· 综　　述 ·

Research progress in 7-ketocholesterol and age-related macular degeneration

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[Abstract] Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in older adults, with early-stage features including subretinal lipid deposits and progressing to retinal geographic atrophy and choroidal neovascularization in advanced stages. The dysregulation of ocular lipid metabolism, oxidative stress, and inflammation are critical risk factors for AMD pathogenesis. 7-ketocholesterol (7-KC), a hallmark of ocular lipid metabolism disorders, is a significant component of subretinal lipid deposits in AMD patients, exhibiting toxicity to retinal cells and exacerbating lipotoxic aging. This review elaborates on the biosynthesis and metabolism of 7-KC in the retina, investigates its detoxification mechanisms by examining its binding proteins, and summarizes recent progress on kinase signaling pathways induced by 7-KC through inflammatory cytokines and intracellular effectors. The aim is to pinpoint potential pharmacological targets, nutritional compounds, and synthetic molecules to explore the potential of targeting 7-KC for AMD treatment.

[Key words] Age-related macular degeneration; 7-ketocholesterol; Aging; Binding protein; Inflammation; Pharmacological targets

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7-酮基胆甾醇与年龄相关性黄斑变性疾病研究进展

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【摘要】 年龄相关性黄斑变性（AMD）是老年人不可逆性视力丧失的首要原因，早期以视网膜下脂质沉积为特征，晚期可进展为视网膜下地图样萎缩及脉络膜新生血管形成。眼部脂质代谢紊乱、氧化应激及炎症反应是 AMD 发病的关键危险因素。7-酮基胆甾醇（7-KC）作为眼部脂质代谢紊乱的标志性物质，是 AMD 患者视网膜下脂质沉积的重要组成部分，对视网膜细胞具有毒性作用，且可加重视网膜细胞的脂毒性衰老进程。本文阐述 7-KC 在视网膜中的生物合成与代谢，通过分析其潜在结合蛋白探讨视网膜内 7-KC 的解毒机制，并总结其通过炎性细胞因子及细胞内效应分子介导的激酶信号通路研究进展，旨在明确针对 7-KC 调控的潜在药物靶点、营养活性物质及合成分子，为探索 7-KC 靶向治疗 AMD 的潜力提供依据。

【关键词】 年龄相关性黄斑变性；7-酮基胆甾醇；衰老；结合蛋白；炎症；药物靶点

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Cholesterol is one of the most abundant molecules in mammalian tissues and cells. Its precise regulation is essential for maintaining cellular homeostasis and viability. Cellular cholesterol homeostasis is maintained through a sophisticated balance between three key pathways: *de novo* biosynthesis, receptor-mediated uptake from exogenous sources, and active efflux mechanisms[1-2]. Dysregulation of cholesterol levels has been strongly linked to age-related diseases, including atherosclerosis and age-related macular degeneration (AMD)[3] and Alzheimer disease[4-5]. AMD is the leading cause of irreversible blindness in the elderly population. Early stages are characterized by elevated levels of 7-ketocholesterol (7-KC) within retinal lipid deposits known as drusen, which constitute significant risk factors for progression to late-stage AMD, which manifests as two distinct phenotypes: exudative (neovascular) AMD and geographic atrophy. Exudative AMD is characterized by choroidal neovascularization (CNV), a pathological process driven by angiogenic factors such as vascular endothelial growth factor (VEGF). This results in extravasation of fluid, lipids, and blood into retinal tissues, representing a leading global cause of irreversible visual impairment[6-8]. In the healthy retina, cholesterol is mainly derived from local *de novo* synthesis, with a smaller portion obtained from extra-retinal uptake through low-density lipoproteins (LDL)[7,9]. The highly pro-oxidative microenvironment of the retina renders cholesterol particularly susceptible to oxidation, resulting in the formation of oxysterols[10], which exhibit greater biological activity than cholesterol and significantly influence cholesterol metabolism, synthesis, and reverse cholesterol transport[13]. However, specific oxysterols, particularly 7-KC, exhibit pronounced cytotoxicity in retinal cell populations. Age-dependent accumulation of 7-KC in the subretinal space may drive pathogenic processes underlying retinal degeneration, including AMD[11-12]. 7-KC exhibits pro-oxidant, pro-inflammatory, and pro-angiogenic properties, while demonstrating potent cytotoxicity through programmed cell death induction[13]. These pathogenic effects likely contribute significantly to retinal pigment epithelium (RPE) injury in AMD. Retinal 7-KC formation primarily occurs through autoxidation processes analogous to those observed in atherosclerotic lesions[5,8].

1　Biosynthesis and Metabolism of 7-KC in the Retina

1.1　Biosynthesis of 7-ketocholesterol in the retina

The retina, a highly specialized neural tissue organized into ten distinct layers, contains abundant cholesterol distributed throughout its cellular structure. Photoreceptors, the light-sensing neurons of the retina, play indispensable roles in phototransduction and require continuous cholesterol provision to maintain the dynamic cycle of outer segment shedding, phagocytosis by RPE, and subsequent regeneration. Consequently, retinal cholesterol homeostasis represents a sophisticated regulatory system involving three interdependent processes: *de novo* biosynthesis, receptor-mediated uptake from systemic circulation, and intricate intraretinal transport mechanisms[14]. Early experiments in rats, which involved the intravitreal injection of ^{3}H-acetate followed by monitoring its incorporation into cholesterol, demonstrated cholesterol formation in the neural retina within six hours, confirming the existence of a local cholesterol synthesis pathway. However, the absolute rates of cholesterol synthesis were not measured. Using deuterated water ([^{2}H] water) and subsequent mass spectrometry analysis of cholesterol isotopomer distribution, researchers found that more than 70% of retinal cholesterol arises from local synthesis. Mice fed cholesterol-enriched diets for two weeks exhibited similar cholesterol uptake proportions. However, following the administration of simvastatin, a local cholesterol biosynthesis inhibitor, retinal cholesterol levels decreased[15]. Recent studies have identified cholesterol biosynthetic genes such as 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) in RPE cells, with the expression showing circadian variation, confirming cholesterol synthesis in these cells[16]. Besides *de novo* synthesis, the neural retina can absorb cholesterol from blood-borne lipoproteins by crossing the blood-retina barrier. Research has demonstrated that after intravenous injection of human low-density lipoprotein (LDL) particles "doped" with cholestatrienol, cholesterol fluorescence was observed in the choroid, RPE, and distal outer neural retina within 2 hours after injection, and in 6 hours, cholesterol was present in the entire neural retina, suggesting that cholesterol carried by LDL can be absorbed by the retina and distributed extensively[9]. Experiments showed that LDL is absorbed by ARPE-19 cells through the LDL receptor (LDLR). In LDLR-knockout mice, lipid accumulation was observed in Bruch membrane. It is confirmed that systemic abnormal lipid metabolism can affect the eye[17].

Interestingly, recent single-cell sequencing of human retinas derived from human embryonic stem cells revealed that enzymes in the cholesterol mevalonate pathway are not enriched in photoreceptors[18]. Computational models of retinal sterol homeostasis indicate that photoreceptor cholesterol is predominantly obtained from external sources rather than synthesized locally[19]. Synthesized cholesterol predominantly exists in two forms: esterified cholesterol and unesterified cholesterol, which are interconvertible. Esterified cholesterol is the preferred form for transport and energy storage, while unesterified cholesterol is essential for maintaining cell membrane integrity and serves as a precursor for bile acid, vitamin D, and steroid synthesis.

Retinal cholesterol undergoes oxidation via both enzymatic (e.g., cytochrome P450) and non-enzymatic (reactive oxygen species) pathways. In AMD, histochemical analyses of subretinal

deposits demonstrate substantial accumulation of unesterified cholesterol and cholesteryl esters—lipid species recognized as molecular markers of retinal aging and pathological dysfunction[20]. 7-KC, a prominent form of unesterified cholesterol, is primarily formed through non-enzymatic pathways in the retina. Two primary mechanisms mediate the formation of 7-KC: the singlet oxygen mechanism and free radical mechanisms[21]. The singlet oxygen mechanism leads to the production of 6-hydroperoxides, which rearrange to form 7-hydroperoxides. This compound then oxidizes other molecules and is further rearranged into 7-hydroxycholesterol and 7-KC. This process is light-dependent and requires appropriate photosensitizers to mediate cholesterol oxidation. Although hematoporphyrin is an effective photosensitizer for cholesterol oxidation, studies using retinal extracts from monkeys and light-damaged aging rats have failed to identify intermediates related to this mechanism[22]. Research has shown that in photo-damaged regions of albino rats, particularly in mitochondria-rich areas, 7-KC levels significantly increase. Liquid chromatography-mass spectrometry (LC-MS) analyses of oxidative intermediates revealed that 7-KC is generated through free radical-mediated pathways involving reactive oxygen species (ROS) and reactive nitrogen species, which are byproducts of cellular metabolism[23]. In the retinal microenvironment, labile iron catalyzes lipid peroxidation in photoreceptor outer segments, particularly under bright light exposure. Cytochrome c, while primarily a mitochondrial electron transport protein, can serve as a source of redox-active iron. Through Fenton chemistry, hydrogen peroxide oxidation facilitates iron release from cytochrome c. Another significant source of retinal 7-KC is circulating lipoproteins, when retinal LDL metabolism is impaired, LDL particles undergo oxidative modification to form ox-LDL. Notably, 7-KC constitutes the predominant oxysterol species in ox-LDL, representing approximately 30% of total oxysterol content[24]. Emerging evidence indicates that 7-KC may also derive from gut microbiota-mediated cholesterol metabolism, where microbial oxidation products are absorbed via enterohepatic circulation and subsequently contribute to systemic pro-inflammatory states[25]. Dietary intake, particularly from poorly stored animal-derived foods, is another potential source of 7-KC, significantly increasing the body´s oxysterol load, while the liver typically metabolizes most dietary 7-KC, impaired liver function may result in incomplete metabolism[26]. While direct evidence connecting dietary 7-KC to ocular accumulation remains lacking, excessive intake may potentially contribute to elevated intraocular 7-KC levels through systemic circulation.

As a developmental extension of the central nervous system, the retina exhibits unique cholesterol uptake mechanisms that differ significantly from those in the brain. While the brain relies almost exclusively on endogenous cholesterol synthesis due to the impermeability of the blood-brain barrier (BBB) to circulating lipoproteins, oxidized sterols demonstrate notable BBB penetrance and can progressively accumulate in neural tissue[27]. 7-KC has been identified in the neuropathology of age-related neurodegenerative disorders, including Alzheimer disease. While its exact origin remains uncertain, potentially deriving from either peripheral circulation (via BBB transgression) or local oxidation of brain cholesterol presence in neural tissue demonstrates significant neurotoxic potential. Similarly, large accumulations of 7-KC have been observed in subretinal deposits in AMD, but whether 7-KC can cross the blood-retinal barrier has not been reported. This raises the hypothesis that 7-KC may similarly compromise the blood-retinal barrier. Experimental evidence demonstrates that oxidized sterols can disrupt BBB integrity, particularly under pathological mechanical stress[28]. Such oxidative damage increases vascular permeability, permitting toxic compounds to infiltrate the normally protected neural parenchyma. This process may establish a self-perpetuating cycle wherein: initial barrier dysfunction facilitates 7-KC influx, accumulated 7-KC exacerbates oxidative damage, and progressive barrier deterioration accelerates neuronal degeneration.

1.2 Metabolism of 7-KC in the retina

7-KC undergoes metabolism through three principal pathways: two distinct hydroxylation routes and one sulfonation pathway. Notably, the eye appears to be uniquely capable of executing all three metabolic transformations. In retinal tissue, the cytochrome P450 enzymes CYP27A1 and CYP46A1 mediate the hydroxylation of 7-KC[29]. CYP27A1, a mitochondrial protein, is found in the inner segment of photoreceptors, RPE, Müller cells, and choroidal capillary endothelial cells. In humans, the expression of CYP27A1 in the RPE is 2－4 times higher than that in the neural retina, where it converts 7-KC into 27-hydroxycholesterol[21]. Deficiency of CYP27A1 leads to cerebrotendinous xanthomatosis, which is associated with ocular abnormalities such as cataracts[30], retinal degeneration, optic disc pallor, and retinal atrophy[31]. In contrast, CYP46A1 is primarily expressed in the neural retina, with lower expression levels in the RPE. The expression of CYP46A1 in the retinas of both humans and monkeys is severalfold lower than that of CYP27A1, making CYP27A1 the primary enzyme responsible for 7-KC metabolism. The sulfonation pathway represents an alternative metabolic route for 7-KC detoxification. Through the action of SULT2B1b, an enzyme notably overexpressed in 293T cell models, 7-KC undergoes conversion to 7-ketocholesterol-3-sulfate, resulting in partial attenuation of its cytotoxic effects. Notably, SULT2B1b has not been detected in the human neural retina despite comprehensive profiling. Although three metabolic pathways for 7-KC have been identified in retinal tissue, conventional oxysterol metabolites , including 24- hydroxycholesterol

and 27-hydroxycholesterol, remain undetectable. Instead, 7-KC accumulates progressively with aging and demonstrates strong pathological associations with major ocular disorders, particularly AMD, glaucoma, and cataract formation.

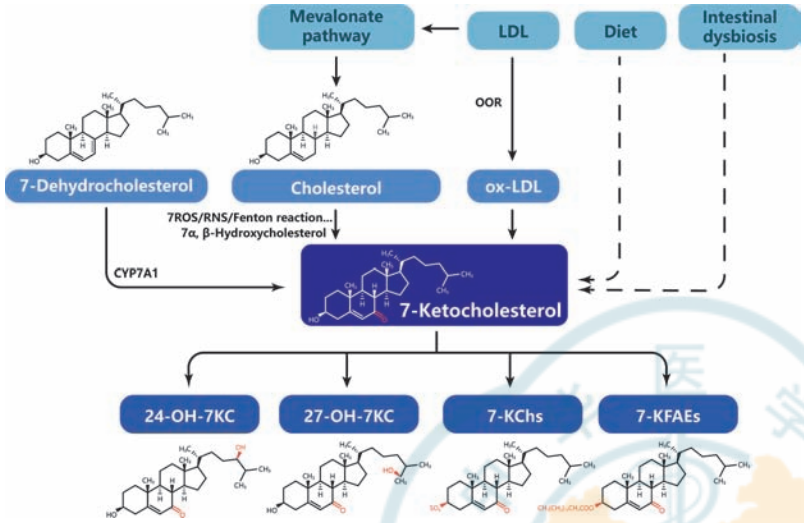

Figure 1　Schematic representation of 7-ketocholesterol synthesis and metabolism
This representation was from *pubchem. ncbi. nlm. nih. gov.* 7-ketocholesterol can be synthesized through enzymatic reactions, where 7-dehydrocholesterol is converted into 7-ketocholesterol via cytochrome P450 reactions, which can be metabolized by the liver. It can also be produced through cholesterol autoxidation and oxidized low-density lipoprotein oxidation. Part of 7-ketocholesterol may arise from diet and gut microbiota imbalance. 7-ketocholesterol metabolism also occurs via hydroxylation and sulfation. The primary hydroxylating enzyme, CYP27A1, converts 7KC to 27-OH-7KC[24]. CYP46A1 converts 7-ketocholesterol to 24-OH-7KC[27]; sulfation by the enzyme SULT2B1b converts 7-ketocholesterol into 7KChs[26]. 7-ketocholesterol can also be converted into 7KC-fatty acid esters in cells via cytosolic phospholipase A2α and sterol O-acyltransferase[42]

2　Pathophysiological Properties of 7-KC in AMD

AMD represents the predominant cause of irreversible vision loss in aging populations. Accumulating evidence demonstrates that dysregulated ocular cholesterol homeostasis plays a central role in AMD pathogenesis. Early disease stages are pathologically characterized by subretinal drusen deposits, which contain a complex mixture of lipids (including cholesterol and oxysterols) and proteinaceous material[32-33]. 7-KC, a major constituent of pathological lipid deposits, emerges as an active mediator of AMD progression. This oxysterol exhibits dual pro-oxidant and cytotoxic properties, functioning as a potent inducer of oxiapoptophagy, a combined oxidative stress-induced cell death pathway involving oxidation, apoptosis, and autophagy[34-37]. 7-KC is now emerging as a fine-tuning regulator involved in various aging-related biological processes. In AMD, 7-KC contributes to disease progression through multifaceted mechanisms: metabolic dysregulation, inflammatory activation, oxidative stress amplification, mitochondrial and peroxisomal dysfunction, cellular senescence induction, programmed cell death initiation, pathological neovascularization, and fibrotic remodeling. These diverse actions underscore the critical influence of oxysterol imbalance on AMD pathogenesis[38-41].

2.1　Accumulation of 7-KC in retina

Studies in rodent models have revealed age-dependent accumulation of 7-KC in retinal tissues. Immunohistochemical analyses of sclerochoroidal flat-mount preparations demonstrate significantly greater 7-KC deposition in aged (24-month-old) CX3CR1GFP/+ mice compared to young (2-month-old) controls, with particularly prominent localization in the outer retina and choroidal layers[42]. Complementary LC-MS/MS analyses in non-human primates have demonstrated progressive age-related accumulation of 7-KC across critical ocular compartments, with particularly pronounced deposition in RPE, choroid, and Bruch membrane[43]. In humans, 7-KC accumulates in the RPE/choroid region as a consequence of aging and is found at significantly higher levels in drusen deposits compared to healthy adults, with concentrations ranging from 200 to 17, 000 pmol/nmol in the RPE/choroid[44]. In some elderly individuals, the levels of 7-KC in the RPE/choroid tissues are similar to those found in atherosclerotic plaques, and the 7-KC level in serum also increased 1.8 times in AMD patients[45], suggesting that it may be a potential biomarker.

2.2　Potential binding proteins for 7-KC

Targeted binding proteins of 7-KC are critical area of research, particularly for understanding its clearance and detoxification mechanisms. Identifying potential binding proteins for 7-KC may provide insight into its intracellular pathological effects and be essential for its elimination. Several potential binding proteins have been identified, including caveolin-1 (Cav-1), oxysterol-binding proteins, and liver X receptors (LXRs).

Cav-1 has been found in various cell types, which is associated with lipid rafts on the cellsurface and cholesterol-rich Golgi membranes[46]. Cav-1 serves a critical role in cholesterol binding and membrane organization, facilitating both cholesterol aggregation and membrane interactions through its scaffolding domain. Within ocular tissues, Cav-1 dysregulation has been mechanistically linked to disrupted lipid homeostasis and aberrant activation of inflammatory signaling pathways, particularly in retinal degenerative diseases[47]. Increased Cav-1 expression is observed in the serum of smokers, enhancing the phagocytic capacity of RPE cells in smokers[48]. Transgenic mice with elevated Cav-1 expression exhibit significantly attenuated choroidal vascular

fibrosis[49]. Mechanistically, impaired Cav-1/VEGFR2 interaction in RPE cells results in cytoplasmic VEGF-VEGFR binding, triggering activation of both the MAPK/ERK1/2 and PLCγ/Akt signaling cascades. This aberrant signaling promotes pathological retinal neovascularization[50-51]. Biochemical studies demonstrate that VEGFR2 localizes to Cav-1-enriched membrane microdomains, where it forms functional complexes with endothelial nitric oxide synthase. Importantly, 7-KC disrupts this spatial organization, significantly reducing Cav-1/endothelial nitric oxide synthase co-localization in endothelial cells[52]. 7-KC has been found to accumulate in Cav-1-enriched regions when smooth muscle cells are exposed to oxidized low-density lipoprotein[53]. This 7-KC-induced dissociation suggests a potential mechanism for impaired NO signaling in vascular pathologies and Cav-1 is the potential protein.

Oxysterol-binding proteins are a family of proteins involved in lipid metabolism, vesicular trafficking, and signal transduction by binding phosphoinositides[54]. During aging, oxysterol-binding protein 2 (OSBP2) exhibits predominant and nearly exclusive expression within the macular region. Emerging evidence suggests that OSBP2 and its oxysterol ligands play a significant role in the pathogenesis of age-related retinal disorders[55]. Research has shown that OSBP2 has a strong binding affinity for 7-KC, forming a complex that recruits and activates JAK2. This interaction triggers signal transducer and activator of transcription 3-phosphorylation, ultimately promoting endothelial dysfunction through pro-inflammatory cytokine production and vascular permeability alterations[56].

LXRs are ligand-activated transcription factors that play a key role in cholesterol homeostasis. They heterodimerize with retinoid X receptors to regulate the expression of genes involved in sterol and fatty acid homeostasis. There are two isoforms of LXRs: LXRα and LXRβ, which are highly expressed in the neural retina and RPE of human donors. LXRs promote cholesterol efflux via ABC transporters (ABCA1 and ABCG1) and regulate the expression of inflammatory genes in ARPE-19 cells[57-58]. 7-KC induces the expression of VEGF and ABCA1 through an LXR-dependent mechanism. LXR activation decreases apoptosis induced by 7-KC in RPE cells and inhibits CNV[59]. However, further research is necessary to fully understand the role of 7-KC and LXRs in health and disease.

Retinoic acid-related orphan receptor α (RORα) is a member of the nuclear hormone receptor family, including RORα, β, and γ, and is expressed in the choroid and retina of mice. RORα regulates several homeostatic pathways, including lipid metabolism, oxidative stress, and inflammatory pathways in the retina. Previous studies indicated that genetic variations in RORα were associated with neovascular AMD and RORα transcriptionally regulates multiple AMD-relevant pathways such as angiogenesis, lipid metabolism, and inflammatory and complement pathways[60]. Research observed that the absence of RORα results in increased expression of VEGFR2 and TNFα in the choroid/RPE complex. In a laser-induced CNV mouse model, RORα expression significantly increases in the choroid/RPE complex following laser treatment[61], correlating with increased 7-KC expression around the laser-induced CNV. In HEK293T cells, 7-KC acts as an inverse agonist of RORα and γ, leading to reduced gene transcription. Although there is no direct evidence of their interaction in the retina, 7-KC may play a role in inhibiting RORα's angiogenic function, leading to increased VEGF expression and promoting neovascularization[62].

Another potential binding protein of 7-KC is the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor involved in multiple cellular responses. AHR is expressed in the retina and ocular veins, playing a crucial role in regulating the function of photoreceptors and RPE cells. It has been confirmed that AHR directly regulates the accumulation of subretinal deposits and plays a role in their removal[63]. A decrease in AHR activity is related to the aging of RPE cells, when AHR expression decreases, it promotes the expression of VEGF and CCL2, exacerbating tissue inflammation, CNV, and fibrosis[63-64]. Research has shown that AHR activation can prevent RPE cell death induced by lipid peroxidation products[65-66].

The transporter protein (TSPO) plays an important role in cholesterol efflux from RPE cells and regulates mitochondrial oxidative stress. TSPO expression decreases with aging, which may lead to intracellular cholesterol accumulation and potentially promote AMD progression[67-68]. Knockout of the TSPO gene in the retina leads to a significant increase in pro-inflammatory cytokines and ROS, and activates microglia[69]. It is consistent with our observation that increased 7-KC accumulation promotes microglial activation. The TSPO activator etifoxine improves functional abnormalities in RPE cells, reduces lipid accumulation, and enhances the expression of cholesterol metabolism and transport enzymes. It also decreases ROS levels in RPE cells and reduces inflammatory cytokine levels in both RPE cells and serum[70]. In contrast to the effects of 7-KC, TSPO may play a significant role in the metabolism of 7-KC and act as a potential effector in inhibiting 7-KC's pathological effects.

Recent studies demonstrate that cytosolic phospholipase A2α and sterol O-acyltransferase 1 mediate the esterification of 7-KC into 7-KC-fatty acid esters in ARPE-19 cells, and high-density lipoprotein (HDL)-facilitated efflux promotes the extracellular clearance of these 7-KC-fatty acid esters[71]. Although other proteins related to lipid metabolism, such as SREBP, NPC1, FXR, ChEH, and EBI2, have been identified as potential sterol-binding partners, their specific roles in 7-KC metabolism remain uncharacterized and thus are excluded from this analysis due to

insufficient experimental evidence[72].

The analysis of the interactions between 7-KC and its potential binding proteins, along with studies on their mechanisms, provides an essential foundation for understanding its pathophysiological effects. 7-KC likely plays a significant role in inducing cellular inflammation, oxidative stress, cell aging, and apoptosis.

2.3 7-KC and inflammation

Inflammation is the body´s protective response to harmful stimuli, but excessive or chronic inflammation can contribute to the development and progression of various diseases with aging[73]. 7-KC is a potent oxysterol in pro-inflammatory response on retinal cells, like RPE cells, macrophages, vascular endothelial cells, and Müller cells, inducing the release of inflammatory cytokines such as VEGF, IL-1β, IL-6, and IL-8. Experimental studies demonstrate that intravitreal injection of 7-KC in 20-week-old Sprague-Dawley rats leads to preferential accumulation at the RPE apical surface, resulting in reduced microvilli, morphological abnormalities, and impaired phagocytosis of photoreceptor outer segments (POS)[38]. *In vitro*, hRPE cells treated with 7-KC showed the decreased expression of ZO-1, cadherin, β-catenin, and impedance (measuring transepithelial resistance), which contributes to the damage of the retinal outer barrier and expression of inflammatory cytokines. This phenomenon is consistent with the higher levels of apoptosis, autophagy, endoplasmic reticulum (ER) stress, disruption of tight junctions, impairment of cell polarity, and cell death observed in RPE cells in AMD patients[32]. The interaction of 7-KC with TLR-4 activates EGFR-related signaling pathways that integrate these pathways in ARPE-19 cells, enhances NLRP3 expression, and upregulates NF-κB, inducing increased release of IL-6, IL-8, IL-1β and VEGF[74-75]. Besides, 7-KC disturbs RPE cells phagocytosis of the outer segment of photoreceptor and induced inflammation via the ERK signaling pathway and the JNK phosphorylation and p38MAPK signaling pathways activated by 7-KC for promoting the inflammatory cytokines release[38,76]. In addition to activating the classical inflammatory signaling pathways, 7-KC can also induce mitochondrial dysfunction, which represents abnormal cellular energy expenditure and oxidative stress, accelerating the aging process.

2.4 7-KC-induced mitochondrial dysfunction and oxidative stress

RPE cell degeneration represents a primary pathogenic event in AMD. *In vitro* studies of patient-derived AMD, RPE cultures demonstrate characteristic ultrastructural and functional alterations: the accumulation of lipid droplets and glycogen granules, reduced mitochondrial activity, increased mitochondrial fragmentation, and heightened sensitivity to oxidative stress. Furthermore, ARPE-19 cells depleted of mitochondrial DNA show increased expression of genes encoding drusen components, with gene expression patterns resembling those observed in AMD. Mitochondrial dysfunction in RPE cells leads to impaired ATP generation, triggering a compensatory metabolic adaptation wherein photoreceptors increase glucose supply to fuel RPE glycolysis. Comparative metabolic profiling of RPE cell lines, including primary human RPE cultures from AMD donors, demonstrates significant disease-associated alterations in energy production, and corresponding increases in glycolytic flux[77]. Low doses of 7-KC have been shown to alter the bioenergetics of RPE cells by increasing mitochondrial fission and elevating TOM20 and oxidative phosphorylation protein levels, although mitochondrial activity and membrane potential remain unchanged. Following 7 days of *in vitro* culture, metabolic flux analysis demonstrated a significant increase in total ATP production rates, primarily driven by enhanced glycolysis[38]. Thus, 7-KC may influence early aging stages by affecting cellular energy metabolism. When mitochondrial regulatory balance is disrupted, and mitochondrial DNA suffers oxidative damage due to high doses of 7-KC, abnormalities arise in the subunits of respiratory chain complexes. This leads to increased expression of lipid transport and inflammation related genes, promoting ROS generation[78]. 7-KC favors mitochondrial dysfunctions and induces a drop of transmembrane mitochondrial potential in 158N cells[79]. The mitochondrial free radical aging theory (MFRTA) proposes that cellular oxidative

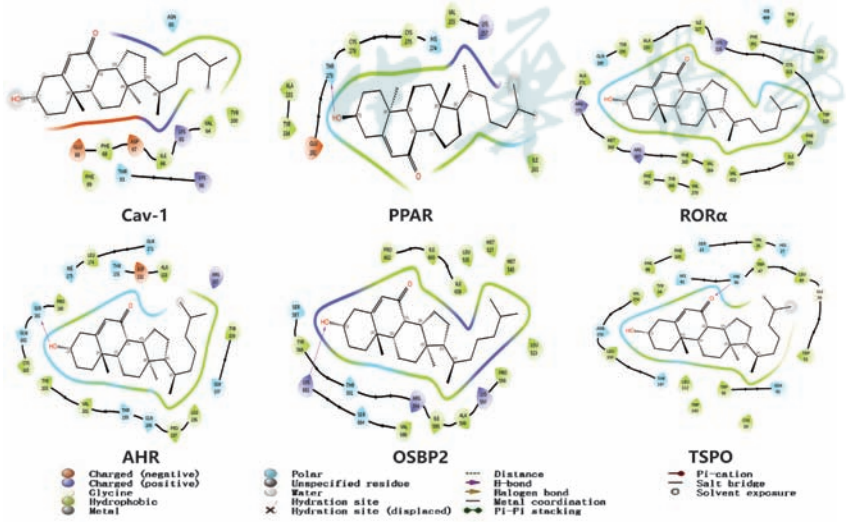

Figure 2　Binding patterns and interactions of 7-ketocholesterol with six possible binding proteins　Specific protein-ligand binding patterns and molecular interactions were shown. All structural data are derived from UniProt. The CAV-1 structure is encoded by AF-Q03135-F1, the PPAR structure by AF-Q07869, the OSBP2 structure by AF-Q969R2-F1, the Ahr structure by AF-P35869-F1, the RORα structure by AF-P35398-F1, the TSPO structure by AF-P30536-F1

damage, such as DNA damage caused by ROS, is a major driver of aging[80]. Research has shown increased ROS and reactive nitrogen species production in ARPE-19 cells exposed to 7-KC (dissolved in ethanol), accompanied by decreased mitochondrial membrane potential and mitochondrial DNA damage. ROS production is a key consequence of 7-KC exposure, further exacerbating cellular deterioration and ultimately leading to cell death (necrosis, autophagic cell death, ferroptosis). Peroxisome is a conserved organelle in eukaryotic cells and an important hub for regulating redox and lipid homeostasis in mammals. It plays a key role in the metabolism of cellular lipids and reactive oxygen species, which is very important for human health and development. Patients with peroxisomal anomalies have visual impairment. In RPE of AMD patients, it has been found that mitochondrial changes are related to the proliferation of peroxisome[81]. In other cells, it has been found that 7-KC can change the function of cell peroxisomes[82]. The potential effect of 7-KC on the peroxisomes of ultimate cells (e.g., nerve cells, epithelial cells) is not known, but is probabilistically concerned.

2.5 7-KC and cell death

Cell death is a crucial physiological process that maintains normal cellular functions by eliminating damaged cells and preventing abnormal cell proliferation. However, excessive cell death can lead to tissue dysfunction and accelerate the aging process. In AMD, dysfunctional and dead cells accumulate significantly in the retina[32]. 7-KC is closely associated with cell death as a toxic substance, contributing to apoptosis induced by ER stress, autophagy, and cellular senescence.

The ER is a membrane-bound organelle contiguous with the nuclear membrane. It consists of two regions: the smooth ER, which participates in detoxification and lipid synthesis. The rough ER, which is involved in protein synthesis. ER stress can be triggered by genetic and environmental factors, including ischemia, hypoxia, oxidative stress, aging, and genetic mutations. This stress activates the unfolded protein response. Under conditions of prolonged or intense ER stress, unfolded protein response can induce apoptosis via the C/EBP homologous protein (CHOP) pathway[83]. Research has shown that 7-KC induces ER stress, characterized by increased levels of CHOP and 78-kDa glucose-regulated protein (GRP78). When Müller cells were treated with 20 μmmol/L 7-KC, elevated levels of CHOP, GRP78, and phosphorylated eIF2α were observed, along with upregulation of NOX4 and SOD2, Stearic acid has been shown to inhibit the release of inflammatory cytokines and ER stress induced by 7-KC, although the exact mechanism remains unclear[84]. Further studies indicate that 7-KC activates multiple signaling pathways in ARPE-19 cells, ER stress markers (CHOP and GRP78) are increased through the TLR4 receptor, with involvement of the NF-κB pathway; this stress may be partially suppressed by rapamycin[85]. However, inhibitors of TLR4, such as CLI-095, reduce the expression of inflammatory factors but do not alleviate ER stress.

Cell cycle arrest represents a hallmark feature of cellular senescence. Research indicates that treating hRPE cells with 10 μmmol/L 7-KC leads to cell cycle arrest and activation of the mTOR-SASP signaling pathway, accompanied by increased expression of senescence markers, including p16 and p21. Additionally, 7-KC treatment increases histone H3 phosphorylation and decreases Lamin-B1 levels, suggesting that 7-KC promotes cellular senescence and the senescence-associated secretory phenotype (SASP) in hRPE cells[75]. Autophagy and apoptosis are protective anti-aging mechanisms in cells, but excessive stress can impair autophagic processes and promote apoptosis, thereby disrupting the cell´s self-protective mechanisms. Treatment of ARPE-19 cells with 7-KC increases the level of p62, a selective autophagy substrate, suggesting potential impairment of autophagic degradation. However, in hRPE cells, 7-KC treatment did not promote the levels of caspase-1 or caspase-3 but significantly increased the expression of anti-apoptotic proteins BCL-2 and BCL-x, at higher concentrations (30－40 μmmol/L), 7-KC activated caspase-8, caspase-12, and caspase-3 in hRPE cells, but did not activate caspase-9. These findings suggest that 7-KC induces apoptosis primarily through the Fas-mediated extrinsic apoptotic pathway and the ER stress pathway, rather than the mitochondrial pathway[86]. Treating R28 cells, a cell line expressing glial and neural retinal markers, with 20 μmmol/L 7-KC resulted in significantly increased apoptosis, correlating with increased activities of caspase-8 and caspase-3. This effect could be partially mitigated by competitive LDL treatment, suggesting a protective role in apoptosis[87]. Similarly, 661W cells, a photoreceptor-derived cell line, exhibited increased apoptosis when treated with 20 μmmol/L 7-KC. Additionally, treatment of Müller cells with 20 μmmol/L 7-KC resulted in increased expression of PARP, caspase-3, and BAX, further promoting apoptosis[88].

The biological impact of 7-KC on retinal cells exhibits concentration-dependent biphasic effects. At subtoxic concentrations (<10 μmmol/L), 7-KC may stimulate protective mechanisms, such as compensatory mitochondrial energy metabolism, enabling cells to adapt to changes in the microenvironment. At intermediate concentrations (10－20 μmmol/L), 7-KC promotes inflammation, ER stress, and mitochondrial dysfunction. Supraphysiological concentrations (>20 μmmol/L), 7-KC exerts cytotoxic effects, promoting apoptosis and necrosis in retinal cells.

2.6 CNV and fibrosis

CNV and subretinal fibrosis constitute the principal pathological processes underlying irreversible vision loss in AMD patients. The pathophysiological progression of CNV is driven by the

overexpression of vascular endothelial growth factor (VEGF) and related pro-angiogenic factors (such as PlGF, Ang-2), and sustained activation of matrix metalloproteinases, which collectively promote endothelial cell migration, vascular permeability, and extracellular matrix remodeling[89-90]. Pathological angiogenesis, driven by dysregulated macrophage polarization, represents a critical mechanism in age-related diseases, including AMD, atherosclerosis and cancer[91-92]. During this process, macrophages can transition from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, which promotes angiogenesis through VEGF and PDGF secretion, while M1-derived inflammatory mediators (e. g., TNF-α) disrupt retinal barriers, both of which contribute to CNV formation[93-94]. Researchers observed that melatonin treatment significantly attenuates CNV by regulating macrophage polarization through inhibition of the RhoA/ROCK signaling pathway[95]. 7-KC has been established as a potent modulator of macrophage polarization, research has indicated that 7-KC induces premature senescence in immature macrophages through ABCA1 transporter dysregulation, leading to intracellular lipid accumulation and polarization into an abnormal, alternatively activated phenotype that promotes pathological angiogenesis[96]. Concurrently, 7-KC accumulates in the retinal pigment epithelium (RPE) and choroidal regions, co-localizing with microglial cells, and significantly contributes to pathological angiogenesis in age-related diseases. Furthermore, 7-KC uptake decreases the expression of neurotrophic factors in microglial cells, exerts pro-inflammatory effects through *NLRP3* gene expression, and drives the secretion of IL-1β, IL-18, and VEGF. These changes are significantly associated with the pathobiology of CNV.

In AMD, multimodal histopathology and optical coherence tomography imaging studies have identified choroidal endothelial cells (CECs) loss as a pathognomonic early feature. The subsequent activation and migratory behavior of remaining CECs characterized by VEGFR2 upregulation and MMP, mediated basement membrane degradation, represent critical determinants of neovascular AMD (nAMD) progression and associated vision loss[97]. 7-KC significantly enhances fibrosis in laser-induced choroidal lesions without eliciting cytotoxic effects, as demonstrated by upregulation of fibrotic marker (αSMA and FAP) in the RPE/choroid regions and downregulation of endothelial markers (VE-cadherin and VEGFR2). These findings establish 7-KC as a potent inducer of choroidal endothelial-mesenchymal transition (EndMT). Complementary *in vitro* studies confirm that 7-KC-treated CECs undergo TGF-β/SMAD3-mediated EndMT and exhibit enhanced migratory capacity through Rac1 GTPase activation[75, 98]. However, hRPE cells exposed to 7-KC did not manifest increased mesenchymal markers.

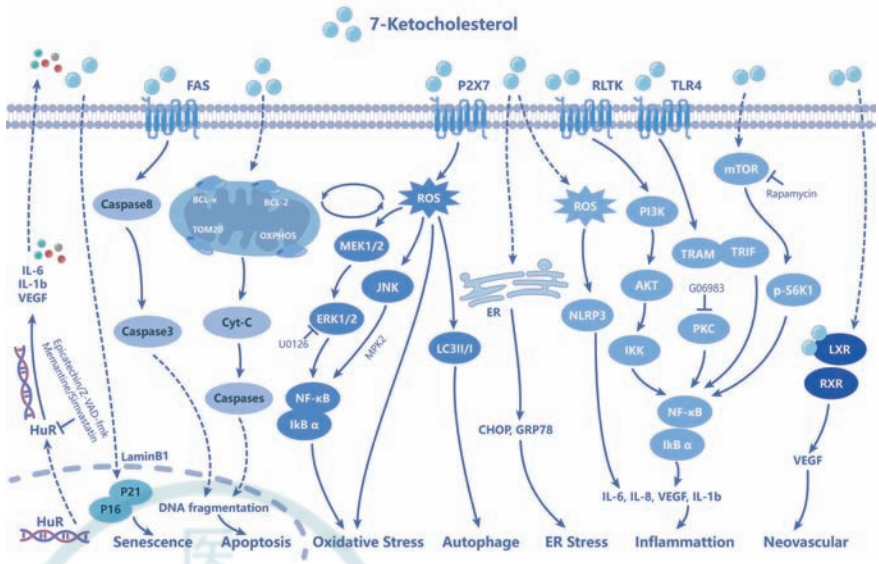

Figure 3　Schematic diagram of the influence of 7-ketocholesterol exposure on retinal-related signal pathway　The data are mainly described in microglia/macrophages[11, 13, 89], vascular endothelial cells[15], retinal pigment epithelial cells[55, 64, 80-81] and neuroretinal-related cells. Gray represents apoptosis-related signal pathway. Light blue and dark blue represent the inflammation and neovascularization-related signal pathways. Blue represents the ROS oxidative stress-related signal pathway. Light brown represents the apoptosis-related signal pathway. Cyan represents the changes of aging proteins

3　Antagonists of 7-KC

The growing understanding of 7-KC's pathological role has led to the development of two primary therapeutic strategies: 7-KC clearance and pathway modulation. Several compounds have been reported as antagonists to 7-KC-induced RPE dysfunction.

Stearic acid (SA), a saturated fatty acid, has been shown to play a role in cell proliferation, inflammation, and cell death in retinal pigment epithelial (RPE) cells. SA partially mitigates the inflammatory response and ER stress induced by 7-KC in ARPE-19 cells, offering protective effects against cell death at very low concentrations *in vitro*. Moreover, SA has been shown to reduce the area of 7-KC-induced CNV by up to 67% *in vivo*, making it one of the most effective antagonists of 7-KC identified[99]. Other molecules known for their plasma lipid-lowering properties, such as statins (e. g., atorvastatin, simvastatin, and lovastatin), which inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, have also been found to alleviate oxidative stress in various degenerative diseases. Studies indicate that statins can restore phagocytic activity in

ARPE-19 cells challenged with oxidized low-density lipoprotein, while also reducing oxidative stress and VEGF levels in the retina.

Docosahexaenoic acid, a naturally occurring molecule well-known for its antioxidant properties, has shown protective functions against 7-KC-induced cytotoxicity in multiple cell types. Additionally, oleic acid offers slight protection against 7-KC-treated ARPE-19 cells, although it does not reduce inflammatory responses. Research suggests that a Mediterranean diet, rich in omega-3 and omega-9 polyunsaturated fatty acids (including α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, and oleic acid), may confer protection against 7-KC-induced cytotoxicity in ARPE-19 cells[100-101].

Antioxidants, such as resveratrol, a compound with notable antioxidant properties, have been observed to prevent 7-KC-induced cell death in ARPE-19 cells, although the effects of resveratrol may vary in other cell types[78].

Furthermore, biodegradation of 7-KC by microorganisms presents a promising approach to counteract its cytotoxicity[102]. Research has demonstrated that enzymes derived from microorganisms, such as Nocardia, Rhodococcus, and Pseudomonas aeruginosa, can degrade 7-KC through the action of cholesterol oxidase, which plays a central role in this process[103]. Further investigation into microbial degradation of 7-KC is ongoing, which may provide novel methods for its clearance.

At present, the clinical research that directly targets 7-KC is still in the early stage, but some clinical trials aimed at its downstream pathways (such as oxidative stress, inflammatory body activation or LXR receptor regulation) may indirectly affect the pathological effect of 7-KC. Research observed that vitamin E can indirectly reduce the level of 7-KC by reducing lipid peroxidation, but this experiment did not directly detect the change in 7-KC (unpublished data). Cyclarity therapeutics developed a new compound, UDP-003, which can selectively remove 7-KC, restore the macrophage phenotype of foam cells, rebuild phagocytic function, reduce the accumulation of reactive oxygen species (ROS) and lipid droplets, and promote the urine excretion of 7-KC[104].

Hydroxypropyl-β-cyclodextrin has demonstrated significant efficacy in preclinical models, reducing retinal 7-KC deposition by 60% through enhanced cellular efflux. This compound is currently undergoing phase Ⅰ/Ⅱa clinical trials (NCT04839016) to evaluate the safety of intravitreal administration in humans[75]. Pathway inhibition strategies focus on interrupting 7-KC-mediated damage cascades. NLRP3 inflammasome inhibitors like MCC950 have proven effective in blocking 7-KC-induced IL-1β release by 72% in experimental models, prompting development of optimized ophthalmic formulations[105]. Notably, the ROCK inhibitor Netarsudil, already approved for glaucoma treatment, has shown additional promise *in vitro* by effectively suppressing 7-KC driven EndMT[106]. These developments position 7-KC as a compelling multi-target intervention candidate.

However, the translation to clinical practice faces several challenges, including the optimization of drug delivery methods and the development of effective combination therapies with existing treatment modalities.

4 Conclusions

7-KC is implicated in retinal cellular dysfunction through multiple mechanisms, including mitochondrial dysfunction, oxidative stress, ER stress, and cell death. These effects lead to the secretion of inflammatory factors by surrounding cells, thereby promoting neovascularization and fibrosis. In AMD patients, 7-KC is predominantly found in soft drusen deposits above Bruch´s membrane, where it interacts directly with RPE cells. This interaction disrupts tight junctions between RPE cells, compromising the blood-retinal barrier. Furthermore, 7-KC activates microglial cells and macrophages, inducing a pro-inflammatory phenotype that exacerbates choroidal neovascularization and promotes endothelial-mesenchymal transition. Consequently, 7-KC is involved in the pathogenesis of age-related diseases such as AMD. Although 7-KC is the primary oxysterol found in drusen, it is relatively stable and difficult to clear. Several natural molecules have been identified as antagonists to 7-KC-induced inflammation and cytotoxicity, both *in vitro* and *in vivo*. However, the mechanisms of action and modes of cell death induced by 7-KC may vary depending on cell type, delivery methods, or animal models. Resolving these discrepancies is crucial for a better understanding of the broader effects of 7-KC. Our research team currently focus on discovering and characterizing proteins that bind to and interact with 7-KC, which will enhance our understanding of 7-KC metabolism and potentially lead to new methods for its degradation.

In conclusion, these studies suggest that7-KC may act as an "age-related" risk factor in degenerative diseases such as AMD. However, significant work remains to be done in several areas, including the development of more efficient delivery systems targeting 7-KC, identification of binding proteins within cellular membranes and the cytoplasm, and the discovery of more effective antagonists with therapeutic potential to counteract 7-KC-induced cytotoxicity.

Conflict of interest: None declared

References

[1] Luo J, Yang H, Song BL. Mechanisms and regulation of cholesterol homeostasis[J]. Nat Rev Mol Cell Biol, 2020, 21(4): 225-245. DOI: 10.1038/s41580-019-0190-7.

[2] de Medina P, Silvente-Poirot S, Poirot M. Oxysterols are potential

physiological regulators of ageing[J]. Ageing Res Rev, 2022, 77: 101615. DOI: 10.1016/j.arr.2022.101615.
[3] Griffiths WJ, Wang Y. Oxysterol research: a brief review[J]. Biochem Soc Trans, 2019, 47(2): 517-526. DOI: 10.1042/BST20180135.
[4] Liu S, Li X, Fan P, et al. The potential role of transcription factor sterol regulatory element binding proteins (SREBPs) in Alzheimer's disease[J]. Biomed Pharmacother, 2024, 180: 117575. DOI: 10.1016/j.biopha.2024.117575.
[5] Anderson A, Campo A, Fulton E, et al. 7-Ketocholesterol in disease and aging[J]. Redox Biol, 2020, 29: 101380. DOI: 10.1016/j.redox.2019.101380.
[6] Yan Q, Ding Y, Weeks DE, et al. AMD genetics: methods and analyses for association, progression, and prediction[J]. Adv Exp Med Biol, 2021, 1256: 191-200. DOI: 10.1007/978-3-030-66014-7_7.
[7] Ramachandra Rao S, Fliesler SJ. Cholesterol homeostasis in the vertebrate retina: biology and pathobiology[J]. J Lipid Res, 2021, 62: 100057. DOI: 10.1194/jlr.TR120000979.
[8] Ghzaiel I, Sassi K, Zarrouk A, et al. Sources of 7-ketocholesterol, metabolism and inactivation strategies: food and biomedical applications[J]. Redox Exp Med, 2022, 1: R40-R56. DOI: 10.1530/REM-22-0005.
[9] Mast N, El-Darzi N, Li Y, et al. Quantitative characterizations of the cholesterol-related pathways in the retina and brain of hamsters[J]. J Lipid Res, 2023, 64(7): 100401. DOI: 10.1016/j.jlr.2023.100401.
[10] Masson E, Serrano J, Leger-Charnay E, et al. Cholesterol and oxysterols in retinal neuron-glia interactions: relevance for glaucoma[J]. Front Ophthalmol (Lausanne), 2023, 3: 1303649. DOI: 10.3389/fopht.2023.1303649.
[11] Duan Y, Gong K, Xu S, et al. Regulation of cholesterol homeostasis in health and diseases: from mechanisms to targeted therapeutics[J]. Signal Transduct Target Ther, 2022, 7(1): 265. DOI: 10.1038/s41392-022-01125-5.
[12] Zhang X, Alhasani RH, Zhou X, et al. Oxysterols and retinal degeneration[J]. Br J Pharmacol, 2021, 178(16): 3205-3219. DOI: 10.1111/bph.15391.
[13] Vigne S, Pot C. Implication of oxysterols and phytosterols in aging and human diseases[J]. Adv Exp Med Biol, 2024, 1440: 231-260. DOI: 10.1007/978-3-031-43883-7_12.
[14] Ana RD, Gliszczyńska A, Sanchez-Lopez E, et al. Precision medicines for retinal lipid metabolism-related pathologies[J]. J Pers Med, 2023, 13(4): 635. DOI: 10.3390/jpm13040635.
[15] Mast N, Bederman IR, Pikuleva IA. Retinal cholesterol content is reduced in simvastatin-treated mice due to inhibited local biosynthesis albeit increased uptake of serum cholesterol[J]. Drug Metab Dispos, 2018, 46(11): 1528-1537. DOI: 10.1124/dmd.118.083345.
[16] Fliesler SJ, Bretillon L. The ins and outs of cholesterol in the vertebrate retina[J]. J Lipid Res, 2010, 51(12): 3399-3413. DOI: 10.1194/jlr.R010538.
[17] Storti F, Raphael G, Griesser V, et al. Regulated efflux of photoreceptor outer segment-derived cholesterol by human RPE cells[J]. Exp Eye Res, 2017, 165: 65-77. DOI: 10.1016/j.exer.2017.09.008.
[18] Collin J, Zerti D, Queen R, et al. CRX expression in pluripotent stem cell-derived photoreceptors marks a transplantable subpopulation of early cones[J]. Stem Cells, 2019, 37(5): 609-622. DOI: 10.1002/stem.2974.
[19] Zekavat SM, Lu J, Maugeais C, et al. An in silico model of retinal cholesterol dynamics (RCD model): insights into the pathophysiology of dry AMD[J]. J Lipid Res, 2017, 58(7): 1325-1337. DOI: 10.1194/jlr.M074088.
[20] Deng Y, Qiao L, Du M, et al. Age-related macular degeneration: epidemiology, genetics, pathophysiology, diagnosis, and targeted therapy[J]. Genes Dis, 2022, 9(1): 62-79. DOI: 10.1016/j.gendis.2021.02.009.
[21] Heo GY, Bederman I, Mast N, et al. Conversion of 7-ketocholesterol to oxysterol metabolites by recombinant CYP27A1 and retinal pigment epithelial cells[J]. J Lipid Res, 2011, 52(6): 1117-1127. DOI: 10.1194/jlr.M014217.
[22] Helmschrodt C, Becker S, Schröter J, et al. Fast LC-MS/MS analysis of free oxysterols derived from reactive oxygen species in human plasma and carotid plaque[J]. Clin Chim Acta, 2013, 425: 3-8. DOI: 10.1016/j.cca.2013.06.022.
[23] Rodriguez IR, Fliesler SJ. Photodamage generates 7-keto- and 7-hydroxycholesterol in the rat retina via a free radical-mediated mechanism[J]. Photochem Photobiol, 2009, 85(5): 1116-1125. DOI: 10.1111/j.1751-1097.2009.00568.x.
[24] Mutemberezi V, Guillemot-Legris O, Muccioli GG. Oxysterols: from cholesterol metabolites to key mediators[J]. Prog Lipid Res, 2016, 64: 152-169. DOI: 10.1016/j.plipres.2016.09.002.
[25] Griffiths WJ, Abdel-Khalik J, Hearn T, et al. Current trends in oxysterol research[J]. Biochem Soc Trans, 2016, 44(2): 652-658. DOI: 10.1042/BST20150255.
[26] Guardiola F, Codony R, Addis PB, et al. Biological effects of oxysterols: current status[J]. Food Chem Toxicol, 1996, 34(2): 193-211. DOI: 10.1016/0278-6915(95)00094-1.
[27] Hu ZL, Yuan YQ, Tong Z, et al. Reexamining the causes and effects of cholesterol deposition in the brains of patients with Alzheimer's disease[J]. Mol Neurobiol, 2023, 60(12): 6852-6868. DOI: 10.1007/s12035-023-03529-y.
[28] Czuba E, Steliga A, Lietzau G, et al. Cholesterol as a modifying agent of the neurovascular unit structure and function under physiological and pathological conditions[J]. Metab Brain Dis, 2017, 32(4): 935-948. DOI: 10.1007/s11011-017-0015-3.
[29] Pikuleva IA. Challenges and opportunities in P450 research on the Eye[J]. Drug Metab Dispos, 2023, 51(10): 1295-1307. DOI: 10.1124/dmd.122.001072.
[30] Vejux A, Samadi M, Lizard G. Contribution of cholesterol and oxysterols in the physiopathology of cataract: implication for the development of pharmacological treatments[J]. J Ophthalmol, 2011, 2011: 471947. DOI: 10.1155/2011/471947.
[31] Köroğlu M, Karakaplan M, Gündüz E, et al. Cerebrotendinous xanthomatosis patients with late diagnosed in single orthopedic clinic: two novel variants in the *CYP27A1* gene[J]. Orphanet J Rare Dis, 2024, 19(1): 53. DOI: 10.1186/s13023-024-03082-4.
[32] Fleckenstein M, Schmitz-Valckenberg S, Chakravarthy U. Age-related macular degeneration: a review[J]. JAMA, 2024, 331(2): 147-157. DOI: 10.1001/jama.2023.26074.
[33] Singh M, Negi R, Alka, et al. Age-related macular degeneration (AMD): pathophysiology, drug targeting approaches, and recent developments in nanotherapeutics[J]. Medicina (Kaunas), 2024, 60(10): 1647. DOI: 10.3390/medicina60101647.
[34] Nury T, Zarrouk A, Yammine A, et al. Oxiapoptophagy: a type of cell death induced by some oxysterols[J]. Br J Pharmacol, 2021, 178(16): 3115-3123. DOI: 10.1111/bph.15173.
[35] Yammine A, Ghzaiel I, Pires V, et al. Cytoprotective effects of α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, oleic acid and α-tocopherol on 7-ketocholesterol - induced oxiapoptophagy: Major roles of PI3-K/PDK-1/Akt signaling pathway and glutathione peroxidase activity in cell rescue[J]. Curr Res Toxicol, 2024, 6: 100153. DOI: 10.1016/j.crtox.2024.100153.
[36] Brahmi F, Nury T, Debbabi M, et al. Evaluation of antioxidant, anti-inflammatory and cytoprotective properties of ethanolic mint extracts from algeria on 7-ketocholesterol-treated murine RAW 264.7 macrophages[J]. Antioxidants (Basel), 2018, 7(12): 184. DOI: 10.3390/antiox7120184.
[37] Saraev DD, Wu Z, Kim HH, et al. Intramolecular H-atom transfers in alkoxyl radical intermediates underlie the apparent oxidation of lipid

hydroperoxides by Fe(Ⅱ)[J]. ACS Chem Biol, 2023, 18(9): 2073-2081. DOI: 10.1021/acschembio.3c00412.
[38] Yang C, Xie L, Gu Q, et al. 7-ketocholesterol disturbs RPE cells phagocytosis of the outer segment of photoreceptor and induces inflammation through ERK signaling pathway[J]. Exp Eye Res, 2019, 189: 107849. DOI: 10.1016/j.exer.2019.107849.
[39] Nury T, Zarrouk A, Ragot K, et al. 7-ketocholesterol is increased in the plasma of X-ALD patients and induces peroxisomal modifications in microglial cells: potential roles of 7-ketocholesterol in the pathophysiology of X-ALD[J]. J Steroid Biochem Mol Biol, 2017, 169: 123-136. DOI: 10.1016/j.jsbmb.2016.03.037.
[40] Nury T, Sghaier R, Zarrouk A, et al. Induction of peroxisomal changes in oligodendrocytes treated with 7-ketocholesterol: attenuation by α-tocopherol[J]. Biochimie, 2018, 153: 181-202. DOI: 10.1016/j.biochi.2018.07.009.
[41] Ghzaiel I, Maaloul S, Ksila M, et al. *In vitro* evaluation of the effects of 7-ketocholesterol and 7β-hydroxycholesterol on the peroxisomal status: prevention of peroxisomal damages and concept of pexotherapy[J]. Adv Exp Med Biol, 2024, 1440: 437-452. DOI: 10.1007/978-3-031-43883-7_21.
[42] Indaram M, Ma W, Zhao L, et al. 7-ketocholesterol increases retinal microglial migration, activation, and angiogenicity: a potential pathogenic mechanism underlying age-related macular degeneration[J]. Sci Rep, 2015, 5: 9144. DOI: 10.1038/srep09144.
[43] Moreira EF, Larrayoz IM, Lee JW, et al. 7-ketocholesterol is present in lipid deposits in the primate retina: potential implication in the induction of VEGF and CNV formation[J]. Invest Ophthalmol Vis Sci, 2009, 50(2): 523-532. DOI: 10.1167/iovs.08-2373.
[44] Rodriguez IR, Clark ME, Lee JW, et al. 7-ketocholesterol accumulates in ocular tissues as a consequence of aging and is present in high levels in drusen[J]. Exp Eye Res, 2014, 128: 151-155. DOI: 10.1016/j.exer.2014.09.009.
[45] Wang B, Shen Y, Zhai L, et al. Atherosclerosis-associated hepatic secretion of VLDL but not PCSK9 is dependent on cargo receptor protein Surf4[J]. J Lipid Res, 2021, 62: 100091. DOI: 10.1016/j.jlr.2021.100091.
[46] Enyong EN, Gurley JM, De Ieso ML, et al. Caveolar and non-caveolar caveolin-1 in ocular homeostasis and disease[J]. Prog Retin Eye Res, 2022, 91: 101094. DOI: 10.1016/j.preteyeres.2022.101094.
[47] Huang G, Chen L, Lin Y, et al. Comparative proteomic analysis of retinal hypoxia-ischemia in an acute ocular hypertension model using tandem mass tag-based quantitative proteomics[J]. Exp Eye Res, 2024, 247: 110063. DOI: 10.1016/j.exer.2024.110063.
[48] Cai B, Zhang Z, Sun S, et al. A pilot application of an iTRAQ-based proteomics screen estimates the effects of cigarette smokers´ serum on rpe cells with AMD high-risk alleles[J]. Transl Vis Sci Technol, 2022, 11(2): 15. DOI: 10.1167/tvst.11.2.15.
[49] Shimizu H, Yamada K, Suzumura A, et al. Caveolin-1 promotes cellular senescence in exchange for blocking subretinal fibrosis in age-related macular degeneration[J]. Invest Ophthalmol Vis Sci, 2020, 61(11): 21. DOI: 10.1167/iovs.61.11.21.
[50] Courtaut F, Scagliarini A, Aires V, et al. VEGF-R2/caveolin-1 pathway of undifferentiated ARPE-19 retina cells: a potential target as anti-VEGF-A therapy in wet amd by resvega, an omega-3/polyphenol combination[J]. Int J Mol Sci, 2021, 22(12): 6590. DOI: 10.3390/ijms22126590.
[51] Zhang R, Dang Y. The recent advances in the function and mechanism of caveolin-1 in retinal neovascularization[J]. Curr Drug Targets, 2024, 25(7): 465-472. DOI: 10.2174/0113894501310201240403065930.
[52] Tran J, Magenau A, Rodriguez M, et al. Activation of endothelial nitric oxide (eNOS) occurs through different membrane domains in endothelial cells[J/OL]. PLoS One, 2016, 11(3): e0151556[2025-04-22]. https://pubmed.ncbi.nlm.nih.gov/26977592/. DOI: 10.1371/journal.pone.0151556.
[53] Ingueneau C, Huynh-Do U, Marcheix B, et al. TRPC1 is regulated by caveolin-1 and is involved in oxidized LDL-induced apoptosis of vascular smooth muscle cells[J]. J Cell Mol Med, 2009, 13(8B): 1620-1631. DOI: 10.1111/j.1582-4934.2008.00593.x.
[54] Moreira EF, Jaworski C, Li A, et al. Molecular and biochemical characterization of a novel oxysterol-binding protein (OSBP2) highly expressed in retina[J]. J Biol Chem, 2001, 276(21): 18570-18578. DOI: 10.1074/jbc.M011259200.
[55] Torrini M, Marchese C, Vanzetti M, et al. Mutation analysis of oxisterol-binding-protein gene in patients with age-related macular degeneration[J]. Genet Test, 2007, 11(4): 421-426. DOI: 10.1089/gte.2007.0021.
[56] Romeo GR, Kazlauskas A. Oxysterol and diabetes activate STAT3 and control endothelial expression of profilin-1 via OSBP1[J]. J Biol Chem, 2008, 283(15): 9595-9605. DOI: 10.1074/jbc.M710092200.
[57] Hammer SS, Beli E, Kady N, et al. The mechanism of diabetic retinopathy pathogenesis unifying key lipid regulators, sirtuin 1 and liver X receptor[J]. EBioMedicine, 2017, 22: 181-190. DOI: 10.1016/j.ebiom.2017.07.008.
[58] Ghosh S, Ghzaiel I, Vejux A, et al. Impact of oxysterols in age-related disorders and strategies to alleviate adverse effects[J]. Adv Exp Med Biol, 2024, 1440: 163-191. DOI: 10.1007/978-3-031-43883-7_9.
[59] Wu T, Wei X, Dang K, et al. Liver X receptor agonist inhibits oxidized low-density lipoprotein induced choroidal neovascularization via the NF-κB signaling pathway[J]. J Clin Med, 2023, 12(4): 1674. DOI: 10.3390/jcm12041674.
[60] Choudhary M, Ismail EN, Yao PL, et al. LXRs regulate features of age-related macular degeneration and may be a potential therapeutic target[J/OL]. JCI Insight, 2020, 5(1): e131928[2025-04-22]. https://pubmed.ncbi.nlm.nih.gov/31829999/. DOI: 10.1172/jci.insight.131928.
[61] Liu CH, Yemanyi F, Bora K, et al. Genetic deficiency and pharmacological modulation of RORα regulate laser-induced choroidal neovascularization[J]. Aging (Albany NY), 2023, 15(1): 37-52. DOI: 10.18632/aging.204480.
[62] Akula M, McNamee SM, Love Z, et al. Retinoic acid related orphan receptor α is a genetic modifier that rescues retinal degeneration in a mouse model of Stargardt disease and dry AMD[J]. Gene Ther, 2024, 31(7-8): 413-421. DOI: 10.1038/s41434-024-00455-z.
[63] Choudhary M, Safe S, Malek G. Suppression of aberrant choroidal neovascularization through activation of the aryl hydrocarbon receptor[J]. Biochim Biophys Acta Mol Basis Dis, 2018, 1864(5 Pt A): 1583-1595. DOI: 10.1016/j.bbadis.2018.02.015.
[64] Hammond CL, Roztocil E, Gupta V, et al. More than meets the eye: the aryl hydrocarbon receptor is an environmental sensor, physiological regulator and a therapeutic target in ocular disease[J]. Front Toxicol, 2022, 4: 791082. DOI: 10.3389/ftox.2022.791082.
[65] Ji J, Xiong C, Yang H, et al. The aryl hydrocarbon receptor: a crucial mediator in ocular disease pathogenesis and therapeutic target[J]. Exp Eye Res, 2024, 249: 110144. DOI: 10.1016/j.exer.2024.110144.
[66] Schustak J, Han H, Bond K, et al. Phenotypic high-throughput screening identifies aryl hydrocarbon receptor agonism as common inhibitor of toxin-induced retinal pigment epithelium cell death[J/OL]. PLoS One, 2024, 19(4): e0301239[2025-04-22]. https://pubmed.ncbi.nlm.nih.gov/38635505/. DOI: 10.1371/journal.pone.0301239.
[67] Qu S, Lin H, Pfeiffer N, et al. Age-related macular degeneration and mitochondria-associated autoantibodies: a review of the specific pathogenesis and therapeutic strategies[J]. Int J Mol Sci, 2024, 25(3): 1624. DOI: 10.3390/ijms25031624.
[68] Biswas L, Zhou X, Dhillon B, et al. Retinal pigment epithelium

cholesterol efflux mediated by the 18 kDa translocator protein, TSPO, a potential target for treating age-related macular degeneration[J]. Hum Mol Genet, 2017, 26(22): 4327-4339. DOI: 10.1093/hmg/ddx319.

[69] Alamri A, Biswas L, Watson DG, et al. Deletion of TSPO resulted in change of metabolomic profile in retinal pigment epithelial cells[J]. Int J Mol Sci, 2019, 20(6): 1387. DOI: 10.3390/ijms20061387.

[70] Biswas L, Ibrahim KS, Li X, et al. Effect of a TSPO ligand on retinal pigment epithelial cholesterol homeostasis in high-fat fed mice, implication for age-related macular degeneration[J]. Exp Eye Res, 2021, 208: 108625. DOI: 10.1016/j.exer.2021.108625.

[71] Lee JW, Huang JD, Rodriguez IR. Extra-hepatic metabolism of 7-ketocholesterol occurs by esterification to fatty acids via cPLA2α and SOAT1 followed by selective efflux to HDL[J]. Biochim Biophys Acta, 2015, 1851(5): 605-619. DOI: 10.1016/j.bbalip.2015.01.007.

[72] Ksila M, Ghzaiel I, Sassi K, et al. Therapeutic applications of oxysterols and derivatives in age-related diseases, infectious and inflammatory diseases, and cancers[J]. Adv Exp Med Biol, 2024, 1440: 379-400. DOI: 10.1007/978-3-031-43883-7_19.

[73] Hansman DS, Du J, Casson RJ, et al. Eye on the horizon: the metabolic landscape of the RPE in aging and disease[J]. Prog Retin Eye Res, 2025, 104: 101306. DOI: 10.1016/j.preteyeres.2024.101306.

[74] Huang JD, Amaral J, Lee JW, et al. 7-ketocholesterol-induced inflammation signals mostly through the TLR4 receptor both *in vitro* and *in vivo*[J/OL]. PLoS One, 2014, 9(7): e100985[2025-04-22]. https://pubmed.ncbi.nlm.nih.gov/25036103/. DOI: 10.1371/journal.pone.0100985.

[75] Wang H, Ramshekar A, Cung T, et al. 7-ketocholesterol promotes retinal pigment epithelium senescence and fibrosis of choroidal neovascularization via IQGAP1 phosphorylation-dependent signaling[J]. Int J Mol Sci, 2023, 24(12): 276. DOI: 10.3390/ijms241210276.

[76] Saha S, Profumo E, Togna AR, et al. Lupeol counteracts the proinflammatory signalling triggered in macrophages by 7-keto-cholesterol: new perspectives in the therapy of atherosclerosis[J]. Oxid Med Cell Longev, 2020, 2020: 1232816. DOI: 10.1155/2020/1232816.

[77] Ferrington DA, Ebeling MC, Kapphahn RJ, et al. Altered bioenergetics and enhanced resistance to oxidative stress in human retinal pigment epithelial cells from donors with age-related macular degeneration[J]. Redox Biol, 2017, 13: 255-265. DOI: 10.1016/j.redox.2017.05.015.

[78] Yammine A, Zarrouk A, Nury T, et al. Prevention by dietary polyphenols (resveratrol, quercetin, apigenin) against 7-ketocholesterol-induced oxiapoptophagy in neuronal N2a cells: potential interest for the treatment of neurodegenerative and age-related diseases[J]. Cells, 2020, 9(11): 2346. DOI: 10.3390/cells9112346.

[79] Leoni V, Nury T, Vejux A, et al. Mitochondrial dysfunctions in 7-ketocholesterol-treated 158N oligodendrocytes without or with α-tocopherol: Impacts on the cellular profil of tricarboxylic cycle-associated organic acids, long chain saturated and unsaturated fatty acids, oxysterols, cholesterol and cholesterol precursors[J]. J Steroid Biochem Mol Biol, 2017, 169: 96-110. DOI: 10.1016/j.jsbmb.2016.03.029.

[80] Barja G. The mitochondrial free radical theory of aging[J]. Prog Mol Biol Transl Sci, 2014, 127: 1-27. DOI: 10.1016/B978-0-12-394625-6.00001-5.

[81] Lakkaraju A, Boya P, Csete M, et al. How crosstalk between mitochondria, lysosomes, and other organelles can prevent or promote dry age-related macular degeneration[J]. Exp Eye Res, 2026, 251: 110219. DOI: 10.1016/j.exer.2024.110219.

[82] Badreddine A, Zarrouk A, Karym EM, et al. Argan oil-mediated attenuation of organelle dysfunction, oxidative stress and cell death induced by 7-ketocholesterol in murine oligodendrocytes 158N[J]. Int J Mol Sci, 2017, 18(10): 2220. DOI: 10.3390/ijms18102220.

[83] Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response[J]. Nat Rev Mol Cell Biol, 2020, 21(8): 421-438. DOI: 10.1038/s41580-020-0250-z.

[84] Huang JD, Amaral J, Lee JW, et al. Sterculic acid antagonizes 7-ketocholesterol-mediated inflammation and inhibits choroidal neovascularization[J]. Biochim Biophys Acta, 2012, 1821(4): 637-646. DOI: 10.1016/j.bbalip.2012.01.013.

[85] Yang L, Yu P, Chen M, et al. Mammalian target of rapamycin inhibitor rapamycin alleviates 7-ketocholesterol induced inflammatory responses and vascular endothelial growth factor elevation by regulating MAPK pathway in human retinal pigment epithelium cells[J]. J Ocul Pharmacol Ther, 2022, 38(2): 189-200. DOI: 10.1089/jop.2021.0082.

[86] Luthra S, Fardin B, Dong J, et al. Activation of caspase-8 and caspase-12 pathways by 7-ketocholesterol in human retinal pigment epithelial cells[J]. Invest Ophthalmol Vis Sci, 2006, 47(12): 5569-5575. DOI: 10.1167/iovs.06-0333.

[87] Son SH, Goo YH, Chang BH, et al. Perilipin 2 (PLIN2)-deficiency does not increase cholesterol-induced toxicity in macrophages[J/OL]. PLoS One, 2012, 7(3): e33063[2025-04-22]. https://pubmed.ncbi.nlm.nih.gov/22427949/. DOI: 10.1371/journal.pone.0033063.

[88] Pfeffer BA, Xu L, Porter NA, et al. Differential cytotoxic effects of 7-dehydrocholesterol-derived oxysterols on cultured retina-derived cells: dependence on sterol structure, cell type, and density[J]. Exp Eye Res, 2016, 145: 297-316. DOI: 10.1016/j.exer.2016.01.016.

[89] González-Zamora J, Hernandez M, Recalde S, et al. Matrix metalloproteinase 10 contributes to choroidal neovascularisation[J]. Biomedicines, 2022, 10(7): 1557. DOI: 10.3390/biomedicines10071557.

[90] Sheth JU, Stewart MW, Narayanan R, et al. Macular neovascularization[J]. Surv Ophthalmol, 2025, 70(4): 653-675. DOI: 10.1016/j.survophthal.2024.08.003.

[91] Zhang YY, Shi YN, Zhu N, et al. Autophagy: a killer or guardian of vascular smooth muscle cells[J]. J Drug Target, 2020, 28(5): 449-455. DOI: 10.1080/1061186X.2019.1705312.

[92] Brahmi F, Vejux A, Sghaier R, et al. Prevention of 7-ketocholesterol-induced side effects by natural compounds[J]. Crit Rev Food Sci Nutr, 2019, 59(19): 3179-3198. DOI: 10.1080/10408398.2018.1491828.

[93] Rosmus DD, Wieghofer P. Guardians of the eye: new tales about retinal microglia and other ocular macrophages[J]. Neural Regen Res, 2022, 17(6): 1275-1277. DOI: 10.4103/1673-5374.327335.

[94] Zandi S, Nakao S, Chun KH, et al. ROCK-isoform-specific polarization of macrophages associated with age-related macular degeneration[J]. Cell Rep, 2015, 10(7): 1173-1186. DOI: 10.1016/j.celrep.2015.01.050.

[95] Cui K, Tang X, Hu A, et al. Therapeutic benefit of melatonin in choroidal neovascularization during aging through the regulation of senescent macrophage/microglia polarization[J]. Invest Ophthalmol Vis Sci, 2023, 64(11): 19. DOI: 10.1167/iovs.64.11.19.

[96] Li G, Park HJ, Suh JH, et al. 7-ketocholesterol plays a key role in cholesterol-induced hepatitis via macrophage and neutrophil infiltration[J]. J Nutr Biochem, 2024, 125: 109552. DOI: 10.1016/j.jnutbio.2023.109552.

[97] Gill K, Yoo HS, Chakravarthy H, et al. Exploring the role of granzyme B in subretinal fibrosis of age-related macular degeneration[J]. Front Immunol, 2024, 15: 1421175. DOI: 10.3389/fimmu.2024.1421175.

[98] Wang H, Ramshekar A, Kunz E, et al. 7-ketocholesterol induces endothelial-mesenchymal transition and promotes fibrosis: implications in neovascular age-related macular degeneration and treatment[J].

Angiogenesis, 2021, 24(3): 583-595. DOI: 10.1007/s10456-021-09770-0.
[99] Pariente A, Pérez-Sala Á, Ochoa R, et al. Identification of 7-ketocholesterol-modulated pathways and sterculic acid protective effect in retinal pigmented epithelium cells by using genome-wide transcriptomic analysis[J]. Int J Mol Sci, 2023, 24(8): 7459. DOI: 10.3390/ijms24087459.
[100] Yammine A, Nury T, Vejux A, et al. Prevention of 7-ketocholesterol-induced overproduction of reactive oxygen species, mitochondrial dysfunction and cell death with major nutrients (polyphenols, ω3 and ω9 unsaturated fatty acids) of the mediterranean diet on N2a neuronal cells[J]. Molecules, 2020, 25(10): 2296. DOI: 10.3390/molecules25102296.
[101] Debbabi M, Zarrouk A, Bezine M, et al. Comparison of the effects of major fatty acids present in the Mediterranean diet (oleic acid, docosahexaenoic acid) and in hydrogenated oils (elaidic acid) on 7-ketocholesterol-induced oxiapoptophagy in microglial BV-2 cells[J]. Chem Phys Lipids, 2017, 207(Pt B): 151-170. DOI: 10.1016/j.chemphyslip.2017.04.002.
[102] Haubrich BA. Microbial sterolomics as a chemical biology tool[J]. Molecules, 2018, 23(11): 2768. DOI: 10.3390/molecules23112768.
[103] Ghosh S, Khare SK. Biodegradation of 7-Ketocholesterol by rhodococcus erythropolis MTCC 3951: process optimization and enzymatic insights[J]. Chem Phys Lipids, 2017, 207(Pt B): 253-259. DOI: 10.1016/j.chemphyslip.2017.05.008.
[104] Bhargava P, Dinh D, Teramayi F, et al. Selective removal of 7-ketocholesterol by a novel atherosclerosis therapeutic candidate reverts foam cells to a macrophage-like phenotype[J]. Atherosclerosis, 2025, 409: 119217. DOI: 10.1016/j.atherosclerosis.2025.119217.
[105] Naaber P, Tserel L, Kangro K, et al. Protective antibodies and T cell responses to omicron variant after the booster dose of BNT162b2 vaccine[J]. Cell Rep Med, 2022, 3(8): 100716. DOI: 10.1016/j.xcrm.2022.100716.
[106] Jonas JB, Jonas RA, Jonas SB, et al. Bruch's membrane and Brücke's muscle in the pars plana region[J/OL]. Acta Ophthalmol, 2024, 102(1): e53-e59[2025-04-26]. https://pubmed.ncbi.nlm.nih.gov/38185862/. DOI: 10.1111/aos.15678.

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本刊对论文中统计学方法描述的要求

研究论文如有量化测试指标时须有统计学分析的内容，并在方法部分提供统计学方法的描述，反应变量为单变量时请提供测量指标数据资料的性质（如计量数据资料及计数数据资料的表达方式）、多个样本计量数据资料正态分布检验方法的名称及方差齐性检验方法的名称、实（试）验设计方法及与之相匹配的统计学设计（如配对设计、成组设计、交叉设计、析因设计、正交设计等）、与统计设计相应的统计方法名称（如 t 检验、方差分析）以及检验水准。选择方差分析统计设计时应根据单因素或多因素设计选择正确的方法，不宜简单套用单因素方差分析。反应变量为双变量时，应根据实（试）验设计正确选择简单直线相关分析、回归分析或其他方法，不宜简单套用直线相关分析。统计学的检验水准请提供为双侧检验或单侧检验。论文结果部分的统计学分析内容可用相应的图表表达。

统计学符号的著录执行 GB/T 3358.1—2009/ISO 3534-1：2006《统计学词汇及符号》的有关规定，统计学符号一律采用斜体，如样本量用 n；中位数用英文斜体大写 M，样本均数的标准误用英文小写 $\sigma_{\bar{x}}$，t 检验用英文小写 t，F 检验用英文大写 F，卡方检验用希文小写 χ^2，相关系数用英文小写 r，秩相关分析相关系数用 r_s，确定系数用 R^2，自由度用希文小写 υ；概率用英文大写 P；检验水准用 α。统计结果的解释和表达采用对比组或比较对象之间差异有统计学意义的描述方法，而不用对比组之间差异具有显著性（或非常显著性）的描述。论文的统计学分析结果提倡提供统计学检验量值和 P 值的具体数据，如不能提供 P 值的具体数据时，必须提供统计学检验量值如 χ^2 值、t 值、F 值等。当涉及总体参数（如总体均数、总体率等）时，在给出显著性检验结果的同时，请给出 95% 可信区间（CI）。

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