and other antioxidants [70]. In parallel with AREDS, the Rotterdam Eye Study, a population-based cohort study, suggested that zinc status, above median intake of vitamins E, C and beta-carotene, was associated with a 35% reduced risk of incident AMD [60]. This was further supported by analyses from the Blue Mountains Eye Study, which found that higher dietary zinc intake had a favourable effect on incident AMD [61, 62].

The effect of zinc supplementation may be determined according to genetic background. In the Rotterdam study population, zinc nutrition was beneficial for
patients with early AMD carrying high complement factor H gene (CFH) genetic risk variant [185]. Patients from AREDS study carrying exclusively age-related maculopathy sensitivity 2 (ARMS2) risk alleles, and not CFH, derived maximum benefit from zinc-containing AREDS formula [186, 187] (although others found errors in the data used to support the initial claim of genotype-treatment interaction [188]). It is still not known at which stage of disease the protective effects of zinc may be important, or when the potential negative interactions with genetic and/or other risk factors become significant. Furthermore, there have been some conflicting results amongst zinc supplementation trials and epidemiological studies [189-193]. Therefore, the role of zinc in the pathogenesis of AMD needs to be further investigated.

**Zinc and complement in AMD**

Dysregulation of the innate immune system can lead to autologous tissue damage and development of degenerative diseases. Complement has been recently recognized as a key player of the innate immune system, which, in addition to defending the host against pathogen infection, also coordinates various events during inflammation, and bridges innate and adaptive immune response [194]. There are three complement pathways: the classic pathway, the lectin pathway and the alternative pathway [194]. All three pathways converge at the formation of the C3 convertase which cleaves C3, and trigger a cascade of events leading to the formation of a membrane attack complex (MAC), which destroy pathogens or damaged ‘self’ cells, by opsonisation and/or lytic destruction [194].

Since the identification that the CFH polymorphism (in a region known to bind heparin and C-reactive protein) was found to be strongly associated with AMD [195], the complement component of innate immunity has been heavily implicated in the pathogenesis of AMD [196]. Several studies have found evidence of deposition of complement proteins in drusen, a focus of inflammatory activity, including complement components C3a and C5a [197], C5 and C5b-9 terminal complement complex (TCC) [198], as well as fluid-phase complement regulators (complement factor H-CFH, vitronectin and clusterin) and membrane-bound complement inhibitors (complement receptor 1-CR1, also called CD35, and membrane cofactor protein-MCP, also called CD46) [199, 200]. Notably, CFH binding to mononuclear phagocytes was shown to curb the CD47-mediated elimination of resident immune cells in a murine retinal model system [201]. Thus the presence of mutant CFH may inhibit CD47-mediated resolution of retinal inflammation, driven by resident macrophages and disrupt homeostasis in the subretinal space. CFH, and its Tyr402 mutant form, is possibly of most interest in relation to zinc, sub-RPE deposit formation and AMD [202-206]. It was shown almost 30 years ago that millimolar concentrations of zinc induced the oligomerisation of CFH and rendered it inactive in experimental ‘test tube’ conditions [207, 208]. Later, it was found that there was no need for millimolar extracellular zinc levels to trigger oligomerisation and inhibition of CFH. Large oligomers were formed in the test tube at levels higher than 20 µM zinc, and at zinc levels of 200 µM, more than 85% of CFH is oligomeric and in its fully
inhibited form [151]. Therefore, we hypothesise that similar oligomerization occur in the Bruch’s membrane, in AMD. It has been shown that inactivation of CFH and the uncontrolled activation of the alternative pathway, resulting in secondary C3 deficiency, is part of the pathological process leading to AMD [209]. In addition, Hageman et al [203] provided evidence that CFH, together with C3b/iC3b, membrane attack complex and C5b-9, are constituents of sub-RPE deposits. Therefore, the potential to release exceptionally high levels of zinc from the RPE, through injury to this cell layer, and the fact that the Bruch’s membrane contains high concentrations of zinc in AMD, [103] suggests that zinc could potentially induce the kind of pathological protein aggregation described above [151, 210, 211]. As oligomerized CFH will have attenuated complement inhibitor function, the RPE and the choroid will be at sustained risk for alternative pathway-mediated complement attack. Within the complement system, zinc may affect more than just CFH. Zinc may bind to a number of complement proteins [212] and affect complement activity in several different ways [213-216]. In summary, these data seem to support the proposal that complement-mediated inflammation is a major pathological driver of AMD [203]. Whether the Tyr402His mutation will serve as an additional zinc binding site requires more experimental proof.

Zinc as modulator of humoral and cellular immunity: implications for inflammation in the retina

In addition to its impact on several biological processes discussed earlier, zinc can function as an anti-inflammatory agent. Zinc is essential for the development and maintenance of both the innate and adaptive compartments of the immune system and, therefore, has a key role in the modulation of inflammation. Aging is known to affect immune function in the older population, mainly adaptive immunity characterised by the T cell-mediated immune response [217], and these changes have been termed “immunosenesence”. Immunosenesence may lead to immune dysregulation, which can result in an increased production of pro-inflammatory cytokines, a status known as “inflamm-aging”[218]. With regards to cellular immunity, both zinc deficiency and supplementation have important effects on the development and function of T cells, B cells, NK cells, monocytes, and macrophages [218]. Rapid as well as delayed changes in readily exchangeable zinc (free Zn) and the zinc proteome are crucial in determining activation of immune cells, cytokine responses, signalling and nutritional immunity [219]. In particular, age-related effects on T-cell cytokine signalling and T-cell activation induced cell death have been observed, which may be modulated in vitro by zinc [220].

As mentioned previously, mild zinc deficiency is common in older people and is characterised by a decline of serum or plasma zinc levels with age [218]. Zinc supplementation studies in the elderly suggest that replacing the zinc deficit in this population results in decreased incidence of infections and decreased generation of inflammatory cytokines [164]. A study which determined the effects of zinc deficiency and age on the induction of inflammatory responses, using an in vitro cell culture system and an aged mouse model showed that zinc deficiency, particularly the
reduction in intracellular zinc in immune cells, was associated with increased inflammation with age [221]. Furthermore, it was demonstrated that reduced Zip 6 zinc transporter expression enhanced proinflammatory response, and age-specific Zip 6 dysregulation correlated with an increase in Zip 6 promoter methylation [221]. Finally, this study showed that restoring zinc status, via dietary supplementation, reduced aged-associated inflammation [221]. These data suggest that cellular zinc levels, which may be subject to epigenetic regulation, contribute to increased susceptibility to inflammation with age and that dietary zinc supplements may help to counteract these effects in zinc-deficient older people.

It is well known that almost all age-related degenerative diseases involve chronic inflammation, including those that occur in immune-privileged tissues, such as the retina and the brain [222]. Ocular immune privilege may be described as: ‘a complex phenomenon that involves multiple components, starting with sequestration behind an efficient blood–retina barrier, through active local inhibition by soluble and surface-bound molecules that actively inhibit activation and function of adaptive and innate immune cells, and culminating in systemic regulation via induction of T regulatory cells.’ [223]. Failure of ocular immunological tolerance may lead to classical autoimmune-type inflammation, manifesting as inflammatory disease of the uvea (uveitis). Inflammation of the choroid, the posterior section of the uvea, is often associated with inflammation of the overlying retina.

T-helper (Th)1 and Th17 subsets, characterised by expression of T-bet (Tbx21) and retinoic-acid related orphan receptor (ROR) γ-t (Rorc) transcription factors and secretion of the ‘signature’ pro-inflammatory cytokines, interferon (IFN)-γ and IL-17, respectively, are thought to be causal agents in the pathogenesis of autoimmunity [224, 225]. Th17 effector cells may be induced in parallel to Th1, and, like Th1, polarized Th17 cells have the capacity to cause inflammation and autoimmune disease [225]. The Th2 effector subset, characterised by the transcription factor, GATA-3, and production of cytokines IL-4, IL-5, and IL-13, was initially described around the same time as the Th1 subset [226], and before the Th17 subset was discovered. Although both Th1 and Th2 have important roles in host defence (with Th2 being particularly important in allergic responses and the clearance of extracellular pathogens), only the Th1 subset has been widely implicated in autoimmune inflammation [225, 226].

Thymic-derived and peripherally-induced ‘regulatory’ T-cells (Treg) are an important physiological immune mechanism to suppress autoreactive T-cells and other sources of endogenous inflammation. The identification of the Forkhead family transcription factor, (Fox)P3 [227, 228] and its specific expression in CD4+CD25+ T cells, a specialised subset of T-cells with capacity to suppress inflammation [229], has defined FoxP3 expression as a key phenotypic marker of Treg. These phenotypically categorised CD4+CD25+FoxP3+ Treg suppress inflammation through direct cell-to-cell interactions and anti-inflammatory cytokine production, such as IL-10 and transforming growth factor β (TGF-β), in addition to other mechanisms [224, 230-233]. FoxP3 is considered the Treg ‘master transcription factor’ because it is critically required for Treg-cell development and function, and for suppressing autoimmunity [229, 234-236]. The FoxP3 protein contains a forkhead (FKH) domain.
at the C terminus, critical for nuclear localisation and DNA binding, an N terminus transcriptional repressor domain, in addition to C2H2 zinc finger and leucine zipper domains, which mediate DNA binding and dimerization (Figure 4) [237]. Interestingly, electrophoretic mobility shift assay (EMSA) experiments have confirmed that both the DNA-binding FKH domain and an intact leucine-zipper domain, which mediates homo-multimerization of FOXP3, are required for DNA binding, whereas the zinc-finger domain is dispensable [238, 239]. Thus, the molecular basis by which the zinc finger domain might influence FOXP3- regulated gene transcription and Treg suppressive function (if indeed it does) is still to be elucidated.

Figure 4: Cartoon representation of the FOXP3 protein, which contains a large (∼181 aa) amino-terminal repressor domain region, required for transcriptional activation and repression, a central C2H2 zinc-finger domain, to which no specific function has yet been ascribed, a leucine-zipper domain implicated in multimer formation and suppressor function, and a C-terminal forkhead (FKH) domain that mediates DNA-binding by FOX proteins. FOXP3 is preferentially expressed in T regulatory (Treg) cells and is critical for their immunosuppressive function.

Much of our knowledge of the pathogenesis of human chorio-retinal inflammation has derived from animal models. Experimental autoimmune uveitis (EAU) is induced by immunising animals, most commonly, rodents, with a retinal antigen (Ag), such as interphotoreceptor retinoid binding protein (IRBP) or retinal soluble Ag (S-Ag) [233, 240] (Figure 5).

Figure 5: Cross-sectional histological specimens of murine retina ([241]; reproduced with permission), demonstrating the anatomical cellular organization of the retina/RPE/choroid complex as previously shown in Figure 1. Figure 5(a) shows normal murine retina with organised anatomical cellular layers, whereas Figure 5(b) depicts inflamed retina from a murine model of EAU, demonstrating dark pink subretinal deposits/infiltrate originating in the choroid layer, with disruption of the RPE layer and overall disorganisation of retinal anatomical layers.
These retinal Ags are involved in the visual cycle and are typically unique to the eye, therefore serving as targets for the immune system in EAU. The blood-retinal barrier can be an effective barrier to small molecules but it is not a very effective barrier to cells. For example, circulating IFN-γ producing Th1 cells and monocytes, which have been activated systemically, are able to cross the blood vessels and penetrate the barrier in the context of inflammation [242, 243]. When previously ‘unknown’ retinal Ag are exposed to the immune system, a break in immune tolerance may occur and then an inflammatory response ensues.

Inflammation in EAU has been shown to be mediated by the Th1 and Th17 T-cell subsets and may be suppressed by Treg [224, 225, 230-233]. A study in rats found that during resolution of the first acute attack of EAU, the number of ocular Tregs increased [244]. Interestingly, the suppressor function of Tregs was weaker in those rats who went on to develop recurrent EAU [244]. In mouse models of EAU, a significantly increased frequency and immunoregulatory action of Treg cells has been associated with the development and regression of EAU, suggesting that CD4+CD25+ Treg cells are induced during EAU and may be involved in its regression [245]. This is further supported by murine EAU evidence demonstrating that retina-specific functionally suppressive FoxP3+ Tregs accumulated in inflamed eyes and persisted for several months after disease remission [246]. Depletion of Tregs at the peak of uveitis delayed resolution and, following resolution, (when mice displayed a low grade chronic inflammation), Treg depletion precipitated disease relapse [246]. One mechanism by which zinc has been shown to influence levels of T-cell mediated inflammation is by inducing a tolerogenic dendritic cell phenotype (characterised by diminishing surface MHC class II (MHCII) and promoting programmed death–ligand (PD-L)1, PD-L2, and the tryptophan degrading enzyme, IDO), which in turn skews the Treg cell–Th17 balance against inflammation [247]. Zinc supplementation has also been found to augment T-reg induction through upregulation of FoxP3 [248] and TGF-β dependent mechanisms [248]. Interestingly, in a murine model of experimental autoimmune encephalitis (EAE), zinc administration diminished EAE scores *in vivo*, reduced Th17 RORγT+ cells and significantly increased inducible Treg cells [249]. Thus, it was suggested that zinc supplementation was capable of inducing tolerance in unwanted immune reactions by increasing Treg cell activity, and zinc has been proposed as a promising future tool for treating autoimmune inflammation, without suppressing the whole immune system [249].

Despite an increased understanding of metabolic and physiologic changes that occur in the retina with age, we still do not know the exact immunological changes that cause or drive AMD progression. In particular, there is a paucity of evidence to suggest that T-cells are a major driver or regulator of chronic inflammation in AMD. ‘Para-inflammation’ first described by Medzhitov, and summarised as ‘a tissue adaptive response to noxious stress or malfunction and has characteristics that are intermediate between basal and inflammatory states’ [250], however, has been proposed as a mechanism for AMD pathogenesis in the retina. In the aging eye, and especially at the level of the RPE, there is an accumulation of the products of oxidative stress, such as reactive oxygen species (ROS), which further drive oxidative stress, and potentially alter the metabolism and health of the RPE [251,
Sources of ROS may be physiological, for example, from the accumulation of lipofuscin fluorophore A2E, a by-product of the visual cycle generated from the phagocytosis of photoreceptor outer segments, or environmental sources of photo-oxidative stress unique to the eye, that is, UV light exposure, in addition to modifiable factors such as cigarette smoking and high dietary fat ingestion, which promote pathological inflammatory activity in response to oxidative stress in the retina [253]. Tissue-resident immune cells and supporting stromal cells, such as the RPE and microglial in the retina and choroid, are capable of controlling as well as mounting immune responses, and it is thought likely that these cells may be acting as key mediators of inflammation in the aging retina [252]. Macrophages and dendritic cells are not normally present in the retina, but reside in the underlying choroid. As in the case of uveitis, where there is a breakdown of the blood–retinal barrier, these immune cells are recruited from the underlying choroid or from the systemic circulation into the retina where they modulate disease [26]. Subretinal migration of microglia is necessary to eliminate visual by-products and to maintain vision, and their impaired migration into or out of the subretinal space promotes the death of photoreceptor cells [26]. Thus, oxidative damage may be an initial trigger for AMD and this increased physiological stress could activate the resident immune cells to further contribute to cell and tissue damage, with resultant loss of function [254]. Oxidative stress should be counterbalanced by mechanisms that attempt to return the cell to a homeostatic state. Microglia may accumulate in the subretinal space as a symptom of inflammatory damage and a beneficial response to injury, but, in AMD, this accumulation of cells and metabolic waste products in the sub-RPE space exacerbates progression of age-related degeneration [26].

Macrophages, in particular, seem to play an important role in AMD pathogenesis [194, 255]. In striking similarity to their presence at atherosclerotic blood vessel sites in cardiovascular disease, macrophages are found at the sites of RPE atrophy, breakdown of Bruch’s membrane, and choroidal neovascularization [194, 256, 257]. With the accumulation of cell damage and oxidative stress in the aging retina, macrophages may become overburdened, much like the overload of lipids in foam cells in atherosclerosis, and furthermore, the macrophage itself is undergoing aging and loss of phagocytic capacity [194]. Contrary to the observed shift of macrophages from the prototypic pro-inflammatory M1 phenotype (which engulf and digest damaged cells and produce proinflammatory factors and generate ROS) to the prototypic M2 anti-inflammatory phenotype, usually associated with aging, in AMD patients, this shift is reversed [194, 258]. In AMD, there is an increase in number of choroidal macrophages, which express complement receptor CR1g [259]. Since an important function of the complement cascade is to coat self and foreign particles with C3-proteins that serve as ligands for phagocytic receptors (opsonisation), this may reflect an attempt to clear an increased number of damaged or apoptotic cells in the aging retina, using macrophages and the complement system [260]. It is known that deficits in zinc adversely impact macrophage function, resulting in dysregulation of phagocytosis and cytokine production [261], and this supports that local availability of zinc may influence levels of retinal inflammation, driven by resident immune cells. With regard to specific cellular interactions of macrophages with zinc, it has been observed, in the context of intracellular
Histoplasma infection, that the pleiotropic cytokine, granulocyte macrophage-colony stimulating factor (GM-CSF), is capable of stimulating macrophages to upregulate expression of zinc exporters, Slc30a4 and Slc30a7, so that the zinc was shuttled away from phagosomes and into the Golgi apparatus [262]. This distinctive zinc sequestration strategy has the effect of elevating phagosomal H⁺ channel function and triggering ROS generation by NADPH oxidase [263]. It is possible that retinal parainflammation might be characterised by similar macrophage effects on zinc homeostasis and ROS generation.

Zinc and the microbiome

In addition to the direct effects of zinc on innate and adaptive immunity described previously, zinc may influence retinal inflammation indirectly via its effects on the microbiome and immune cells in the gut. It is now well recognised that the human immune system has a highly co-evolved relationship with the microbes that inhabit the human intestine, resulting in the maintenance of homeostasis between the host and resident microbes [264]. This relationship with intestinal bacteria, known as the microbiome, is shaped during development and into adulthood, and contributes to the function of the gastrointestinal immune system and play an important role in health and disease throughout life [264]. Dysbiosis, ‘an imbalance in microbiota structure and/or function that disrupts host microorganism homeostasis’, is an emerging feature of many non-communicable inflammatory diseases [265]. If retinal parainflammation develops into a chronic retinal malfunction, and constitutes a shift in the normal homeostasis or ‘balance’ to adapt to the new physiological or metabolic conditions of AMD (as occurs in many other chronic inflammatory disease states), the disruption in the interrelationships between nutrition, the microbiome and host metabolism may be key elements in the disruption of normal homeostasis. The gut microbiome composition may vary according to macro/micronutrient dietary habits, as shown in several studies [266-271]. The redox state also strongly modulates the gut microbiota [272-275]. Different bacterial taxa modulate immune functionality toward a pro or anti-inflammatory pattern, extensively summarized previously [276-278]. Thus, the composition of the microbiota community determines, in part, the level of resistance to infection and susceptibility to inflammatory diseases.

We propose that nutritional zinc and/or zinc availability in the intestine might influence systemic inflammation, and consequently, the immune cells driving local retinal inflammation, through direct interactions with gut microbiota or via effects on resident T-cells in gut mucosa. Zinc is absorbed mainly in the stomach, in the small and large intestine via diffusion- and carrier-mediated mechanism [279]. It is essential for the growth of most organisms, including numerous bacteria, which require zinc uptake systems for growth and virulence [280, 281]. Studies have showed that the microbiota have high-affinity binding and transport systems for zinc, and there is zinc competition between microbiota in the gastrointestinal tract of a host [280]. For example, it has been shown that excess dietary zinc (Zn) substantially alters the gut microbiota, which in the context of colonisation by pathogenic bacteria, such as Clostridium difficile, may exacerbate C. difficile—
associated disease by increasing toxin activity and altering the host immune response [282]. Regarding factors that may influence zinc absorption and local zinc availability, it has been shown that the presence of intestinal mucins facilitates \textit{in vitro} zinc uptake into enterocytes and act as a zinc delivery system for the intestinal epithelium [283]. In a clinical study comparing individuals with rheumatoid arthritis (RA) with healthy subjects, the redox environment, transport and metabolism of iron, sulphur, zinc and arginine were found altered in the microbiota of individuals with RA, along with detectable specific alterations in the gut and oral microbiome, including molecular mimicry of human antigens related to RA, in individuals with the disease [284]. These findings suggest that the microbiome composition could potentially be used as a tool for prognosis and diagnosis of inflammation at body sites distant to the gut.

Murine models demonstrate that intestinal RORγ\textsuperscript{T}+FoxP3\textsuperscript{+} Treg induced \textit{in vivo} by the local microbiota display a stable suppressive phenotype and exist in dynamic balance with pathogenic Th17 [285, 286]. The local microenvironment of the microbiome, which influences the cytokine milieu, has been shown to regulate the Treg/Th17 balance and influence cell plasticity; thus, disruption of this balance may lead to the development of inflammatory disease [286-288]. Since it is known that zinc deficiency may drive Th17 polarization and promote loss of Treg function [155], we suggest that interactions between zinc, T-cells and the microbiome in the gut, may have a role in influencing levels of inflammation at distant tissue sites, including the retina.

\textbf{Potential risks of zinc supplementation and future directions}

Dietary supplements are not subject to the stringent regulation of pharmaceutical drugs and can be purchased without prescription in most countries, which has led to concerns over quality, safety and potential toxicity of zinc supplements. High dose zinc dietary supplementation had been shown to interfere with absorption of dietary copper and iron [163], resulting in their deficiency. In the AREDS trial, zinc oxide was observed to be a gastrointestinal irritant and also a cause of urinary complications [289], with patients taking zinc being hospitalized more often for genitourinary complaints [183]. Furthermore, whilst zinc is an important regulator of inflammation and immune function, it can also suppress the immune system, increasing the risk for certain cancers, including metastatic prostate cancer [2].

The potential safety risks of nutrient supplementation, in addition to questions about efficacy [290, 291] in the context of AMD, have led to a renewed public health interest and research focus on components of a healthy balanced diet and dietary patterns, which provide the necessary required vitamins and micronutrients for optimal visual and body function [60]. In particular, the Mediterranean dietary pattern appears promising in reducing the risk of progression of AMD [292, 293]. In addition to the focus on increasing zinc intake through dietary supplementation in zinc deficient individuals, it is also important to optimise dietary zinc intake through improving absorption of available dietary zinc, for example, by minimising the effect of phytates on zinc absorption. Finally, it is recognized that in order for the clinical
translation of nutritional science on zinc to progress, valid, reliable and feasible biomarkers and surrogate endpoints for measurement of zinc status, such as retinal dark adaptation, will need to be developed for future clinical studies.

References

[1] Prasad, A. S., Clinical, endocrinological and biochemical effects of zinc deficiency. *Clinics in endocrinology and metabolism* 1985, 14, 567-589.
[2] Maret, W., Sandstead, H. H., Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol* 2006, 20, 3-18.
[3] Agriculture and Consumer Protection Department, F. a. O. o. t. U. N., *Human Vitamin and Mineral requirements*, 2002.
[4] Health Education Authority, *A Synopsis of Dietary Reference Values*, 1996.
[5] Age-Related Eye Disease Study 2 Research Group, Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* 2013, 309, 2005-2015.
[6] Age-Related Eye Disease Study Research Group, 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* 2001, 119, 1417-1436.
[7] Bowness, J. M., Morton, R. A., Shakir, M. H., Stubbs, A. L., Distribution of copper and zinc in mammalian eyes. Occurrence of metals in melanin fractions from eye tissues. *Biochem J* 1952, 51, 521-530.
[8] Galin, M. A., Nano, H. D., Hall, T., Ocular zinc concentration. *Invest Ophthalmol* 1962, 1, 142-148.
[9] Rodriguez-Menendez, S., Fernandez, B., Garcia, M., Alvarez, L., Luisa Fernandez, M., Sanz-Medel, A., Coca-Prados, M., Pereiro, R., Gonzalez-Iglesias, H., Quantitative study of zinc and metallothioneins in the human retina and RPE cells by mass spectrometry-based methodologies. *Talanta* 2018, 178, 222-230.
[10] Tam, S. W., Wilber, K. E., Wagner, F. W., Light sensitive zinc content of protein fractions from bovine rod outer segments. *Biochem Biophys Res Commun* 1976, 72, 302-309.
[11] Rosenthal, A., Eckhert, C., *Zinc and Copper in Medicine*, Charles C. Thomas Springfield, Il 1980, pp. 579-633.
[12] Eckhert, C. D., Elemental concentrations in ocular tissues of various species. *Exp Eye Res* 1983, 37, 639-647.
[13] Koumantakis, E., Alexiou, D., Grimanis, A., Kaskarelis, D., Bouzas, A., Zinc, cobalt and selenium concentrations in the premature and full-term newborn eye. *Ophthalmologica* 1983, 186, 41-46.
[14] Ulshafer, R. J., Allen, C. B., Rubin, M. L., Distributions of elements in the human retinal pigment epithelium. *Arch Ophthalmol* 1990, 108, 113-117.
[15] Fabe, J. S., Grahn, B. H., Paterson, P. G., Zinc concentration of selected ocular tissues in zinc-deficient rats. *Biological trace element research* 2000, 75, 43-52.
[16] Bentley, P. J., Grubb, B. R., Effects of a zinc-deficient diet on tissue zinc concentrations in rabbits. *J Anim Sci* 1991, 69, 4876-4882.
[17] Pålsgård, E., Ugarte, M., Rajta, I., Grime, G. W., The role of zinc in the dark-adapted retina studied directly using microPIXE. *7th International Conference on Nuclear Microprobe Technology and Applications; Bordeaux, France; SEP 10-15, 2000* 2001, 181, 489-492.
[18] Wills, N. K., Ramanujam, V. M., Kalariya, N., Lewis, J. R., van Kuijk, F. J., Copper and zinc distribution in the human retina: relationship to cadmium accumulation, age, and gender. Exp Eye Res 2008, 87, 80-88.
[19] Wills, N. K., Kalariya, N., Sadagopa Ramanujam, V. M., Lewis, J. R., Haji Abdollahi, S., Husain, A., van Kuijk, F. J., Human retinal cadmium accumulation as a factor in the etiology of age-related macular degeneration. Exp Eye Res 2009, 89, 79-87.
[20] Erie, J. C., Good, J. A., Butz, J. A., Pulido, J. S., Reduced zinc and copper in the retinal pigment epithelium and choroid in age-related macular degeneration. Am J Ophthalmol 2009, 147, 276-282 e271.
[21] Karcioğlu, Z. A., Zinc in the eye. Surv Ophthalmol 1982, 27, 114-122.
[22] Grahn, B. H., Paterson, P. G., Gottschall-Pass, K. T., Zhang, Z., Zinc and the eye. J Am Coll Nutr 2001, 20, 106-118.
[23] Kolb, H., Fernandez, E., Nelson, R., Webvision: the organization of the retina and visual system. 2007.
[24] Hosoya, K., Tachikawa, M., Inner blood-retinal barrier transporters: role of retinal drug delivery. Pharm Res 2009, 26, 2055-2065.
[25] Cunha-Vaz, J. G., The blood-retinal barriers. Doc Ophthalmol 1976, 41, 287-327.
[26] Ambati, J., Atkinson, J. P., Gelfand, B. D., Immunology of age-related macular degeneration. Nature reviews. Immunology. 2013, 13, 438-451.
[27] Maret, W., Li, Y., Coordination dynamics of zinc in proteins. Chemical reviews 2009, 109, 4682-4707.
[28] McCall, K. A., Huang, C., Fierke, C. A., Function and mechanism of zinc metalloenzymes. J Nutr 2000, 130, 1437s-1446s.
[29] Cousins, R. J., in: Bowman, B. a. a. R., R. M. (Ed.), Present Knowledge in Nutrition, International Life Science Institute (ILSI) Press, DoH (Department of Health), Washington DC, USA 2006, pp. 225-457.
[30] Ha, N., Hellauer, K., Turcotte, B., Mutations in target DNA elements of yeast HAP1 modulate its transcriptional activity without affecting DNA binding. Nucleic Acids Res 1996, 24, 1453-1459.
[31] Beyersmann, D., Haase, H., Functions of zinc in signaling, proliferation and differentiation of mammalian cells. Biometals: an international journal on the role of metal ions in biology, biochemistry, and medicine 2001, 14, 331-341.
[32] Tate, D. J., Jr., Miceli, M. V., Newsome, D. A., Zinc protects against oxidative damage in cultured human retinal pigment epithelial cells. Free radical biology & medicine 1999, 26, 704-713.
[33] Henderson, L. M., Chappell, J. B., Jones, O. T., Superoxide generation by the electrogenic NADPH oxidase of human neutrophils is limited by the movement of a compensating charge. Biochem J 1988, 255, 285-290.
[34] Musset, B., Decoursey, T., Biophysical properties of the voltage gated proton channel H(V)1. Wiley interdisciplinary reviews. Membrane transport and signaling 2012, 1, 605-620.
[35] Cousins, R. J., Liuzzi, J. P., Lichten, L. A., Mammalian zinc transport, trafficking, and signals. J Biol Chem 2006, 281, 24085-24089.
[36] Cassandri, M., Smirnov, A., Novelli, F., Pitoli, C., Agostini, M., Malewicz, M., Melino, G., Raschellà, G., Zinc-finger proteins in health and disease. Cell Death Discovery 2017, 3, 17071.
[37] Prasad, A. S., Effects of zinc deficiency on Th1 and Th2 cytokine shifts. J Infect Dis 2000, 182 Suppl 1, S62-68.
[38] Ibs, K. H., Rink, L., Zinc-altered immune function. J Nutr 2003, 133, 1452S-1456S.
[39] Bogden, J. D., Influence of zinc on immunity in the elderly. J Nutr Health Aging 2004, 8, 48-54.
[40] Mochegiani, E., Giaconia, R., Cipriano, C., Malavolta, M., NK and NKT cells in aging and longevity: role of zinc and metallothioneins. Journal of clinical immunology 2009, 29, 416-425.
[41] Rich, R. R., in: Rich, R. R. (Ed.), Clinical Immunology: Principles and Practice, Elsevier Saunders 2013.
[42] Prasad, A. S., Zinc: mechanisms of host defense. J Nutr 2007, 137, 1345-1349.
[43] Fraker, P. J., Roles for cell death in zinc deficiency. J Nutr 2005, 135, 359-362.
[44] Haase, H., Hebel, S., Engelhardt, G., Rink, L., Zinc ions cause the thimerosal-induced signal of fluorescent calcium probes in lymphocytes. Cell Calcium 2009, 45, 185-191.
[45] Scientific Opinion on the substantiation of health claims related to zinc and function of the immune system (ID 291, 1757), DNA synthesis and cell division (ID 292, 1759), protection of DNA, proteins and lipids from oxidative damage (ID 294, 1758), maintenance of bone (ID 295, 1756), cognitive function (ID 296), fertility and reproduction (ID 297, 300), reproductive development (ID 298), muscle function (ID 299), metabolism of fatty acids (ID 302), maintenance of joints (ID 305), function of the heart and blood vessels (ID 306), prostate function (ID 307), thyroid function (ID 308), acid-base metabolism (ID 360), vitamin A metabolism (ID 361) and maintenance of vision (ID 361) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. 2009, 7, 1229.
[46] Watabe, Y., Baba, Y., Nakauchi, H., Mizota, A., Watanabe, S., The role of Zic family zinc finger transcription factors in the proliferation and differentiation of retinal progenitor cells. Biochem Biophys Res Commun 2011, 415, 42-47.
[47] Schippert, R., Burkhardt, E., Feldkaemper, M., Schaeffel, F., Relative axial myopia in Egr-1 (ZENK) knockout mice. Invest Ophthalmol Vis Sci 2007, 48, 11-17.
[48] Zhang, J., Jin, Z., Bao, Z. Z., Disruption of gradient expression of Zic3 resulted in abnormal intra-retinal axon projection. Development 2004, 131, 1553-1562.
[49] Herrera, E., Brown, L., Aruga, J., Rachel, R. A., Dolen, G., Mikoshiba, K., Brown, S., Mason, C. A., Zic2 patterns binocular vision by specifying the uncrossed retinal projection. Cell 2003, 114, 545-557.
[50] Shi, Y., Li, Y., Zhang, D., Zhang, H., Li, Y., Lu, F., Liu, X., He, F., Gong, B., Cai, L., Li, R., Liao, S., Ma, S., Lin, H., Cheng, J., Zheng, H., Shan, Y., Chen, B., Hu, J., Jin, X., Zhao, P., Chen, Y., Zhang, Y., Lin, Y., Li, X., Fan, Y., Yang, H., Wang, J., Yang, Z., Exome sequencing identifies ZNF644 mutations in high myopia. PLoS Genet 2011, 7, e1002084.
[51] Collin, R. W., Nikopoulos, K., Dona, M., Glissen, C., Hoischen, A., Boonstra, F. N., Poultter, J. A., Kondo, H., Berger, W., Toomes, C., Tahira, T., Mohn, L. R., Blokland, E. A., Hetterschijt, L., Ali, M., Groothuismink, J. M., Duijkers, L., Inglehearn, C. F., Sollfrank, L., Strom, T. M., Uchio, E., van Nouhuys, C. E., Kremer, H., Veltman, J. A., van Wijk, E., Cremers, F. P., ZNF408 is mutated in familial exudative vitreoretinopathy and is crucial for the development of zebrafish retinal vasculature. Proc Natl Acad Sci U S A 2013, 110, 9856-9861.
[52] de Melo, J., Peng, G. H., Chen, S., Blackshaw, S., The Spalt family transcription factor Sall3 regulates the development of cone photoreceptors and retinal horizontal interneurons. Development 2011, 138, 2325-2336.
[53] Dufner-Beattie, J., Weaver, B. P., Geiser, J., Bilgen, M., Larson, M., Xu, W., Andrews, G. K., The mouse acrodermatitis enteropathica gene Slc39a4 (Zip4) is essential for early development and heterozygosity causes hypersensitivity to zinc deficiency. Hum Mol Genet 2007, 16, 1391-1399.
[54] Fukada, T., Civic, N., Furuchi, T., Shimoda, S., Mishima, K., Higashiyama, H., Iida, Y., Asada, Y., Kitamura, H., Yamasaki, S., Hojo, S., Nakayama, M., Ohara, O., Koseki, H., Dos Santos, H. G., Bonafe, L., Ha-Vinh, R., Zankl, A., Unger, S., Kraenzlin, M. E., Beckmann, J. S., Saito, I., Rivolta, C., Ikegawa, S., Superti-Furga, A., Hirano, T., The zinc transporter SLC39A13/ZIP13 is required for connective tissue development; its involvement in BMP/TGF-beta signaling pathways. PLoS One 2008, 3, e3642.
[55] Silverstone, B. Z., Berson, D., Seelenfreund, M. H., Plasma zinc levels in high myopia and retinitis pigmentosa. Metabolic and pediatric ophthalmology 1981, 5, 187-190.
[56] Morrison, S. A., Russell, R. M., Carney, E. A., Oaks, E. V., Zinc deficiency: a cause of abnormal dark adaptation in cirrhotics. The American journal of clinical nutrition 1978, 31, 276-281.
[57] Newsome, D. A., Swartz, M., Leone, N. C., Elston, R. C., Miller, E., Oral zinc in macular degeneration. Arch Ophthalmol 1988, 106, 192-198.
[58] Newsome, D. A., A randomized, prospective, placebo-controlled clinical trial of a novel zinc-monocysteine compound in age-related macular degeneration. Curr Eye Res 2008, 33, 591-598.
[59] Chew, E. Y., Clemons, T. E., Agron, E., Sperduto, R. D., Sangiovanni, J. P., Kurinij, N., Davis, M. D., Long-term effects of vitamins C and E, beta-carotene, and zinc on age-related macular degeneration: AREDS report no. 35. *Ophthalmology* 2013, 120, 1604-1611.e1604.

[60] van Leeuwen, R., Boekhoorn, S., Vingerling, J. R., Witteman, J. C., Klaver, C. C., Hofman, A., de Jong, P. T., Dietary intake of antioxidants and risk of age-related macular degeneration. *Jama* 2005, 294, 3101-3107.

[61] Tan, J. S., Wang, J. J., Flood, V., Rochtchina, E., Smith, W., Mitchell, P., Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology* 2008, 115, 334-341.

[62] Gopinath, B., Liew, G., Russell, J., Cosatto, V., Burlutsky, G., Mitchell, P., Intake of key micronutrients and food groups in patients with late-stage age-related macular degeneration compared with age-sex-matched controls. *Br J Ophthalmol* 2017, 101, 1027-1031.

[63] Smailhodzic, D., van Asten, F., Blom, A. M., Mohlin, F. C., den Hollander, A. I., van de Ven, J. P., van Huet, R. A., Groenewoud, J. M., Tian, Y., Berendschot, T. T., Lechanteur, Y. T., Fauser, S., de Bruijn, C., Daha, M. R., van der Wilt, G. J., Hoing, C. B., Klevering, B. J., Zinc supplementation inhibits complement activation in age-related macular degeneration. *PloS one* 2014, 9, e112682.

[64] Siapich, S. A., Wrubel, H., Albanna, W., Alnawaiseh, M., Hescheler, J., Weiерgraber, M., Luke, M., Schneider, T., Effect of ZnCl2 and chelation of zinc ions by N,N-diethylthiocarbamate (DEDTC) on the ERG b-wave amplitude from the isolated superfused vertebrate retina. *Curr Eye Res* 2010, 35, 322-334.

[65] Chappell, R. L., Anastassov, I., Lugo, P., Ripps, H., Zinc-mediated feedback at the synaptic terminals of vertebrate photoreceptors. *Exp Eye Res* 2008, 87, 394-397.

[66] Redenti, S., Chappell, R. L., Zinc chelation enhances the sensitivity of the ERG b-wave in dark-adapted skate retina. *Biol Bull* 2003, 205, 213-224.

[67] Redenti, S., Chappell, R. L., Zinc chelation enhances the zebrafish retinal ERG b-wave. *Biol Bull* 2002, 203, 200-202.

[68] Chappell, R. L., Redenti, S., Endogenous zinc as a neuromodulator in vertebrate retina: evidence from the retinal slice. *The Biological bulletin* 2001, 201, 265-267.

[69] Zhang, P. B., Xiao, H. Y., Liu, H., Qin, R., Regulation of zinc transporter 3 and carbonic anhydrases 2 and 14 mRNA expression in the retina of rats affected by low dietary zinc. *Genet Mol Res* 2014, 13, 963-971.

[70] Wong, P., Markey, M., Rapp, C. M., Darrow, R. M., Ziesel, A., Organisciak, D. T., Enhancing the efficacy of AREDS antioxidants in light-induced retinal degeneration. *Mol Vis* 2017, 23, 718-739.

[71] Rosenstein, F. J., Chappell, R. L., Endogenous zinc as a retinal neuromodulator: evidence from the skate (Raja erinacea). *Neurosci Lett* 2003, 345, 81-84.

[72] Zhang, D. Q., Ribelayga, C., Mangel, S. C., McMahon, D. G., Suppression by zinc of AMPA receptor-mediated synaptic transmission in the retina. *J Neurophysiol* 2002, 88, 1245-1251.

[73] Sun, Z., Zhang, D. Q., McMahon, D. G., Zinc modulation of hemi-gap-junction channel currents in retinal horizontal cells. *J Neurophysiol* 2009, 101, 1774-1780.

[74] Redenti, S., Chappell, R. L., Neuroimaging of zinc released by depolarization of rat retinal cells. *Vision research* 2005, 45, 3520-3525.

[75] Zhang, D. Q., Sun, Z., McMahon, D. G., Modulation of A-type potassium currents in retinal horizontal cells by extracellular calcium and zinc. *Vis Neurosci* 2006, 23, 825-832.

[76] Luo, D. G., Yang, X. L., Zn2+ differentially modulates signals from red- and short wavelength-sensitive cones to horizontal cells in carp retina. *Brain Res* 2001, 900, 95-102.

[77] Luo, D. G., Li, G. L., Yang, X. L., Zn2+ modulates light responses of color-opponent bipolar and amacrine cells in the carp retina. *Brain Res Bull* 2002, 58, 461-468.

[78] Kaneda, M., Ishii, K., Akagi, T., Tatsukawa, T., Hashikawa, T., Endogenous zinc can be a modulator of glycinerergic signaling pathway in the rat retina. *Journal of molecular histology* 2005, 36, 179-185.
[81] Anastassov, I., Shen, W., Ripps, H., Chappell, R. L., Zinc modulation of calcium activity at the photoreceptor terminal: a calcium imaging study. Exp Eye Res 2013, 112, 37-44.

[82] Bernard, M., Voisin, P., Photoreceptor-specific expression, light-dependent localization, and transcriptional targets of the zinc-finger protein Yin Yang 1 in the chicken retina. J Neurochem 2008, 105, 595-604.

[83] Anastassov, I., Ripps, H., Chappell, R. L., Cytoprotection by endogenous zinc in the vertebrate retina. J Neurochem 2014, 129, 249-255.

[84] Stojanovic, A., Stitham, J., Hwa, J., Critical role of transmembrane segment zinc binding in the structure and function of rhodopsin. J Biol Chem 2004, 279, 35932-35941.

[85] Toledo, D., Cordoni, A., Proietti, M. G., Benfatto, M., del Valle, L. J., Perez, J. J., Garriga, P., Sepulcre, F., Structural characterization of a zinc high-affinity binding site in rhodopsin. Photochemistry and photobiology 2009, 85, 479-484.

[86] Gleim, S., Stojanovic, A., Arehart, E., Byington, D., Hwa, J., Conserved rhodopsin intradiscal structural motifs mediate stabilization: effects of zinc. Biochemistry 2009, 48, 1793-1800.

[87] del Valle, L. J., Ramon, E., Canavate, X., Dias, P., Garriga, P., Zinc-induced decrease of the thermal stability and regeneration of rhodopsin. J Biol Chem 2003, 278, 4719-4724.

[88] Olsson, J. E., Gordon, J. W., Pawlyk, B. S., Roof, D., Hayes, A., Molday, R. S., Mukai, S., Cowley, G. S., Berson, E. L., Dryja, T. P., Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. Neuron 1992, 9, 815-830.

[89] Otteson, D. C., Liu, Y., Lai, H., Wang, C., Gray, S., Jain, M. K., Zack, D. J., Kruppel-like factor 15, a zinc-finger transcriptional regulator, represses the rhodopsin and interphotoreceptor retinoid-binding protein promoters. Invest Ophthalmol Vis Sci 2004, 45, 2522-2530.

[90] Newsome, D. A., Oliver, P. D., Deupree, D. M., Miceli, M. V., Diamond, J. G., Zinc uptake by primate retinal pigment epithelium and choroid. Curr Eye Res 1992, 11, 213-217.

[91] Julien, S., Biesemeier, A., Kokkinou, D., Eibl, O., Schraermeyer, U., Zinc deficiency leads to lipofuscin accumulation in the retinal pigment epithelium of pigmented rats. PLoS One 2011, 6, e29245.

[92] Furumura, M., Solano, F., Matsunaga, N., Sakai, C., Spritz, R. A., Hearing, V. J., Metal ligand-binding specificities of the tyrosinase-related proteins. Biochem Biophys Res Commun 1998, 242, 579-585.

[93] Ha, K. N., Chen, Y., Cai, J., Sternberg, P., Jr., Increased glutathione synthesis through an ARE-Nrf2-dependent pathway by zinc in the RPE: implication for protection against oxidative stress. Invest Ophthalmol Vis Sci 2006, 47, 2709-2715.

[94] Rezaei, K. A., Chen, Y., Cai, J., Sternberg, P., Modulation of Nrf2-dependent antioxidant functions in the RPE by Zip2, a zinc transporter protein. Invest Ophthalmol Vis Sci 2008, 49, 1665-1670.

[95] Kennedy, C. J., Rakocy, P. E., Robertson, T. A., Papadimitriou, J. M., Constable, I. J., Kinetic studies on phagocytosis and lysosomal digestion of rod outer segments by human retinal pigment epithelial cells in vitro. Exp Cell Res 1994, 210, 209-214.

[96] Rajapakse, D., Curtis, T., Chen, M., Xu, H., Zinc Protects Oxidative Stress-Induced RPE Death by Reducing Mitochondrial Damage and Preventing Lysosome Rupture. Oxid Med Cell Longev 2017, 2017, 6926485.

[97] Schraermeyer, U., Heimann, K., Current understanding on the role of retinal pigment epithelium and its pigmentation. Pigment Res 1999, 12, 219-236.

[98] Seo, B. R., Lee, S. J., Cho, K. S., Yoon, Y. H., Koh, J. Y., The zinc ionophore cloquioquin reverses autophagy arrest in chloroquine-treated ARPE-19 cells and in APP/mutant presenilin-1-transfected Chinese hamster ovary cells. Neurobiol Aging 2015, 36, 3228-3238.
Hyun, H. J., Sohn, J. H., Ha, D. W., Ahn, Y. H., Koh, J. Y., Yoon, Y. H., Depletion of intracellular zinc and copper with TPEN results in apoptosis of cultured human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 2001, 42, 460-465.

Ames, B. N., Micronutrient deficiencies. A major cause of DNA damage. *Ann N Y Acad Sci* 1999, 889, 87-106.

Pao, P. J., Emri, E., Abdirahman, S. B., Soorma, T., Zeng, H. H., Hauck, S. M., Thompson, R. B., Lengyel, I., The effects of zinc supplementation on primary human retinal pigment epithelium. *J Trace Elem Med Biol* 2018, 49, 184-191.

Li, H., Wang, H., Wang, F., Gu, Q., Xu, X., Snail involves in the transforming growth factor beta1-mediated epithelial-mesenchymal transition of retinal pigment epithelial cells. *PLoS One* 2011, 6, e23322.

Lengyel, I., Flinn, J. M., Peto, T., Linkous, D. H., Cano, K., Bird, A. C., Lanziriotti, A., Frederickson, C. J., van Kuijk, F. J., High concentration of zinc in sub-retinal pigment epithelial deposits. *Exp Eye Res* 2007, 84, 772-780.

Zeng, S., Hernandez, J., Mullins, R. F., Effects of antioxidant components of AREDS vitamins and zinc ions on endothelial cell activation: implications for macular degeneration. *Invest Ophthalmol Vis Sci* 2012, 53, 1041-1047.

Rink, L., *Zinc in Human Health*, IOS Press 2011.

Krezel, A., Maret, W., Zinc-buffering capacity of a eukaryotic cell at physiological pZn. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry* 2006, 11, 1049-1062.

Wu, S. M., Qiao, X., Noebels, J. L., Localization and modulatory actions of zinc in vertebrate retina. *Vision research* 1993, 33, 2611-2616.

Akagi, T., Kaneda, M., Ishii, K., Hashikawa, T., Differential subcellular localization of zinc in the rat retina. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* 2001, 49, 87-96.

Ugarte, M., Osborne, N. N., Zinc in the retina. *Prog Neurobiol* 2001, 64, 219-249.

Wang, X., Wang, Z. Y., Gao, H. L., Danscher, G., Huang, L., Localization of ZnT7 and zinc ions in mouse retina--immunohistochemistry and selenium autometallography. *Brain research bulletin* 2006, 71, 91-96.

 Hirayama, Y., Histochemical localization of zinc and copper in rat ocular tissues. *Acta histochemica* 1990, 89, 107-111.

Ugarte, M., Osborne, N. N., The localization of endogenous zinc and the in vitro effect of exogenous zinc on the GABA immunoreactivity and formation of reactive oxygen species in the retina. *General pharmacology* 1998, 30, 297-303.

Ugarte, M., Osborne, N. N., The localization of free zinc varies in rat photoreceptors during light and dark adaptation. *Exp Eye Res* 1999, 69, 459-461.

Maret, W., Zinc and sulfur: a critical biological partnership. *Biochemistry* 2004, 43, 3301-3309.

Kochanczyk, T., Drozd, A., Krezel, A., Relationship between the architecture of zinc coordination and zinc binding affinity in proteins--insights into zinc regulation. *Metallomics : integrated biometal science* 2015, 7, 244-257.

Maret, W., Metallothionein redox biology in the cytoprotective and cytotoxic functions of zinc. *Experimental gerontology* 2008, 43, 363-369.

Colvin, R. A., Holmes, W. R., Fontaine, C. P., Maret, W., Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. *Metallomics* 2010, 2, 306-317.

Maret, W., Metallomics: The Science of Biometals and Biometalloids. *Adv Exp Med Biol* 2018, 1055, 1-20.

Liuzzi, J. P., Bobo, J. A., Lichten, L. A., Samuelson, D. A., Cousins, R. J., Responsive transporter genes within the murine intestinal-pancreatic axis form a basis of zinc homeostasis. *Proc Natl Acad Sci U S A* 2004, 101, 14355-14360.
[120] Fukada, T., Kambe, T., Molecular and genetic features of zinc transporters in physiology and pathogenesis. Metallomics : integrated biometal science 2011, 3, 662-674.
[121] Lichten, L. A., Cousins, R. J., Mammalian zinc transporters: nutritional and physiologic regulation. Annual review of nutrition 2009, 29, 153-176.
[122] Gaither, L. A., Eide, D. J., Eukaryotic zinc transporters and their regulation. Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine 2001, 14, 251-270.
[123] Potocki, S., Rowinska-Zyrek, M., Valensin, D., Krzywoszynska, K., Witkowska, D., Luczkowski, M., Kozlowski, H., Metal binding ability of cysteine-rich peptide domain of ZIP13 Zn2+ ions transporter. Inorg Chem 2011, 50, 6135-6145.
[124] Alvarez, L., Gonzalez-Iglesias, H., Garcia, M., Ghosh, S., Sanz-Medel, A., Coca-Prados, M., The stoichiometric transition from Zn6Cu1-metallothionein to Zn7-metallothionein underlies the up-regulation of metallothionein (MT) expression: quantitative analysis of MT-metal load in eye cells. J Biol Chem 2012, 287, 28456-28469.
[125] Maret, W., Cellular zinc and redox states converge in the metallothionein/thionein pair. J Nutr 2003, 133, 1460s-1462s.
[126] Kambe, T., Tsuji, T., Hashimoto, A., Itsumura, N., The Physiological, Biochemical, and Molecular Roles of Zinc Transporters in Zinc Homeostasis and Metabolism. Physiological reviews 2015, 95, 749-784.
[127] Gonzalez-Iglesias, H., Alvarez, L., Garcia, M., Pettrash, C., Sanz-Medel, A., Coca-Prados, M., Metallothioneins (MTs) in the human eye: a perspective article on the zinc-MT redox cycle. Metallomics : integrated biometal science 2014, 6, 201-208.
[128] Bellomo, E., Birla Singh, K., Massarotti, A., Hogstrand, C., Maret, W., The metal face of protein tyrosine phosphatase 1B. Coordination chemistry reviews 2016, 327-328, 70-83.
[129] Laity, J. H., Andrews, G. K., Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). Arch Biochem Biophys 2007, 463, 201-210.
[130] Paolletti, P., Ascher, P., Neyton, J., High-affinity zinc inhibition of NMDA NR1-NR2A receptors. J Neurosci 1997, 17, 5711-5725.
[131] Qian, H., Li, L., Chappell, R. L., Ripps, H., GABA receptors of bipolar cells from the skate retina: actions of zinc on GABA-mediated membrane currents. Journal of neurophysiology 1997, 78, 2402-2412.
[132] Sun, Y., Zhang, Y. T., Gong, H. Q., Liang, P. J., The mechanisms of Zn2+ effects on Ca2+-permeable AMPA receptors on carp retinal horizontal cells. Brain Res 2010, 1345, 103-109.
[133] Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., Kurosaki, T., Yamashita, S., Tokunaga, M., Nishida, K., Hirano, T., Zinc is a novel intracellular second messenger. J Cell Biol 2007, 177, 637-645.
[134] Hogstrand, C., Kille, P., Nicholson, R. I., Taylor, K. M., Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. Trends in molecular medicine 2009, 15, 101-111.
[135] Hershfinkel, M., Moran, A., Grossman, N., Sekler, I., A zinc-sensing receptor triggers the release of intracellular Ca2+ and regulates ion transport. Proc Natl Acad Sci U S A 2001, 98, 11749-11754.
[136] Bettger, W. J., Zinc and selenium, site-specific versus general antioxidation. Canadian journal of physiology and pharmacology 1993, 71, 721-724.
[137] Oomen, H. A., Vitamin A deficiency, xerophthalmia and blindness. Nutrition reviews 1974, 32, 161-166.
[138] Kagi, J. H., Vallee, B. L., The role of zinc in alcohol dehydrogenase. V. The effect of metal-binding agents on the structure of the yeast alcohol dehydrogenase molecule. J Biol Chem 1960, 235, 3188-3192.
[139] Auld, D. S., Bergman, T., Medium- and short-chain dehydrogenase/reductase gene and protein families : The role of zinc for alcohol dehydrogenase structure and function. Cellular and molecular life sciences : CMLS 2008, 65, 3961-3970.
[140] Morrison, S. A., Russell, R. A., Carney, E. A., Oaks, E. V., Zinc deficiency: A cause of abnormal dark adaptation in cirrhotics. Am. J. Clin. Nutr, 1978, 276-281.
[141] Solomons, N. W., Zinc and copper in human nutrition. Prog Clin Biol Res 1981, 67, 97-127.
[142] van Kuijk, F., McPherson, S. W., Roehrich, H., Enhanced Detection of Sub-Retinal Pigment Epithelial Cell Layer Deposits in Human and Murine Tissue: Imaging Zinc as a Biomarker for Age-Related Macular Degeneration (An American Ophthalmological Society Thesis). Trans Am Ophthalmol Soc 2017, 115, T3.
[143] Flinn, J. M., Kakalec, P., Tappero, R., Jones, B., Lengyel, I., Correlations in distribution and concentration of calcium, copper and iron with zinc in isolated extracellular deposits associated with age-related macular degeneration. Metallomics : integrated biometal science 2014, 6, 1223-1228.
[144] Kokkinou, D., Kasper, H. U., Bartz-Schmidt, K. U., Schraermeyer, U., The pigmentation of human iris influences the uptake and storing of zinc. Pigment cell research 2004, 17, 515-518.
[145] Tate, D. J., Newsome, D. A., A novel zinc compound (zinc monocysteine) enhances the antioxidant capacity of human retinal pigment epithelial cells. Curr Eye Res 2006, 31, 675-683.
[146] Ulshafer, R. J., Zinc content in melanosomes of degenerating RPE as measured by X-ray mapping. Prog Clin Biol Res 1989, 314, 131-139.
[147] Biesemeier, A., Julien, S., Kokkinou, D., Schraermeyer, U., Ebil, O., A low zinc diet leads to loss of Zn in melanosomes of the RPE but not in melanosomes of the choroidal melanocytes. Metallomics 2012, 4, 323-332.
[148] van der Schaft, T. L., de Bruijn, W. C., Mooy, C. M., Ketelaars, D. A., de Jong, P. T., Element analysis of the early stages of age-related macular degeneration. Arch Ophthalmol 1992, 110, 389-394.
[149] Kokkinou, D., Kasper, H. U., Schwarz, T., Bartz-Schmidt, K. U., Schraermeyer, U., Zinc uptake and storage: the role of fundus pigmentation. Graefes Arch Clin Exp Ophthalmol 2005, 243, 1050-1055.
[150] Chen, Y., Saari, J. C., Noy, N., Interactions of all-trans-retinol and long-chain fatty acids with interphotoreceptor retinoid-binding protein. Biochemistry 1993, 32, 11311-11318.
[151] Nan, R., Gor, J., Lengyel, I., Perkins, S. J., Uncontrolled zinc- and copper-induced oligomerisation of the human complement regulator factor H and its possible implications for function and disease. J Mol Biol 2008, 384, 1341-1352.
[152] Hussain, A. A., Lee, Y., Marshall, J., High molecular-weight gelatinase species of human Bruch’s membrane: compositional analyses and age-related changes. Invest Ophthalmol Vis Sci 2010, 51, 2363-2371.
[153] Rao, N. A., Mechanisms of inflammatory response in sympathetic ophthalmia and VKH syndrome. Eye (Lond) 1997, 11 ( Pt 2), 213-216.
[154] Sugita, S., Takase, H., Taguchi, C., Imai, Y., Kamoi, K., Kawaguchi, T., Sugamoto, Y., Futagami, Y., Itoh, K., Mochizuki, M., Ocular infiltrating CD4+ T cells from patients with Vogt-Koyanagi-Harada disease recognize human melanocyte antigens. Invest Ophthalmol Vis Sci 2006, 47, 2547-2554.
[155] Kulik, L., Maywald, M., Kloubert, V., Wessels, I., Rink, L., Zinc deficiency drives Th17 polarization and promotes loss of Treg cell function. The Journal of nutritional biochemistry 2018, 63, 11-18.
[156] Prasad, A. S., Impact of the discovery of human zinc deficiency on health. J Trace Elem Med Biol 2014, 28, 357-363.
[157] King, J. C., Zinc: an essential but elusive nutrient. The American journal of clinical nutrition 2011, 94, 679S-684S.
[158] Prasad, A. S., Halsted, J. A., Nadimi, M., Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med 1961, 532-546.
[159] Warth, J. A., Prasad, A. S., Zwas, F., Frank, R. N., Abnormal dark adaptation in sickle cell anemia. J Lab Clin Med 1981, 98, 189-194.
[160] Mahajan, S. K., Prasad, A., Brewer, G. J., Zwas, F. Lee, D. Y., Effect of changes in dietary zinc intake on taste acuity and normal dark adaptation in normal human subjects. J. Trace Elem. Exp. Med. 1992, 33-45.
[161] Castro, P. L., Dominguez, D., Jose Caballero, M., Izquierdo, M., Histochemical localization of zinc in the retina cells of gilthead sea bream (sparus aurata) fed different presentations of zinc. Synapse (New York, N.Y.) 2017, 71.
[162] Prasad, A. S., Zinc deficiency in women, infants and children. J Am Coll Nutr 1996, 15, 113-120.
[163] Lonnerdal, B., Dietary factors influencing zinc absorption. J Nutr 2000, 130, 1378s-1383s.
[164] Prasad, A. S., Zinc: An antioxidant and anti-inflammatory agent: Role of zinc in degenerative disorders of aging. Journal of Trace Elements in Medicine and Biology 2014, 28, 364-371.
[165] Bird, A. C., The Bowman lecture. Towards an understanding of age-related macular disease. Eye (Lond) 2003, 17, 457-466.
[166] Wormald, R., Evans, J., Registration of blind and partially sighted people. Br J Ophthalmol 1994, 78, 733-734.
[167] Leibowitz, H. M., Krueger, D. E., Maunder, L. R., Milton, R. C., Kini, M. M., Kahn, H. A., Nickerson, R. J., Pool, J., Colton, T. L., Ganley, J. P., Loewenstein, J. I., Dawber, T. R., The Framingham Eye Study monograph: An ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. Surv Ophthalmol 1980, 24, 335-610.
[168] Pauleikhoff, D., Chen, J., Bird, A. C., Wessing, A., [The Bruch membrane and choroid. Angiography and functional characteristics in age-related changes]. Ophthalmologe 1992, 89, 39-44.
[169] Bird, A. C., Bressler, N. M., Bressler, S. B., Chisholm, I. H., Coscas, G., Davis, M. D., de Jong, P. T., Klaver, C. C., Klein, B. E., Klein, R., et al., An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. Surv Ophthalmol 1995, 39, 367-374.
[170] Hageman, G. S., Mullins, R. F., Molecular composition of drusen as related to substructural phenotype. Mol Vis 1999, 5, 28.
[171] Lengyel, I., Tufail, A., Hosaini, H. A., Luthert, P., Bird, A. C., Jeffery, G., Association of drusen deposition with choroidal intercapillary pillars in the aging human eye. Invest Ophthalmol Vis Sci 2004, 45, 2886-2892.
[172] Spaide, R. F., Curcio, C. A., Drusen characterization with multimodal imaging. Retina 2010, 30, 1441-1454.
[173] Crabb, J. W., Miyagi, M., Gu, X., Shadrach, K., West, K. A., Sakaguchi, H., Kamei, M., Hasan, A., Yan, L., Rayborn, M. E., Salomon, R. G., Hollyfield, J. G., Drusen proteome analysis: an approach to the etiology of age-related macular degeneration. Proc Natl Acad Sci U S A 2002, 99, 14682-14687.
[174] Boocj, J. C., Baas, D. C., Beisekseeva, J., Gorgels, T. G., Bergen, A. A., The dynamic nature of Bruch's membrane. Prog Retin Eye Res 2010, 29, 1-18.
[175] Balaratnasingam, C., Hoang, Q. V., Inoue, M., Curcio, C. A., Dolz-Marco, R., Yannuzzi, N. A., Dhrami-Gavazi, E., Yannuzzi, L. A., Freund, K. B., Clinical Characteristics, Choroidal Neovascularization, and Predictors of Visual Outcomes in Acquired Vitelliform Lesions. Am J Ophthalmol 2016, 172, 28-38.
[176] Curcio, C. A., Millican, C. L., Basal linear deposit and large drusen are specific for early age-related maculopathy. Arch Ophthalmal 1999, 117, 329-339.
[177] Ferris, F. L., 3rd, Wilkinson, C. P., Bird, A., Chakravarthy, U., Chew, E., Csaky, K., Sadda, S. R., Clinical classification of age-related macular degeneration. Ophthalmology 2013, 120, 844-851.
[178] Donati, G., Emerging therapies for neovascular age-related macular degeneration: state of the art. Ophthalmologica 2007, 221, 366-377.
[179] Leung, K. W., Gvritshvili, A., Liu, Y., Tombran-Tink, J., ZIP2 and ZIP4 mediate age-related zinc fluxes across the retinal pigment epithelium. J Mol Neurosci 2012, 46, 122-137.
[180] Song, J., Lee, S. C., Kim, S. S., Koh, H. J., Kwon, O. W., Kang, J. J., Kim, E. K., Shin, S. H., Lee, J. H., Zinc2+-induced cell death is mediated by the induction of intracellular ROS in ARPE-19 cells. *Curr Eye Res* 2004, 28, 195-201.

[181] Newsome, D. A. M., M. V. Tate Jr., D. Alcock, N. W. Oliver, P. D., Zinc content of human retinal pigment epithelium decreases with age and macular degeneration, but superoxide dismutase activity increases. *The Journal of Trace Elements in Experimental Medicine* 1996, 8.

[182] Ramrattan, R. S., van der Schaft, T. L., Mooy, C. M., de Bruijn, W. C., Mulder, P. G., de Jong, P. T., Morphometric analysis of Bruch’s membrane, the choriocapillaris, and the choroid in aging. *Invest Ophthalmol Vis Sci* 1994, 35, 2857-2864.

[183] Age-Related Eye Disease Study Research, G., 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* 2001, 119, 1417-1436.

[184] Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *Jama* 2013, 309, 2005-2015.

[185] Ho, L., van Leeuwen, R., Witteman, J. C., van Duijn, C. M., Uitterlinden, A. G., Hofman, A., de Jong, P. T., Vingerling, J. R., Klaver, C. C., Reducing the genetic risk of age-related macular degeneration with dietary antioxidants, zinc, and omega-3 fatty acids: the Rotterdam study. *Arch Ophthalmol* 2011, 129, 758-766.

[186] Awh, C. C., Lane, A. M., Hawken, S., Zanke, B., Kim, I. K., CFH and ARMS2 genetic polymorphisms predict response to antioxidants and zinc in patients with age-related macular degeneration. *Ophthalmology* 2013, 120, 2317-2323.

[187] Awh, C. C., Hawken, S., Zanke, B. W., Treatment response to antioxidants and zinc based on CFH and ARMS2 genetic risk allele number in the Age-Related Eye Disease Study. *Ophthalmology* 2015, 122, 162-169.

[188] Assel, M. J., Li, F., Wang, Y., Allen, A. S., Baggerly, K. A., Vickers, A. J., Genetic Polymorphisms of CFH and ARMS2 Do Not Predict Response to Antioxidants and Zinc in Patients with Age-Related Macular Degeneration: Independent Statistical Evaluations of Data from the Age-Related Eye Disease Study. *Ophthalmology* 2018, 125, 391-397.

[189] Evans, J. R., Henshaw, K., Antioxidant vitamin and mineral supplementation for preventing age-related macular degeneration. *Cochrane Database Syst Rev* 2000, CD000253.

[190] Chong, E. W., Wong, T. Y., Kreis, A. J., Simpson, J. A., Guymer, R. H., Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* 2007, 335, 755.

[191] Evans, J. R., Antioxidant vitamin and mineral supplements for age-related macular degeneration. *Cochrane Database Syst Rev* 2002, CD000254.

[192] Cho, E., Stampfer, M. J., Seddon, J. M., Hung, S., Spiegelman, D., Rimm, E. B., Willett, W. C., Hankinson, S. E., Prospective study of zinc intake and the risk of age-related macular degeneration. *Annals of epidemiology* 2001, 11, 328-336.

[193] Risk factors for neovascular age-related macular degeneration. The Eye Disease Case-Control Study Group. *Arch Ophthalmol* 1992, 110, 1701-1708.

[194] Zhuang, Y., Lyga, J., Inflammaging in skin and other tissues - the roles of complement system and macrophage. *Inflamm Allergy Drug Targets* 2014, 13, 153-161.

[195] Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J.-Y., Sackler, R. S., Haynes, C., Henning, A. K., SanGiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., Hoh, J., Complement factor H polymorphism in age-related macular degeneration. *Science (New York, N.Y.)* 2005, 308, 385-389.

[196] Khandhadia, S., Cipriani, V., Yates, J. R. W., Lotery, A. J., Age-related macular degeneration and the complement system. *Immunobiology* 2012, 217, 127-146.

[197] Nozaki, M., Raisler, B. J., Sakurai, E., Sarma, J. V., Barnum, S. R., Lambris, J. D., Chen, Y., Zhang, K., Ambati, B. K., Baffi, J. Z., Ambati, J., Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A* 2006, 103, 2328-2333.
[198] Mullins, R. F., Russell, S. R., Anderson, D. H., Hageman, G. S., Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *Faseb J* 2000, 14, 835-846.

[199] Hageman, G. S., Luthert, P. J., Victor Chong, N. H., Johnson, L. V., Anderson, D. H., Mullins, R. F., An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res* 2001, 20, 705-732.

[200] Johnson, L. V., Leitner, W. P., Staples, M. K., Anderson, D. H., Complement activation and inflammatory processes in Drusen formation and age-related macular degeneration. *Exp Eye Res* 2001, 73, 887-896.

[201] Calippe, B., Augustin, S., Beguier, F., Charles-Messance, H., Poupel, L., Conart, J. B., Hu, S. J., Lavalette, S., Fauvet, A., Rayes, J., Levy, O., Raoul, W., Fitting, C., Denefle, T., Pickering, M. C., Harris, C., Jorieux, S., Sullivan, P. M., Sahel, J. A., Karoyan, P., Sapieha, P., Guillonneau, X., Gautier, E. L., Sennlaub, F., Complement Factor H Inhibits CD47-Mediated Resolution of Inflammation. *Immunity* 2017, 46, 261-272.

[202] Magnusson, K. P., Duan, S., Sigurdsson, H., Petursson, H., Yang, Z., Zhao, Y., Bernstein, P. S., Ge, J., Jonasson, F., Stefansson, E., Helgadottir, G., Zarbiskie, N. A., Jonsson, T., Bjornsson, A., Thorlacius, T., Jonsson, P. V., Thorleifsson, G., Kong, A., Stefansson, H., Zhang, K., Stefansson, K., Gulcher, J. R., CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PloS Med* 2006, 3, e5.

[203] Hageman, G. S., Anderson, D. H., Johnson, L. V., Hancox, L. S., Taiber, A. J., Hardisty, L. I., Hageman, J. L., Stockman, H. A., Borchardt, J. D., Gehrs, K. M., Smith, R. J., Silvestri, G., Russell, S. R., Klaver, C. C., Barbazetto, I., Chang, S., Yannuzzi, L. A., Barile, G. R., Merriam, J. C., Smith, R. T., Olsh, A. K., Bergeron, J., Zernant, J., Merriam, J. E., Gold, B., Dean, M., Allikmets, R., A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A* 2005, 102, 7227-7232.

[204] Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. Y., Sackler, R. S., Haynes, C., Henning, A. K., SanGiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., Hohn, J., Complement factor H polymorphism in age-related macular degeneration. *Science* 2005, 308, 385-389.

[205] Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, K. L., Kwan, S. Y., Nouri Aghdam, M., Gilbert, J. R., Schnetz-Boutaud, N., Agarwal, A., Postel, E. A., Pericak-Vance, M. A., Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005, 308, 419-421.

[206] Edwards, A. O., Ritter, R., 3rd, Abel, K. J., Manning, A., Panhuysen, C., Farrer, L. A., Complement factor H polymorphism and age-related macular degeneration. *Science* 2005, 308, 421-424.

[207] Crossley, L. G., Porter, R. R., Purification of the human complement control protein C3b inactivator. *Biochem J* 1980, 191, 173-182.

[208] Perkins, S. J., Nealis, A. S., Sim, R. B., Oligomeric domain structure of human complement factor H by X-ray and neutron solution scattering. *Biochemistry* 1991, 30, 2847-2857.

[209] Turnberg, D., Botto, M., The regulation of the complement system: insights from genetically-engineered mice. *Mol Immunol* 2003, 40, 145-153.

[210] Nan, R., Farabella, I., Schumacher, F. F., Miller, A., Gor, J., Martin, A. C., Jones, D. T., Lengyel, I., Perkins, S. J., Zinc binding to the Tyr402 and His402 allotypes of complement factor H: possible implications for age-related macular degeneration. *J Mol Biol* 2011, 408, 714-735.

[211] Nan, R., Tetchner, S., Rodriguez, E., Pao, P. J., Gor, J., Lengyel, I., Perkins, S. J., Zinc-induced self-association of complement C3b and Factor H: implications for inflammation and age-related macular degeneration. *J Biol Chem* 2013, 288, 19197-19210.

[212] Blom, A. M., Kask, L., Ramesh, B., Hillarp, A., Effects of zinc on factor I cofactor activity of C4b-binding protein and factor H. *Arch Biochem Biophys* 2003, 418, 108-118.
[226] Mosmann, T. R., Coffman, R. L., TH1 and TH2 cells: different patterns of lymphokine secretion. Mol Immunol 1987, 24, 1345-1350.

[225] Damsker, J. M., Hansen, A. M., Caspi, R. R., Th1 and Th17 cells: adversaries and collaborators. Science 2003, 299, 1057-1061.

[224] Caspi, R., Autoimmunity in the immune privileged eye: pathogenic and regulatory T cells. Int Rev Immunol 2000, 19, 123-152.

[223] Stein-Streilein, J., Streilein, J. W., Anterior chamber associated immune deviation (ACAID): regulation, biological relevance, and implications for therapy. Int Rev Immunol 2003, 21, 123-152.

[222] Franceschi, C., Campisi, J., Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. The journals of gerontology. Series A, Biological sciences and medical sciences 2014, 69 Suppl 1, S4-9.

[221] Varin, A., Larbi, A., Dedoussis, G. V., Kanoni, S., Jajte, J., Rink, L., Monti, D., Malavolta, M., Marcellini, F., Mocchegiani, E., Herbein, G., Fulop, T., In vitro and in vivo effects of zinc on cytokine signalling in human T cells. Experimental gerontology 2008, 43, 472-482.

[220] Varin, A., Larbi, A., Dedoussis, G. V., Kanoni, S., Jajte, J., Rink, L., Monti, D., Malavolta, M., Marcellini, F., Mocchegiani, E., Herbein, G., Fulop, T., In vitro and in vivo effects of zinc on cytokine signalling in human T cells. Experimental gerontology 2008, 43, 472-482.

[219] Subramanian Vignesh, K., Deepe, G. S., Immunological orchestration of zinc homeostasis: The battle between host mechanisms and pathogen defenses. Arch Biochem Biophys 2016, 611, 66-78.

[218] Maywald, M., Rink, L., Zinc homeostasis and immunosenescence. J Trace Elem Med Biol 2015, 69 Suppl 1, 52-55.

[217] Weiskopf, D., Weinberger, B., Grubeck-Loebenstein, B., The aging of the immune system. The journals of gerontology. Series A, Biological sciences and medical sciences 2013, 68, 145-173.

[216] Evans, B. D., Amiraian, K., Effect of zinc on lysis of sheep erythrocytes by guinea pig C4-deficient serum. Mol Immunol 1987, 24, 1345-1350.

[215] Evans, B. D., Amiraian, K., Effect of zinc on lysis of sheep erythrocytes by guinea pig C4-deficient serum. Mol Immunol 1987, 24, 1345-1350.

[214] Downing, P. R., Keller, J. C., Boackle, R. J., Interaction of dental cements with the complement system. Dent Mater 1989, 5, 83-89.

[213] Montgomery, D. W., Chvapil, M., Zukoski, C. F., Effects of zinc chloride on guinea pig complement component activity in vitro: concentration-dependent inhibition and enhancement. Infect Immun 1979, 23, 424-431.
[235] Hori, S., Takahashi, T., Sakaguchi, S., Control of autoimmunity by naturally arising regulatory CD4+ T cells. *Adv Immunol* 2003, 81, 331-371.

[236] Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E., Saulsbury, F. T., Chance, P. F., Ochs, H. D., The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001, 27, 20-21.

[237] Li, B., Samanta, A., Song, X., Furuuruchi, K., Iacono, K. T., Kennedy, S., Katsumata, M., Saouaf, S. J., Greene, M. I., FOXP3 ensembles in T-cell regulation. *Immunological reviews* 2006, 212, 99-113.

[238] Koh, K. P., Sundrud, M. S., Rao, A., Domain requirements and sequence specificity of DNA binding for the forkhead transcription factor FOXP3. *PloS one* 2009, 4, e8109-e8109.

[239] Lopes, J. E., Torgerson, T. R., Schubert, L. A., Anover, S. D., Ocheltree, E. L., Ochs, H. D., Ziegler, S. F., Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. *J Immunol* 2006, 177, 3133-3142.

[240] Agarwal, R. K., Silver, P. B., Casci, R. R., Rodent models of experimental autoimmune uveitis. *Methods Mol Biol* 2012, 900, 443-469.

[241] Gilbert, R., Al-Janabi, A., Tomkis-Netzer, O., Lightman, S., Statins as anti-inflammatory agents: A potential therapeutic role in sight-threatening non-infectious uveitis. *Porto Biomedical Journal* 2017, 2, 33-39.

[242] Xu, H., Manivannan, A., Jiang, H. R., Liversidge, J., Sharp, P. F., Forrester, J. V., Crane, I. J., Recruitment of IFN-gamma-producing (Th1-like) cells into the inflamed retina in vivo is preferentially regulated by P-selectin glycoprotein ligand 1: P/E-selectin interactions. *J Immunol* 2004, 172, 3215-3224.

[243] Xu, H., Manivannan, A., Dawson, R., Crane, I. J., Mack, M., Sharp, P., Liversidge, J., Differentiation to the CCR2+ inflammatory phenotype in vivo is a constitutive, time-limited property of blood monocytes and is independent of local inflammatory mediators. *J Immunol* 2005, 175, 6915-6923.

[244] Ke, Y., Jiang, G., Sun, D., Kaplan, H. J., Shao, H., Ocular Regulatory T Cells Distinguish Monophasic from Recurrent Autoimmune Uveitis. *Invest Ophthalmol Vis Sci* 2008, 49, 3999-4007.

[245] Sun, M., Yang, P., Du, L., Zhou, H., Ren, X., Kijlstra, A., Contribution of CD4+CD25+ T cells to the regression phase of experimental autoimmune uveoretinitis. *Invest Ophthalmol Vis Sci* 2010, 51, 383-389.

[246] Silver, P., Horai, R., Chen, J., Jittayasothorn, Y., Chan, C.-C., Villasril, M., Kesen, M. R., Caspi, R. R., Retina-Specific T Regulatory Cells Bring About Resolution and Maintain Remission of Autoimmune Uveitis. *The Journal of Immunology* 2015, 194, 3011.

[247] George, M. M., Subramanian Vignesh, K., Landero Figueroa, J. A., Caruso, J. A., Deepe, G. S., Jr., Zinc Induces Dendritic Cell Tolerogenic Phenotype and Skews Regulatory T Cell-Th17 Balance. *J Immunol* 2016, 197, 1864-1876.

[248] Maywald, M., Rink, L., Zinc supplementation induces CD4+(+CD25+(+)Foxp3+(+) antigen-specific regulatory T cells and suppresses IFN-gamma production by upregulation of Foxp3 and KLF-10 and downregulation of IRF-1. *European journal of nutrition* 2017, 56, 1859-1869.

[249] Rosenkranz, E., Maywald, M., Hilgers, R. D., Brieger, A., Clarner, T., Kipp, M., Plumakers, B., Meyer, S., Schwerdtle, T., Rink, L., Induction of regulatory T cells in Th1-/Th17-driven experimental autoimmune encephalomyelitis by zinc administration. *The Journal of nutritional biochemistry* 2016, 29, 116-123.

[250] Medzhitov, R., Origin and physiological roles of inflammation. *Nature* 2008, 454, 428.

[251] Nussenblatt, R. B., Ferris, F., 3rd, Age-related macular degeneration and the immune response: implications for therapy. *Am J Ophthalmol* 2007, 144, 618-626.

[252] Nussenblatt, R. B., Liu, B., Li, Z., Age-related macular degeneration: an immunologically driven disease. *Current opinion in investigational drugs (London, England : 2000)* 2009, 10, 434-442.

[253] Datta, S., Cano, M., Ebrahimii, K., Wang, L., Handa, J. T., The impact of oxidative stress and inflammation on RPE degeneration in non-neovascular AMD. *Prog Retin Eye Res* 2017, 60, 201-218.
[254] Chen, M., Xu, H., Parainflammation, chronic inflammation, and age-related macular degeneration. *Journal of leukocyte biology* 2015, 98, 713-725.
[255] McLeod, D. S., Bhuutton, I., Edwards, M. M., Silver, R. E., Seddon, J. M., Lutty, G. A., Distribution and Quantification of Choroidal Macrophages in Human Eyes With Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2016, 57, 5843-5855.
[256] Dastgheib, K., Green, W. R., Granulomatous reaction to Bruch’s membrane in age-related macular degeneration. *Arch Ophthalmol* 1994, 112, 813-818.
[257] Coleman, H. R., Chan, C. C., Ferris, F. L., 3rd, Chew, E. Y., Age-related macular degeneration. *Lancet* 2008, 372, 1835-1845.
[258] Cao, X., Shen, D., Patel, M. M., Tuo, J., Johnson, T. M., Olsen, T. W., Chan, C.-C., Macrophage polarization in the maculae of age-related macular degeneration: a pilot study. *Pathology international* 2011, 61, 528-535.
[259] Xu, H., Chen, M., Forrester, J. V., Para-inflammation in the aging retina. *Prog Retin Eye Res* 2009, 28, 348-368.
[260] Gorgani, N. N., He, J. Q., Katschke, K. J., Jr., Helmy, K. Y., Xi, H., Steffek, M., Hass, P. E., van Lookeren Campagne, M., Complement receptor of the Ig superfamily enhances complement-mediated phagocytosis in a subpopulation of tissue resident macrophages. *J Immunol* 2008, 181, 7902-7908.
[261] Sapkota, M., Knoell, D. L., Essential Role of Zinc and Zinc Transporters in Myeloid Cell Function and Host Defense against Infection. *Journal of Immunology Research*. 2018, 2018, 8.
[262] Subramanian Vignesh, K., Landero Figueroa, J. A., Porollo, A., Caruso, J. A., Deepe, G. S., Jr., Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity* 2013, 39, 697-710.
[263] Subramanian Vignesh, K., Landero Figueroa, Julio A., Porollo, A., Caruso, Joseph A., Deepe, George S., Granulocyte Macrophage-Colony Stimulating Factor Induced Zn Sequestration Enhances Macrophage Superoxide and Limits Intracellular Pathogen Survival. *Immunity* 2013, 39, 697-710.
[264] Silva, M. J., Carneiro, M. B., dos Anjos Pultz, B., Pereira Silva, D., Lopes, M. E., dos Santos, L. M., The multifaceted role of commensal microbiota in homeostasis and gastrointestinal diseases. *Journal of immunology research* 2015, 2015, 321241.
[265] West, C. E., Renz, H., Jenmalm, M. C., Kozyrskyj, A. L., Allen, K. J., Vuillermin, P., Prescott, S. L., The gut microbiota and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies. *The Journal of allergy and clinical immunology* 2015, 135, 3-13; quiz 14.
[266] Clarke, S. F., Murphy, E. F., Nilaweera, K., Ross, P. R., Shannahon, F., O'Toole, P. W., Cotter, P. D., The gut microbiota and its relationship to diet and obesity: new insights. *Gut Microbes* 2012, 3, 186-202.
[267] Andriessen, E. M., Wilson, A. M., Mawambo, G., Dejda, A., Miloudi, K., Sennlaub, F., Sapieha, P., Gut microbiota influences pathological angiogenesis in obesity-driven choroidal neovascularization. *EMBO Mol Med* 2016, 8, 1366-1379.
[268] Rowan, S., Jiang, S., Korem, T., Szymanski, J., Chang, M. L., Szelog, J., Cassalman, C., Dasuri, K., McGuire, C., Nagai, R., Du, X. L., Brownlee, M., Rabbani, N., Thornalley, P. J., Baleja, J. D., Deik, A. A., Pierce, K. A., Scott, J. M., Clish, C. B., Smith, D. E., Weinberger, A., Avnit-Sagi, T., Lotan-Pompan, M., Segal, E., Taylor, A., Involvement of a gut-retina axis in protection against dietary glycemia-induced age-related macular degeneration. *Proc Natl Acad Sci U S A* 2017, 114, E4472-E4481.
[269] Li, L., Krause, L., Somerset, S., Associations between micronutrient intakes and gut microbiota in a group of adults with cystic fibrosis. *Clin Nutr* 2017, 36, 1097-1104.
[270] Molan, A. L., Liu, Z., Plimmer, G., Evaluation of the effect of blackcurrant products on gut microbiota and on markers of risk for colon cancer in humans. *Phytother Res* 2014, 28, 416-422.
[271] Hildebrandt, M. A., Hoffmann, C., Sherrill-Mix, S. A., Keilbaugh, S. A., Hamady, M., Chen, Y. Y., Knight, R., Ahima, R. S., Bushman, F., Wu, G. D., High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009, 137, 1716-1724 e1711-1712.
[272] Qiao, Y., Sun, J., Ding, Y., Le, G., Shi, Y., Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. Appl Microbiol Biotechnol 2013, 97, 1689-1697.

[273] Kruidener, L., Kuiper, I., van Duijn, W., Marklund, S. L., van Hogezand, R. A., Lamers, C. B., Verspaget, H. W., Differential mucosal expression of three superoxide dismutase isoforms in inflammatory bowel disease. J Pathol 2003, 201, 7-16.

[274] Matsuoka, K., Kanai, T., The gut microbiota and inflammatory bowel disease. Semin Immunopathol 2015, 37, 47-55.

[275] Xu, J., Xu, C., Chen, X., Cai, X., Yang, S., Sheng, Y., Wang, T., Regulation of an antioxidant blend on intestinal redox status and major microbiota in early weaned piglets. Nutrition 2014, 30, 584-589.

[276] Belkaid, Y., Hand, T. W., Role of the microbiota in immunity and inflammation. Cell 2014, 157, 121-141.

[277] Clemente, J. C., Manasson, J., Scher, J. U., The role of the gut microbiome in systemic inflammatory disease. BMJ 2018, 360, j5145.

[278] Slingerland, A. E., Schwabkey, Z., Wiesnoski, D. H., Jenq, R. R., Clinical Evidence for the Microbiome in Inflammatory Diseases. Front Immunol 2017, 8, 400.

[279] Skrypnik, K., Suliburska, J., Association between the gut microbiota and mineral metabolism. J Sci Food Agric 2018, 98, 2449-2460.

[280] Giedla, L. M., DiRita, V. J., Zinc competition among the intestinal microbiota. MBio 2012, 3, e00171-00112.

[281] Frassinetti, S., Bronzetti, G., Calavuturo, L., Cini, M., Croce, C. D., The role of zinc in life: a review. J Environ Pathol Toxicol Oncol 2006, 25, 597-610.

[282] Zackular, J. P., Moore, J. L., Jordan, A. T., Juttukonda, L. J., Noto, M. J., Nicholson, M. R., Crews, J. D., Semler, M. W., Zhang, Y., Ware, L. B., Washington, M. K., Chazin, W. J., Caprioli, R. M., Skaar, E. P., Dietary zinc alters the microbiota and decreases resistance to Clostridium difficile infection. Nat Med 2016, 22, 1330-1334.

[283] Maares, M., Keil, C., Koza, J., Straubing, S., Schwerdtle, T., Haase, H., In Vitro Studies on Zinc Binding and Buffering by Intestinal Mucins. International journal of molecular sciences 2018, 19.

[284] Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., Wu, X., Li, J., Tang, L., Li, Y., Lan, Z., Chen, B., Li, Y., Zhong, H., Xie, H., Jie, Z., Chen, W., Tang, S., Xu, X., Wang, X., Cai, X., Liu, S., Xia, Y., Li, J., Qiao, X., Al-Aama, J. Y., Chen, H., Wang, L., Wu, Q. J., Zhang, F., Zheng, W., Li, Y., Zhang, M., Luo, G., Xue, W., Xiao, L., Li, J., Chen, W., Xu, X., Yin, Y., Yang, H., Wang, J., Kristiansen, K., Liu, L., Li, T., Huang, Q., Li, Y., Wang, J., The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015, 21, 895-905.

[285] Ohnmacht, C., Park, J. H., Cording, S., Wing, J. B., Atarashi, K., Obata, Y., Gaboriau-Routhiau, V., Marques, R., Dulauroy, S., Fedoseeva, M., Busslinger, M., Cerf-Bensussan, N., Boneca, I. G., Voehringer, D., Hase, K., Honda, K., Sakaguchi, S., Eberl, G., MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgamma(+) regulatory T cells. Science 2015, 349, 989-993.

[286] Omenetti, S., Pizarro, T. T., The Treg/Th17 Axis: A Dynamic Balance Regulated by the Gut Microbiome. Front Immunol 2015, 6, 639.

[287] Leung, S., Liu, X., Fang, L., Chen, X., Guo, T., Zhang, J., The cytokine milieu in the interplay of pathogenic Th1/Th17 cells and regulatory T cells in autoimmune disease. Cell Mol Immunol 2010, 7, 182-189.

[288] Sefik, E., Geva-Zatorsky, N., Oh, S., Konnikova, L., Zemmour, D., McGuire, A. M., Burzyn, D., Ortiz-Lopez, A., Lobera, M., Yang, J., Ghosh, S., Earl, A., Snapper, S. B., Jupp, R., Kasper, D., Mathis, D., Benoist, C., MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. Science 2015, 349, 993-997.

[289] Johnson, A. R., Munoz, A., Gottlieb, J. L., Jarrard, D. F., High dose zinc increases hospital admissions due to genitourinary complications. J Urol 2007, 177, 639-643.

[290] Evans, J. R., Lawrenson, J. G., Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. The Cochrane database of systematic reviews 2017, 7, Cd000254.
[291] Evans, J. R., Lawrenson, J. G., Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. *Cochrane Database of Systematic Reviews* 2017.

[292] Hogg, R. E., Woodside, J. V., McGrath, A., Young, I. S., Vioque, J. L., Chakravarthy, U., de Jong, P. T., Rahu, M., Seland, J., Soubrane, G., Tomazzoli, L., Topouzis, F., Fletcher, A. E., Mediterranean Diet Score and Its Association with Age-Related Macular Degeneration: The European Eye Study. *Ophthalmology* 2017, 124, 82-89.

[293] Merle, B. M. J., Colijn, J. M., Cougnard-Gregoire, A., de Koning-Backus, A. P. M., Delyfer, M. N., Kiefte-de Jong, J. C., Meester-Smoor, M., Feart, C., Verzijden, T., Samieri, C., Franco, O. H., Korobelnik, J. F., Klaver, C. C. W., Delcourt, C., Mediterranean Diet and Incidence of Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology* 2018.
Zinc is an essential nutrient for human health. It plays key roles in maintaining protein structure and stability, serves as catalytic factor for many enzymes and regulates diverse fundamental cellular processes. Zinc is important in affecting signal transduction and, in particular, in the development and integrity of the immune system, where it affects both innate and adaptive immune responses. The eye, especially the retina/choroid complex, has an unusually high concentration of zinc compared to other tissues. The highest amount of zinc is concentrated in the retinal pigment epithelium (RPE) (RPE/choroid, 292 ± 98.5 µg/g dry tissue), followed by the retina (123 ± 62.2 µg/g dry tissue). The interplay between zinc and inflammation has been explored in other parts of the body but, so far, has not been extensively researched in the eye. Several lines of evidence suggest that ocular zinc concentration decreases with age, especially in the context of age-related disease. Thus, a hypothesis that retinal function could be modulated by zinc nutrition was proposed, and subsequently trialled clinically. In this review, we outline the distribution and the potential role of zinc in the retina-choroid complex, especially in relation to inflammation and immunity, and summarize the clinical studies to date.
Maintaining protein structure and stability

Regulatory role in diverse fundamental cellular processes, such as DNA synthesis, RNA transcription, cell division and activation, apoptosis, cell signalling.

Critical for normal immune function.

**FUNDAMENTAL ZINC FUNCTIONS**

**IMPORTANT DIETARY SOURCES OF ZINC**

- Seafood e.g. oysters
- Dairy foods e.g. cheese
- Red meat e.g. beef steak
- Nuts and legumes e.g. chickpeas

**UK RECOMMENDED DAILY INTAKE OF ZINC**

|          | Men  | Women |
|----------|------|-------|
| Zinc     | 9-10 mg | 7 mg   |

**DIETARY CAUTIONS**

- Recommended daily supplementation: ≤ 25 mg of elemental zinc
- Different zinc formats contain different amounts of elemental zinc and have different absorption levels
- Dietary phytates (found in cereals/whole grains) inhibit zinc absorption levels
- High excess zinc can interfere with metabolism of other metals, such as Cu, Fe