Involvement of Oxysterols

in the Physiopathology of Age Related Macular Degeneration

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Team 'Biochemistry of the Peroxisome, Inflammation and Lipid Metabolism'

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21000 Dijon - FRANCE

Fourth Biennial Symposium on

Age-Related Macular Degeneration

Boston, October 20-22, 2016

From Cholesterol to Oxysterols : a New Story

Cholestérine
1815

Cholestérol
1884

25-hydroxycholesterol
***Oxysterol** Binding Protein (OSBP)*
1977

1928 1934 1964 1975 1985

Eugène Chevreul
(1786 -1889)

Adolf Windaus
Nobel Price 1928

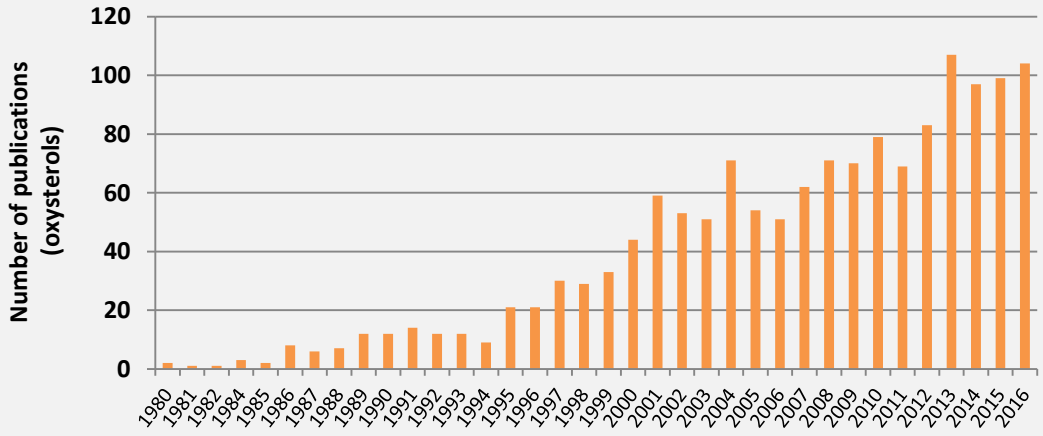
Leary & Willius
Cholesterol & Atherosclerosis

Bloch & Lynen
Cholesterol biosynthesis
Nobel Price 1964

John Warcurp Cornforth
Cholesterol biosynthesis
Nobel Price 1975

Kandutsch A.A. (USA)
Craste de Paulet B. (France)

Brown & Goldstein
Cholesterol biosynthesis
Nobel Price 1985



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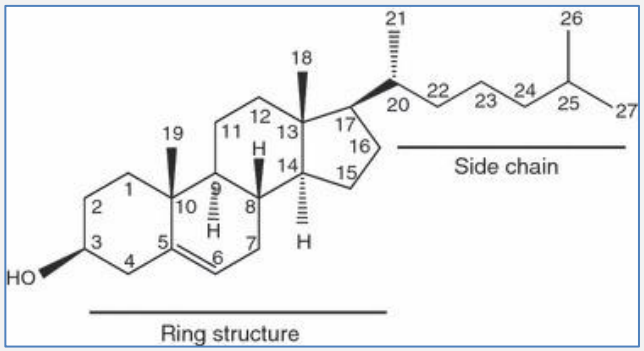
Oxysterols Biogenesis

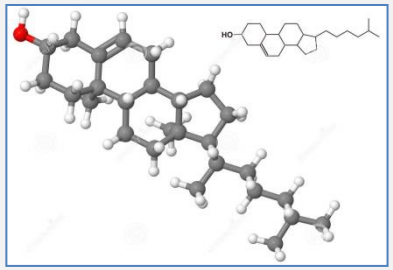


Cholesterol (C27)

- ❑ Steroid backbone is rigid
- ❑ The side chain is flexible

OXYSTEROLS: oxidation products of sterols (including cholesterol)

One or more oxigen atoms



Cholesterol

- ❑ **Auto-oxidation**
- ❑ **Enzymes**
  - **** mainly cytochromes P450 (CYP)***
  - **** Cholesterol 25-hydroxylase***

  $cholesterol + AH_2 + O_2 \rightleftharpoons 25\text{-}hydroxycholesterol + A + H_2O$
- ❑ **Both processes**

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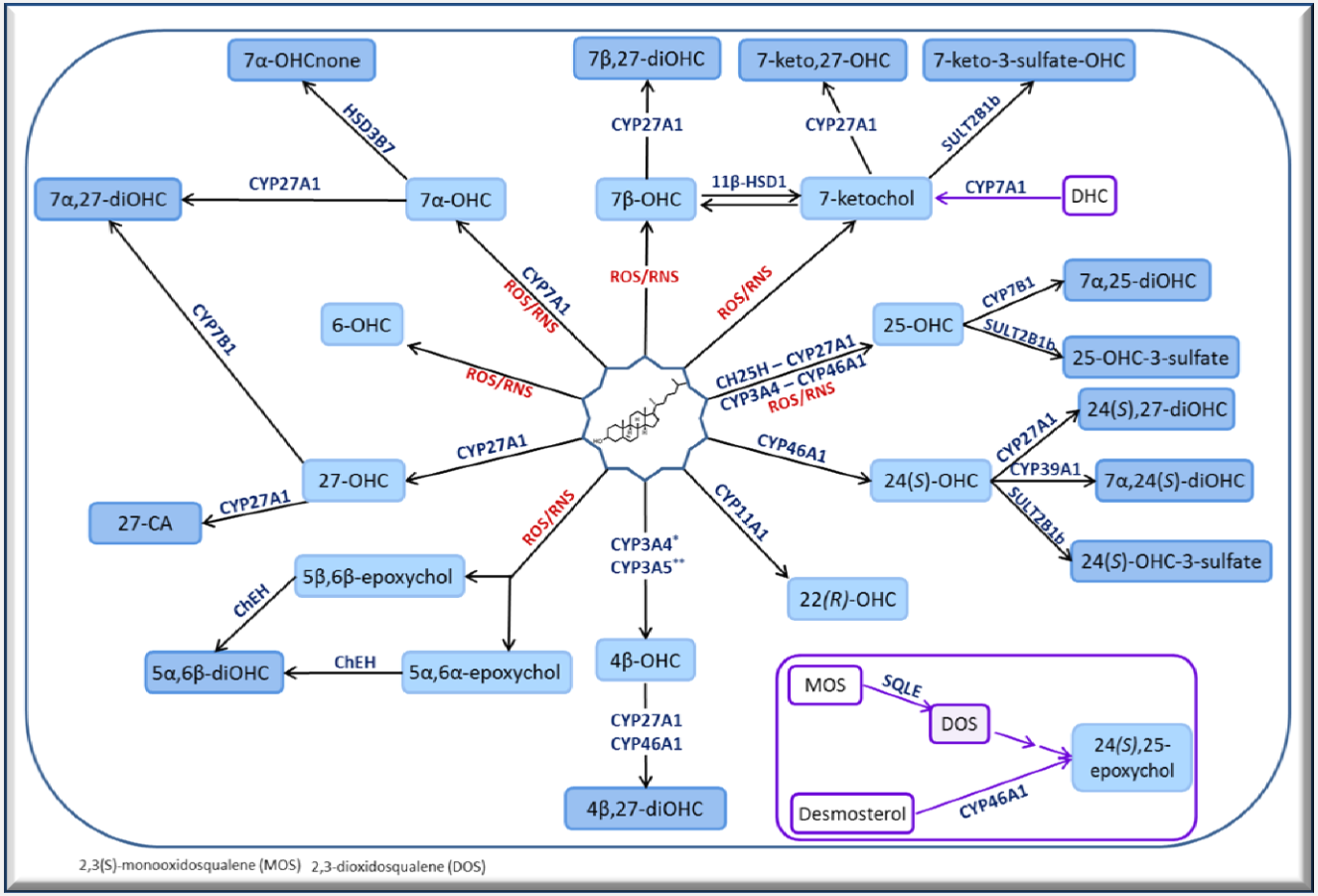
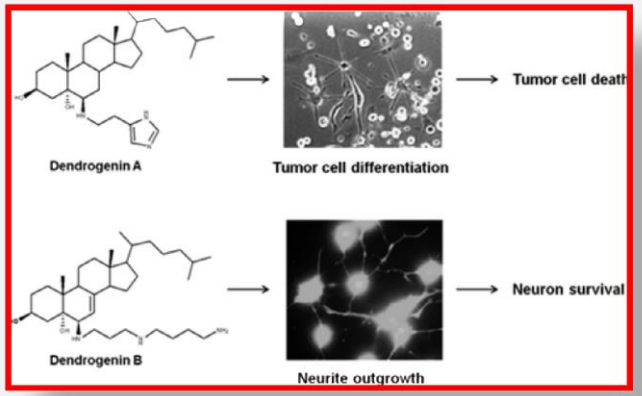
Oxysterol Network / 'Oxysterome'



+ esters

+ cholesterol precursors



❑ **Mutemberezi V. *et al.*, Progress in Lipid Research, 2016**

❑ **de Medina P. *et al.*, J Med Chem 2009;52(23):7765-77.**

✓ ***Aminoalkyoxysterols***

plants, *amphibians* and ancestral *fishes* (dogfish shark *Squalus acanthias*, sea lamprey *Petromyzon marinus)* and *vertebrates* (humans)

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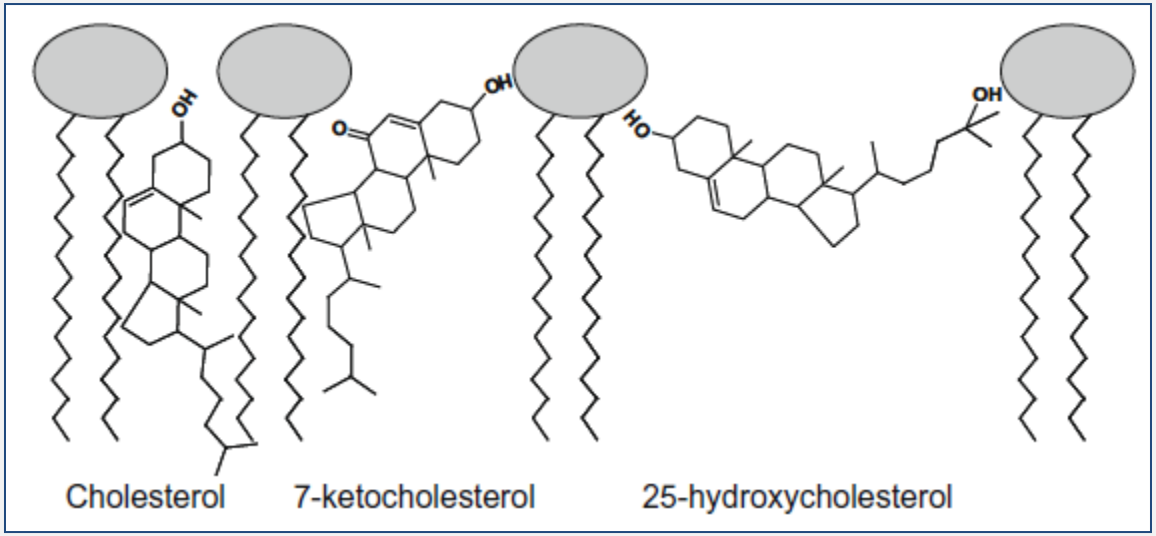
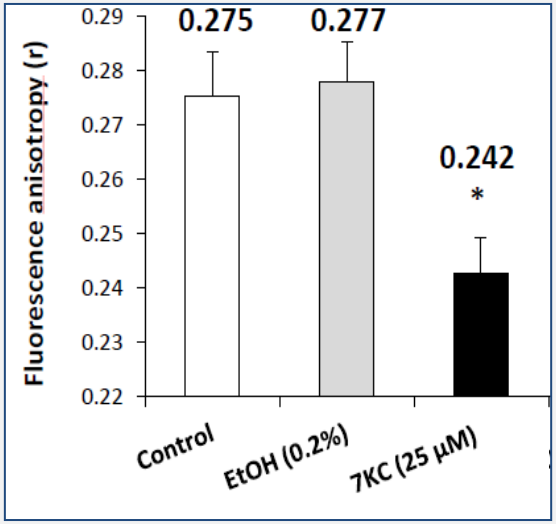
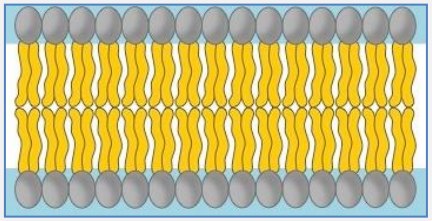
Oxysterols Biophysical Properties





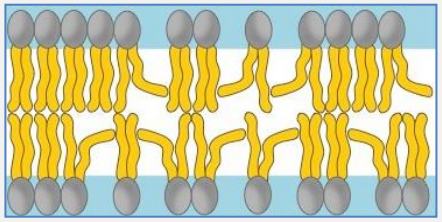
Viscous

❑ Control
❑ EtOH



Fluid

❑ 7KC



Increase of fluidity

- Olkkonen VM & Hynynen R. Mol Aspects Med. 2009; 30(3): 123-33
- Debbabi M , PhD Thesis, 2016

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Molecular Targets of Oxysterols

❑ Nuclear receptors

- LXR α
- LXR β
- ROR α
- ROR ϒ

❑ Cell membrane receptors

- GPR183 (also known as EBI2)

❑ Transport proteins as oxysterol targets

- OSBP
- ORPs
- Aryl hydrocarbon receptor

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Oxysterols Analysis

Biological fluids, cell and tissue extracts

✓**Gas chromatography / mass spectrometry (ng/mL)**

✓ **Liquid chromatography / mass spectrometry (ng/mL)**

✓**'Sub-chip' liquid chromatography / mass spectrometry (fg/mL)**

cholesterol: around 1 mmole/L plasma
oxysterol values in the range of µmole/L plasma
cholesterol / oxysterol : 1 000-10 000
(enzymatic methods are not suitable: only GC/MS or LC/MS)

Highly standardized and calibrated methods are required
Importance of : storage conditions, sample preparations (derivatization)

Fixed cell and tissue samples

✓ **mass spectrometry imaging (3D-analysis)**

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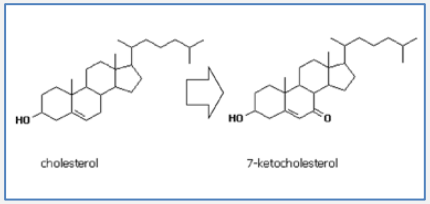
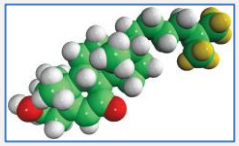
Involvement of Oxysterols in Neurodegenerative and Age Related Diseases



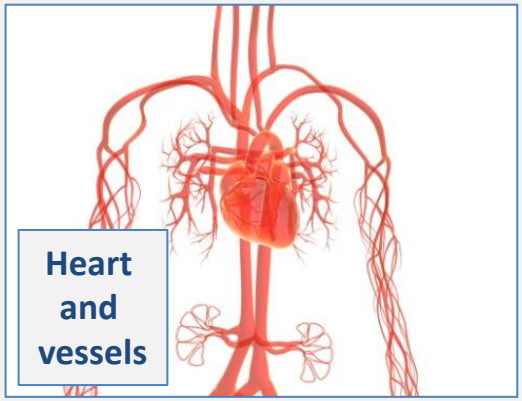
Oxysterols

7-ketocholesterol (7KC)
7β-hydroxycholesterol (7β-OHC)
24S-hydroxycholesterol (24S-OHC)
25-hydroxycholesterol (25-OHC)
……



*** Osteoporosis**
27-OHC, …

*** Atherosclerosis**
*** Cardiovascular diseases**

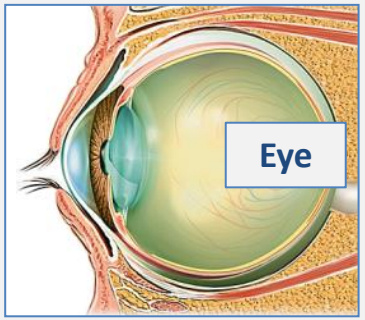


7KC, …

*** X-ALD**
*** Multiple sclerosis**
*** Niemann-Pick disease**
*** Alzheimer's disease**

7KC, 24S-OHC, …

*** Age related Macular Degeneration**
*** Cataract**



7KC, 24S-OHC, …

Javitt NB & Javitt JC, Curr Opin Ophthalmol. 2009
Zarrouk A et al., Ageing Res Rev, 2014

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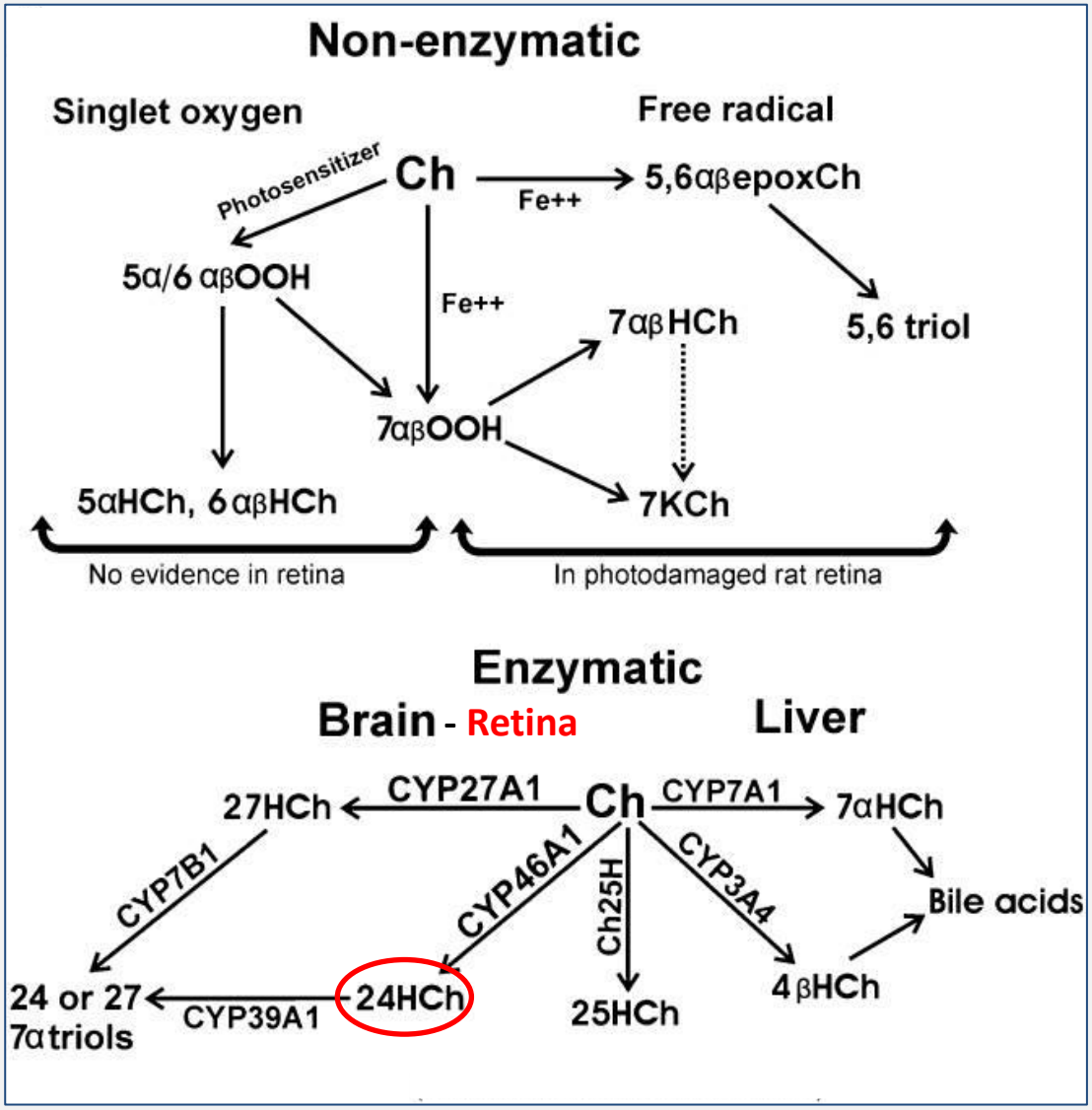
Potential Cholesterol Oxidation Pathway in the Retina



Rodríguez IR, Larrayoz IM, J Lipid Res. 2010; 51(10):2847-62.

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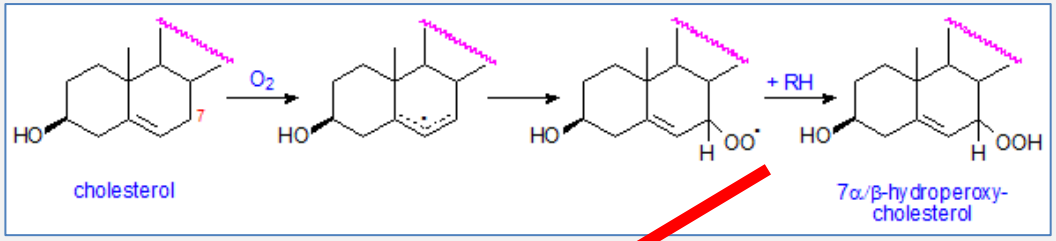
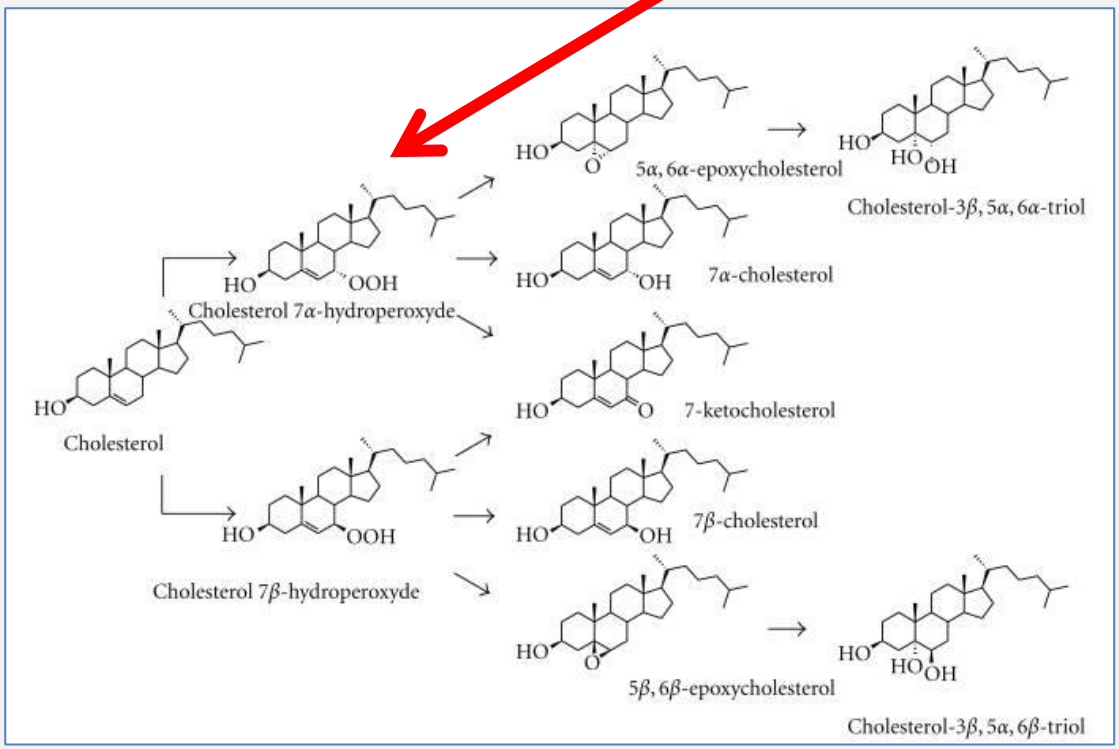
7-Ketocholesterol Biogenesis

❑ *7KC formation is mainly initiated when an hydrogen atom is abstracted at the C7 position by ROS such as $OH^{\bullet}$ or RNS such $ONOO^{\bullet}$*





❑ **Presence of 7KC in AMD lesions suggests:**
- **a rupture of Redox homeostasis in this disease**
- **oxysterol / cholesterol metabolism dysfunctions**

❑ **Signaling pathways associated with the biological activities of 7KC may contribute to identify pharmacological targets**

- Vejux A et al., J Ophtalmol, 2011
http://lipidlibrary.aocs.org/Primer/content.cfm?ItemNumber=39304

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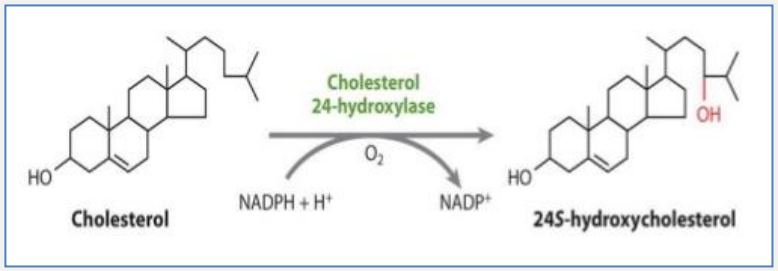
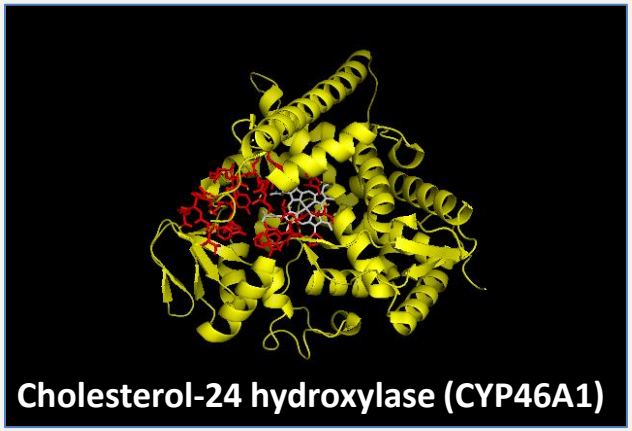
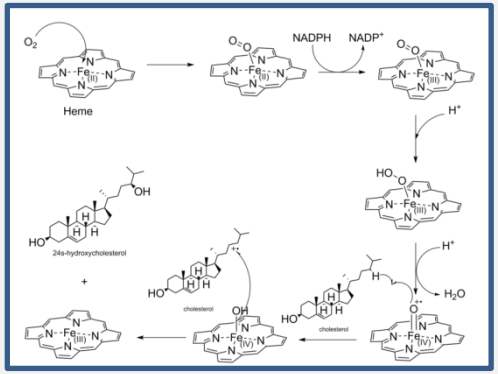
24S-Hydroxycholesterol Biogenesis







- 24S-hydroxycholesterol and CYP-46A1 identified in neural retina

- **CYP46A1 has been suggested as a gene candidate for retinal pathologies**

- Signaling pathways associated with the biological activities of 24S-OHC may contribute to identify pharmacological targets

Bretillon L et al., Curr Eye Res. 2007

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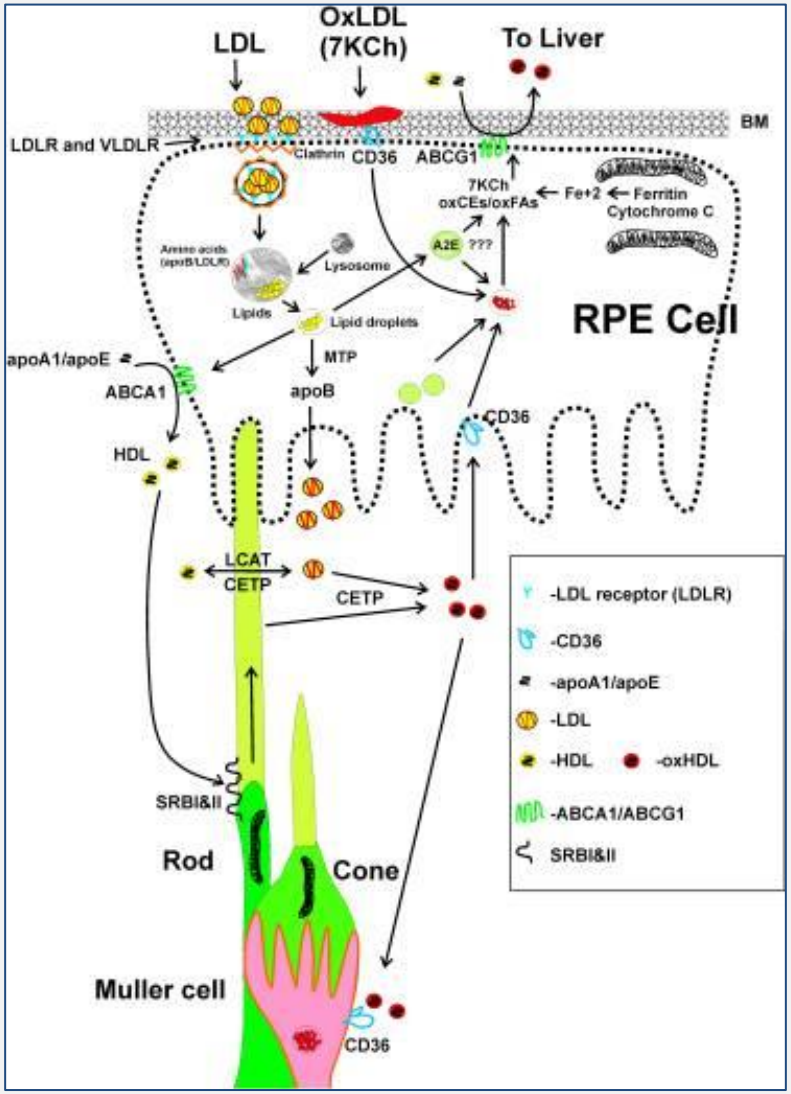
Hypothesis for 7-Ketocholesterol Involvement In Retinal Degeneration



The main source of 7KC is most likely **lipoprotein** deposits in Bruch's membrane (BM).

7KC may also be formed at or near the mitochondria from **Fe^{+2} released from ferritin and/or cytochrome c by light exposure**.

Lipofuscin granules could theoretically generate 7KC.

Unlike cholesterol, **some polyunsaturated fatty acids** like docosahexaenoic acid can be photooxidized directly without the need for iron or a photosensitizer and could generate **oxidized lipids** in the photoreceptor outer segments.

- Tserentsoodol N et al., Mol. Vis. 2006, 12: 1319–1333

- Rodríguez IR, Larrayoz IM, J Lipid Res. 2010; 51(10):2847-62

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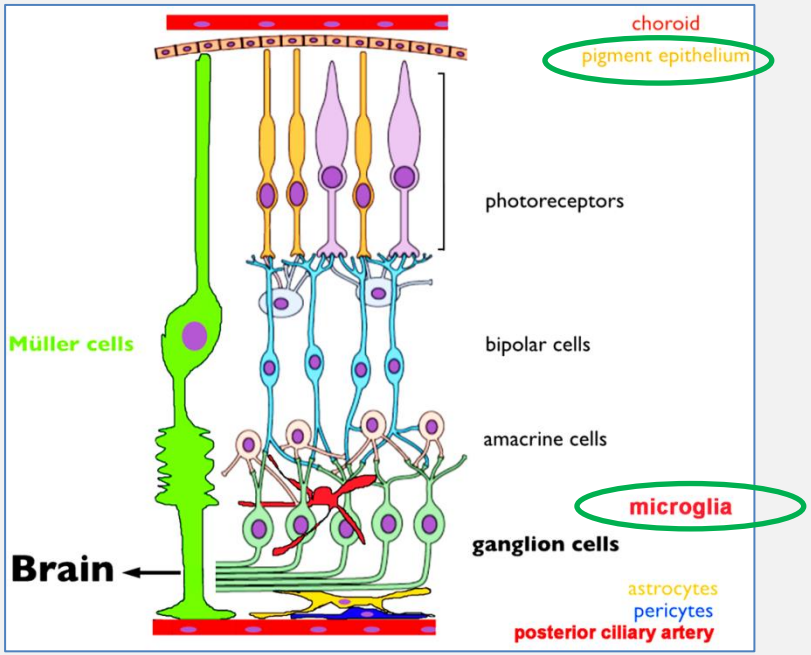
Cell targets of 7-ketocholesterol and 7β-hydroxycholesterol In the Retinal and Associated Side Effects



❑ Major biological activities of 7KC and 7β-OHC *(at elevated concentrations)*

- Cell death induction associated with mitochondrial dysfunctions

- *Activation of* ***apoptosis/autophagy*** *and* ***necroptosis***

- Enhancement of inflammatory cytokines and VEGF secretion

- Induction of lipid dysfunctions (phospholipidosis)

-Stimulation of ROS production (early event)

- Mitochondrial dysfunctions (early events)

- Malvitte L et al., Curr Eye Res. 2008; 33(9): 769-81.
- Dugas B et al. Eur J Nutr. 2010; 49(7): 435-46.

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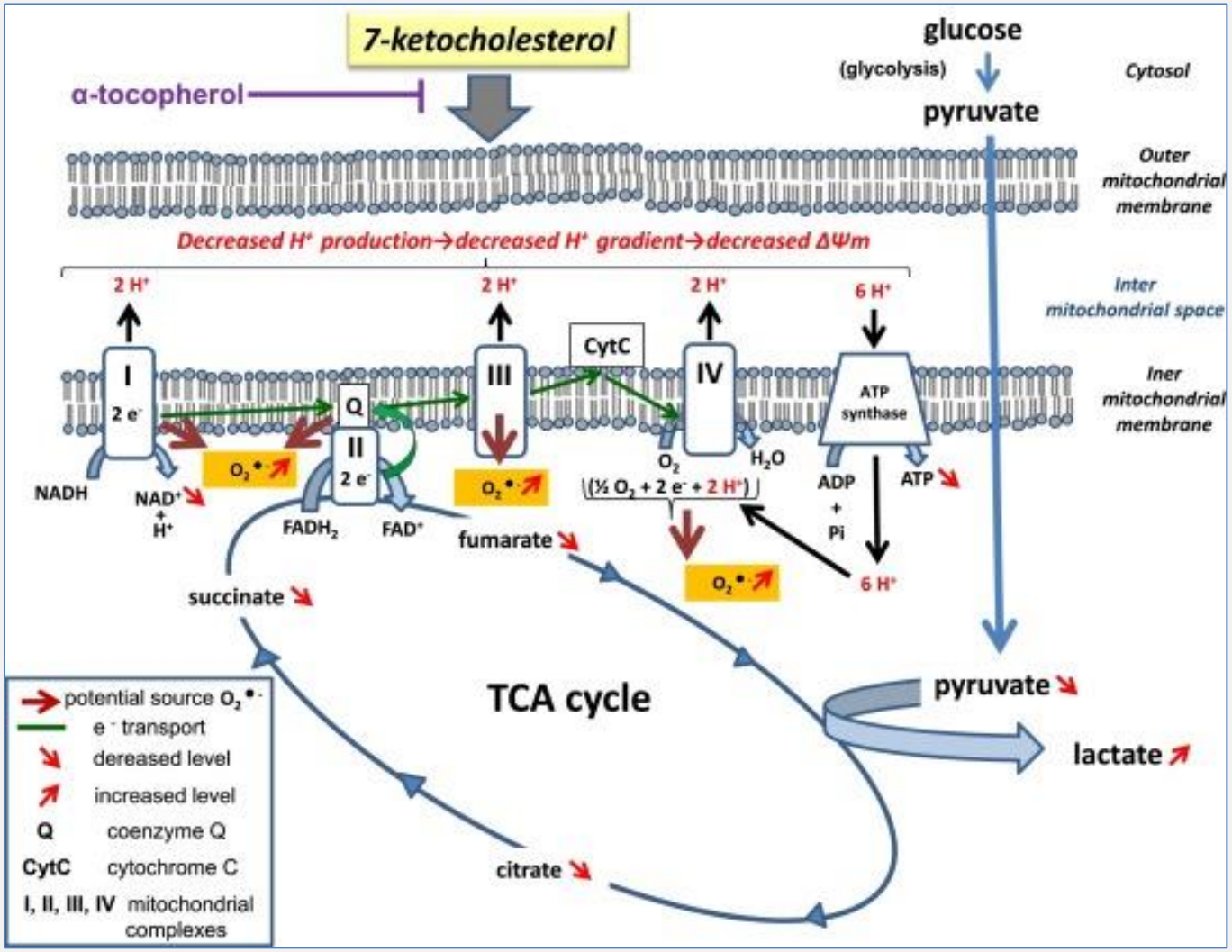
Impact of 7-Ketocholesterol at the Mitochondrial Levels



BV-2 ; murine microglial cells

- Leoni V et al., J Steroid Biochem Mol Biol. 2016, doi: 10.1016/j.jsbmb.2016.03.029.

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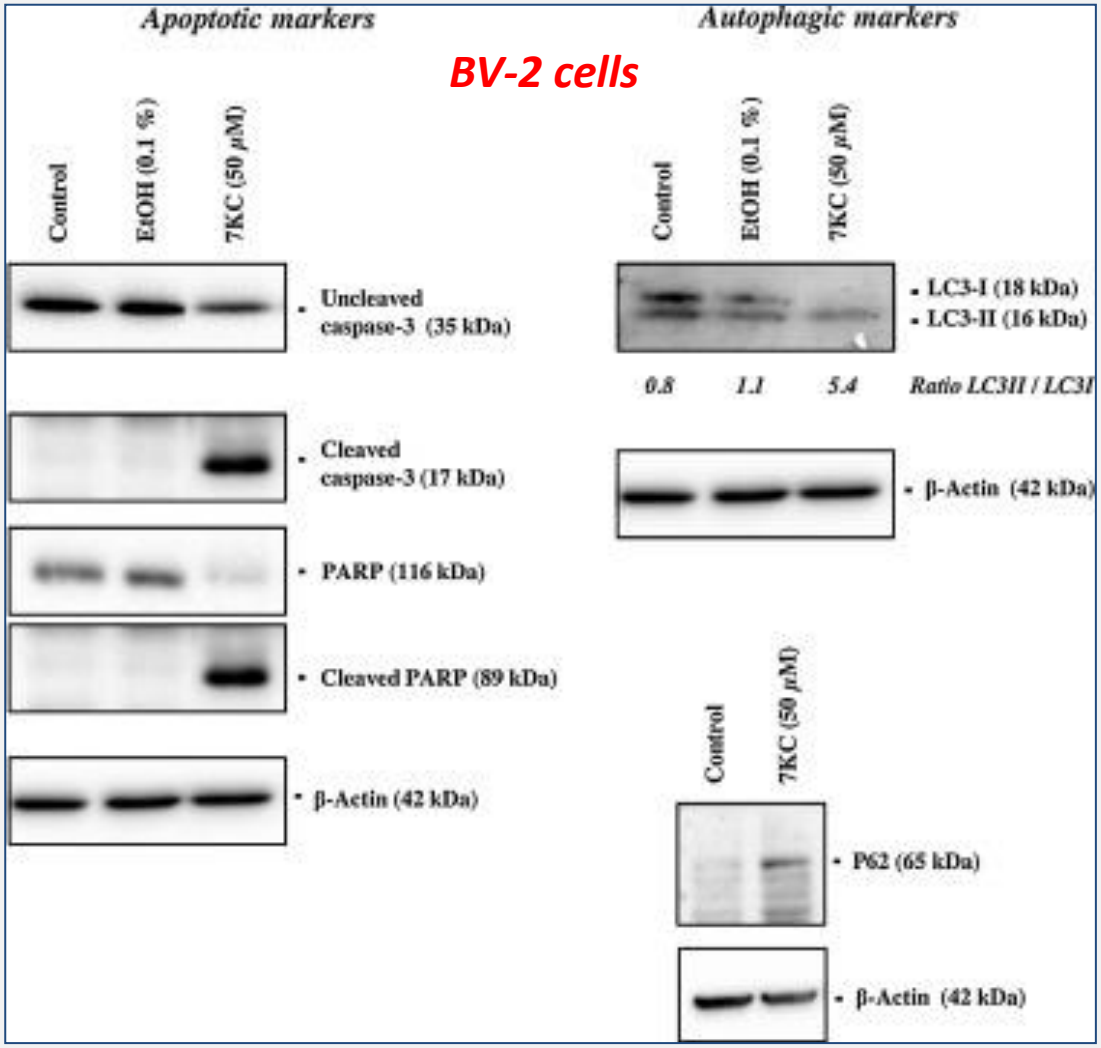
7-Ketocholesterol-Induced Apoptosis and Autophagy (oxiapoptophagy) on Microglial Cells (BV-2): *Caspase-3 Dependent Pathway*



7-ketocholesterol also induces apoptosis on *ARPE-19 cells*

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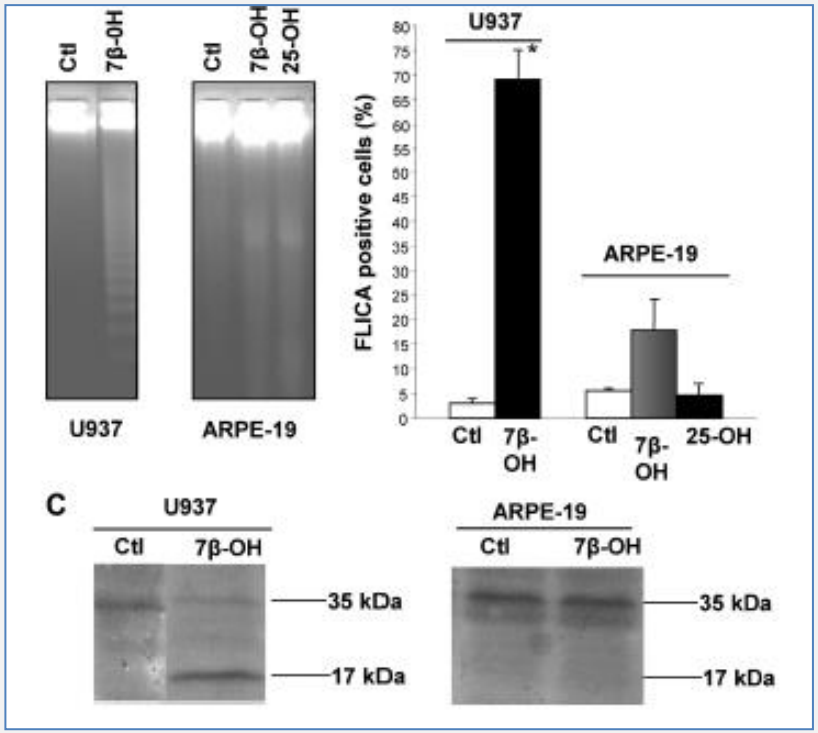
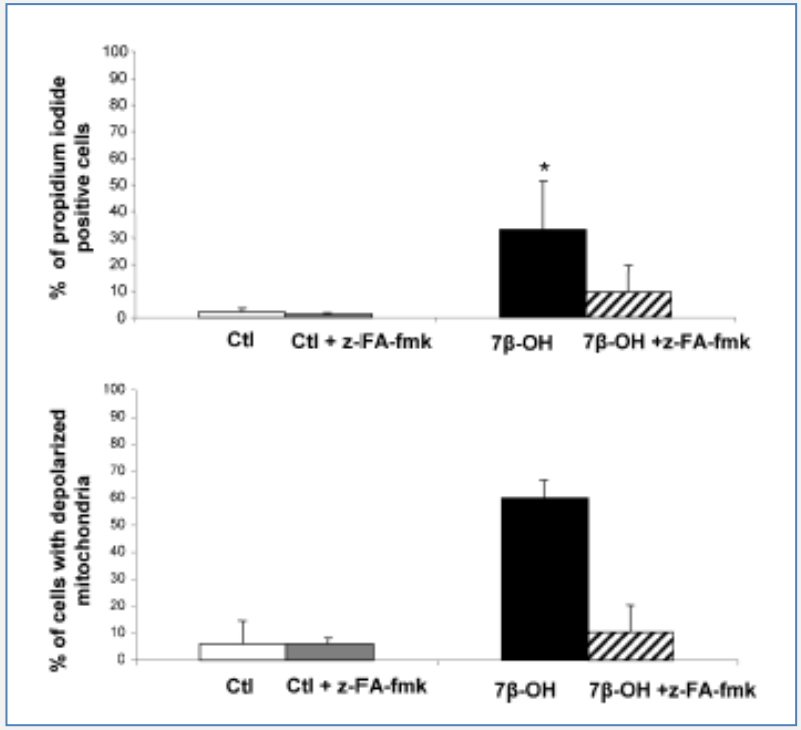
Induction of a Lysosomal Mode of Cell Death On 7β-hydroxycholesterol – Treated ARPE19 cells





Type of cell death depending on the oxysterol considered (?)

7KC accumulates in lipid rafts but not 7β-OHC

Malvitte L et al. Curr Eye Res 2008; 33(9): 769-81.

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7-ketocholesterol, 24S-hydroxycholesterol and 25-hydroxycholesterol Induce inflammatory cytokines secretion and ROS overproduction on primary porcine RPE cells

Taylor & Francis
Taylor & Francis Group

Current Eye Research

ISSN: 0271-3683 (Print) 1460-2202 (Online) Journal homepage: http://www.tandfonline.com/loi/icey20

Oxysterols Induced Inflammation and Oxidation in Primary Porcine Retinal Pigment Epithelial Cells

Corinne Joffre, Laurent Leclère, Bénédicte Buteau, Lucy Martine, Stéphanie Cabaret, Laure Malvitte, Niyazi Acar, Gérard Lizard, Alain Bron, Catherine Creuzot-Garcher & Lionel Bretillon

Joffre C et al., Curr Eye Res. 2007; 32(3): 271-80.

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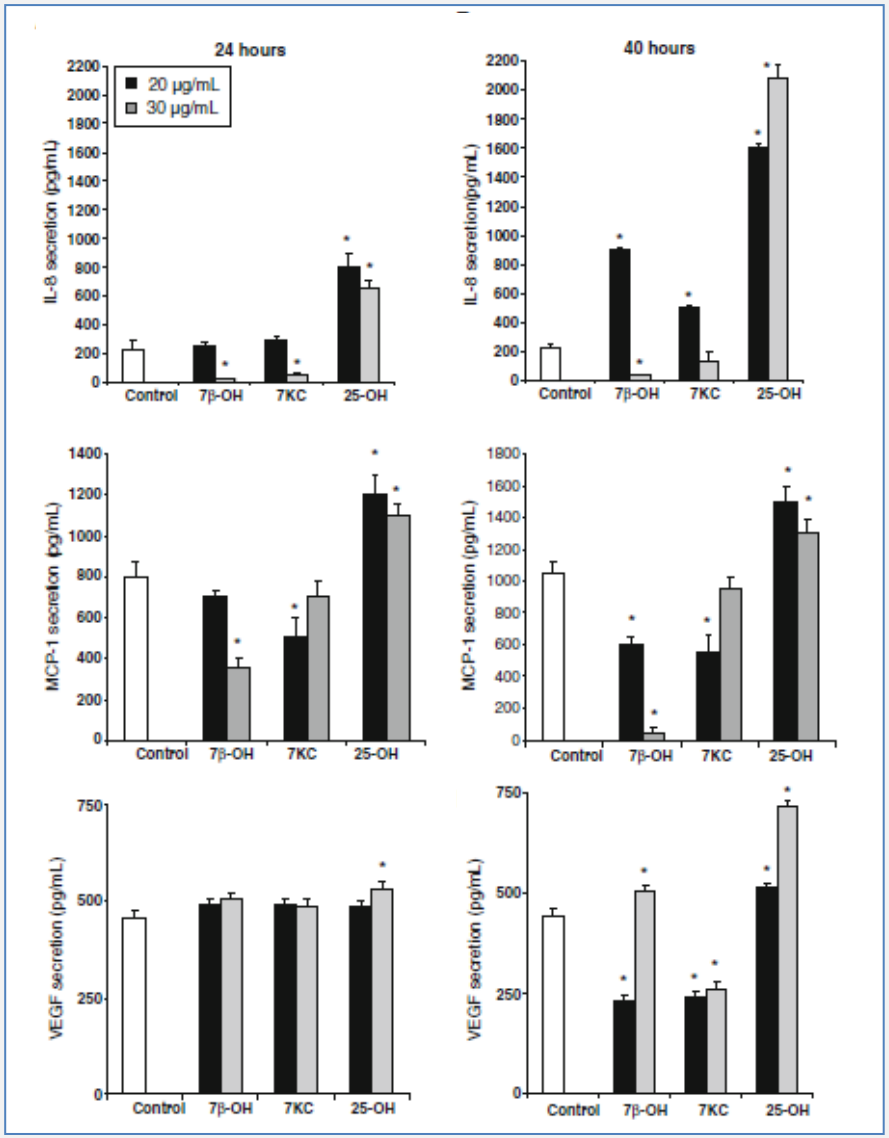
7-Ketocholesterol favors inflammatory cytokines and VEGF Secretion

IL-8

MCP-1

VEGF



ARPE-19 cells (human cells)

Dugas B et al. Eur J Nutr. 2010; 49(7): 435-46.

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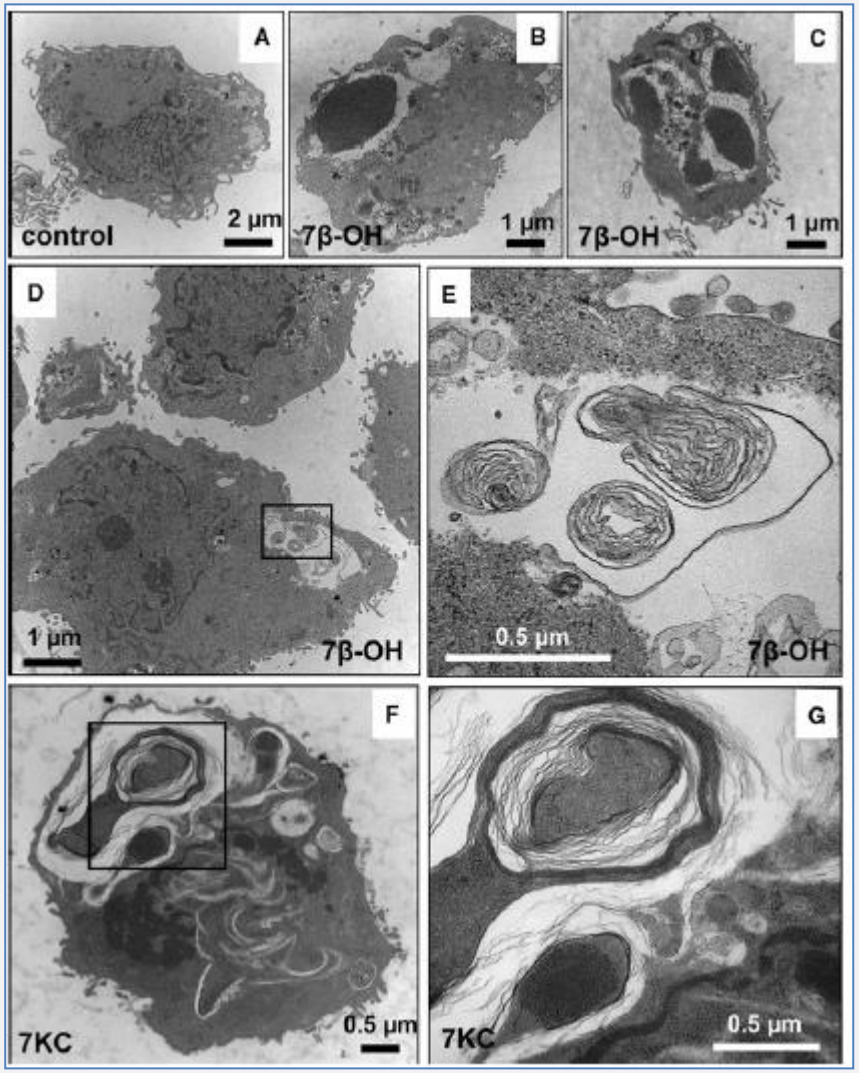
Impact of 7-ketocholesterol and 7β-hydroxycholesterol on lipid metabolism: phospholipidosis

ARPE-19



❑ ***Phospholipidosis induction***

Accumulation of ***cytotoxic oxysterols (7β-OHC, 7KC, 24S-OHC…)*** in acidic vesicles containing high levels of phospholipids; formation of myelin figures which are rich in phospholipids,

❑ ***Myelin figures could constitute useful biomarkers of oxysterols-induced cytotoxicity at the cell and tissue level***

❑ ***Phospholipidosis could be relied to ER stress and autophagy***

Dugas B et al. Eur J Nutr. 2010; 49(7): 435-46.

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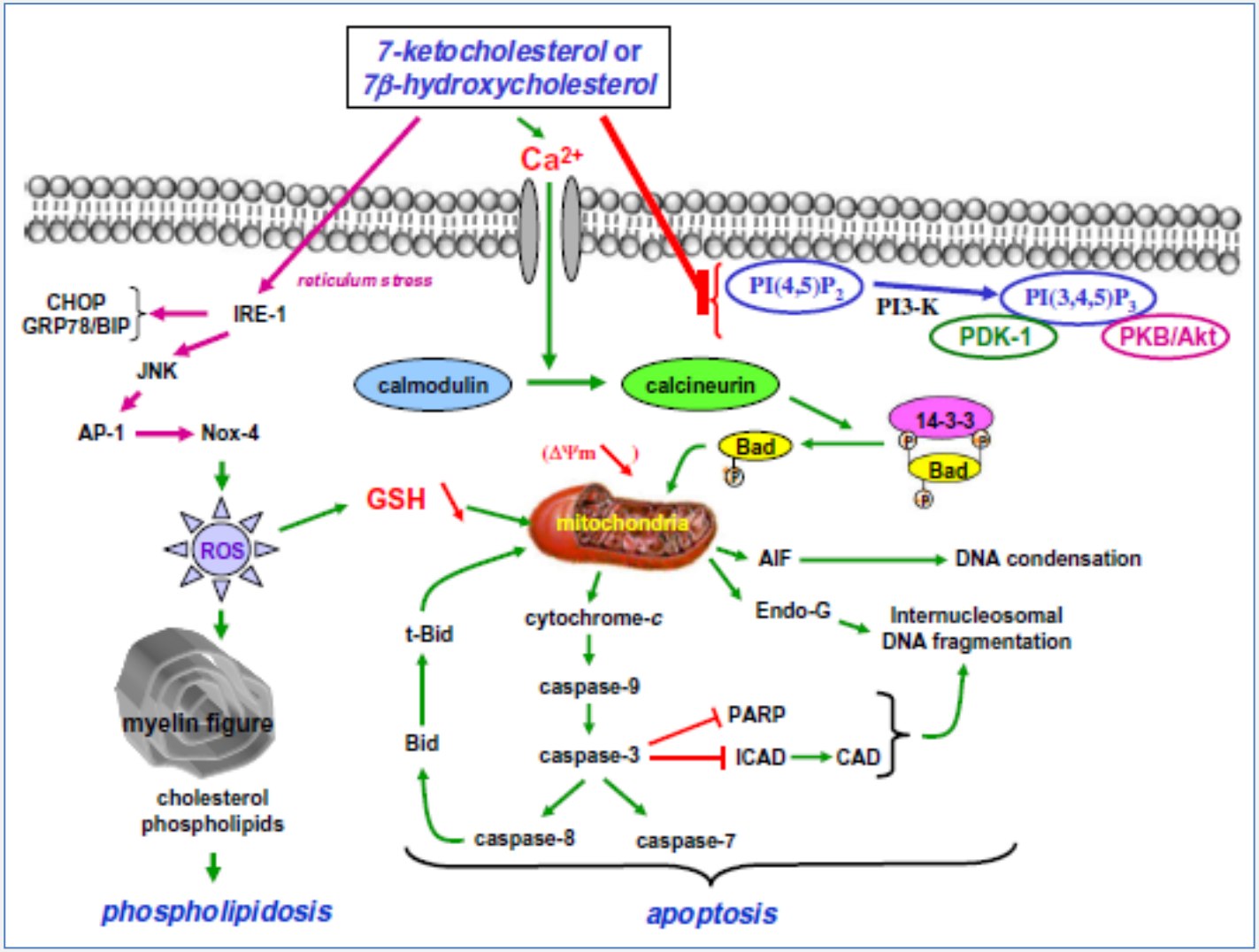
7-Ketocholesterol-and 7β-Hydroxycholesterol Associated Signaling Pathways Contributing to Oxidative Stress, Phospholipidosis and Apoptosis



(U937 cells)

Vejux A & Lizard G Mol Aspects Med. 2009;30(3):153-70.

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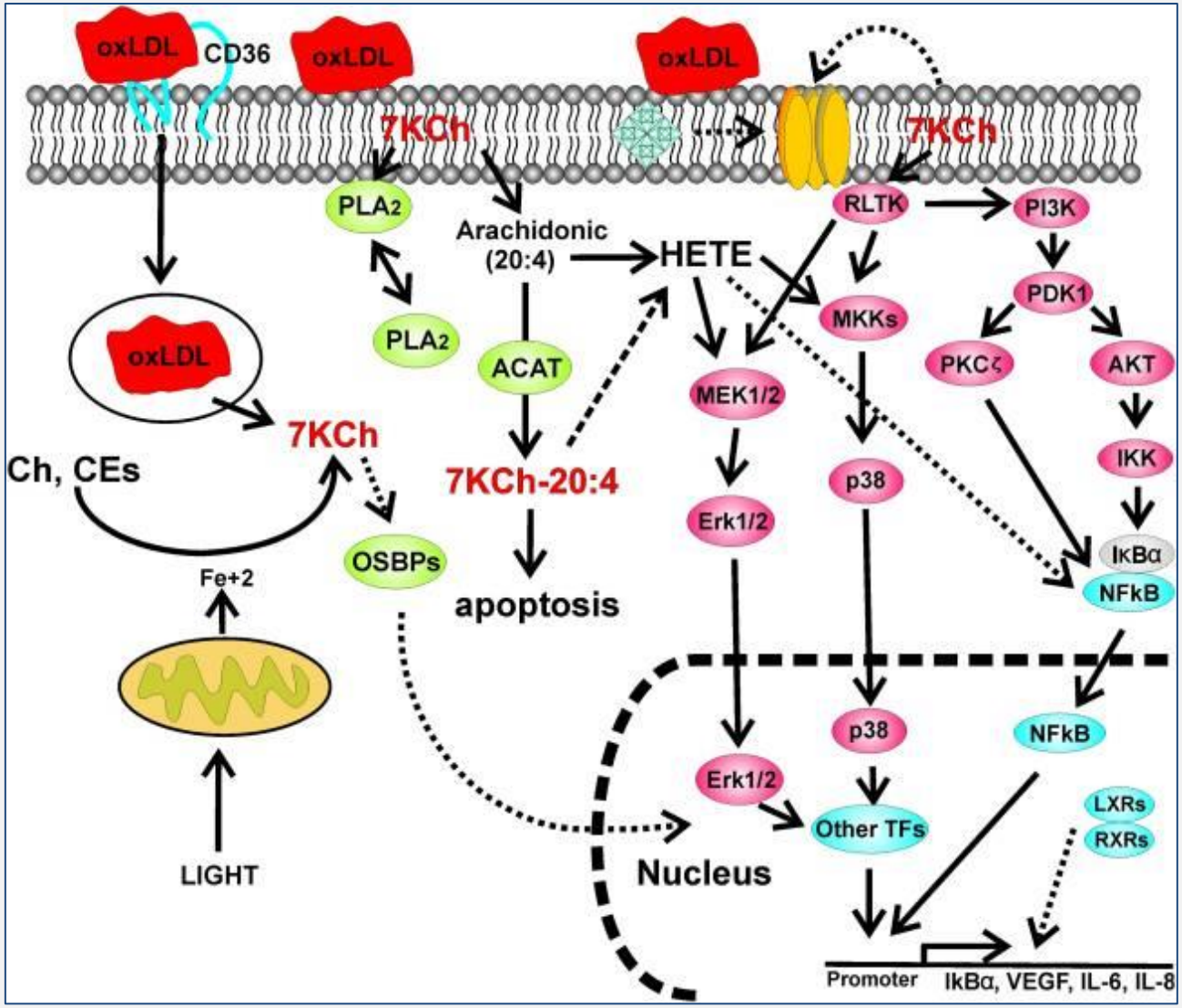
Signaling Pathways Described with 7-Ketocholesterol in ARPE19 cells and in Photodamaged Rat Retina



Conserved mechanisms of 7KC-induced side effects from one cell type to another

Rodríguez IR, Larrayoz IM, J Lipid Res. 2010; 51(10):2847-62.

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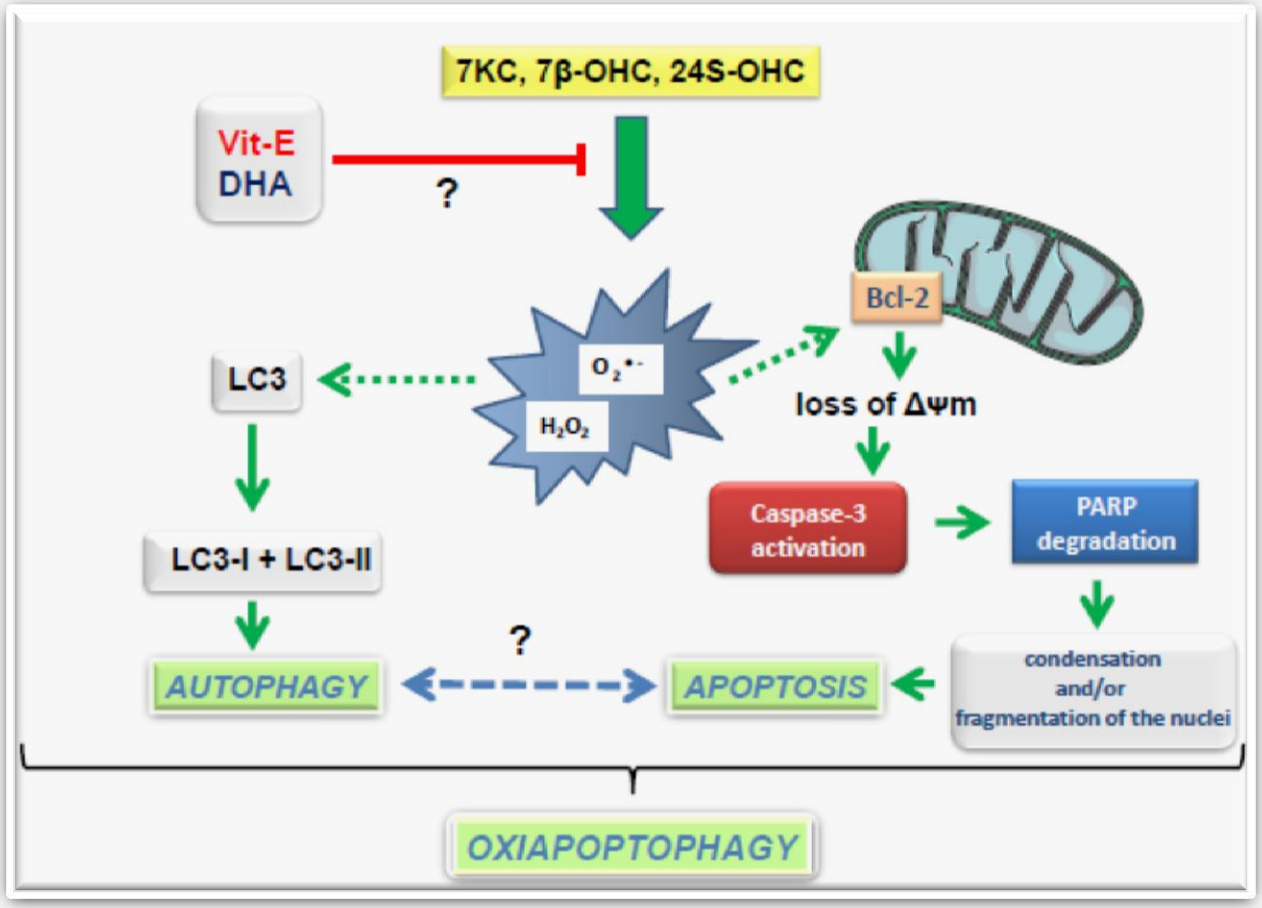
7-Ketocholesterol-Associated Side Effects: the Oxiapoptophagy Hypothesis
Oxidative Stress, Apoptosis and Autophagy (oxiapoptophagy)



❑ the part taken by autophagy (beneficial or detrimental) and the relationships between apoptosis and autophagy are still unknown

Nury T et al., Steroids. 2015; 99(Pt B):194-203.

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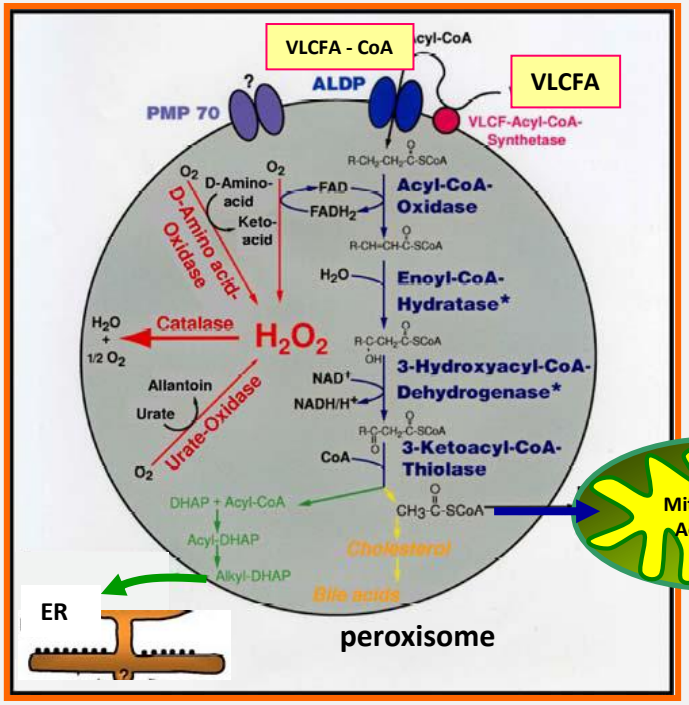
Involvement of Peroxisome in Retinal Dysfunctions



Schrader M & Fahimi HD, Histochem Cell Biol 2008, 129 : 421–440

Peroxisome Biogenesis Disorders, Zellweger Syndrome Spectrum
Steven J Steinberg, Gerald V Raymond, Nancy E Braverman, and Ann B Moser.

« Peroxisome biogenesis disorders, Zellweger syndrome spectrum (PBD, ZSS) is a continuum comprising three phenotypes - Zellweger syndrome (ZS), the most severe; neonatal adrenoleukodystrophy (NALD); and infantile Refsum disease (IRD), the least severe - that were originally described before the biochemical and molecular bases of these disorders had been fully determined. ……. ***Older children have retinal dystrophy***…..»
https://www.ncbi.nlm.nih.gov/books/NBK1448/

Liver DHA is linked to eye diseases

DHA plays an essential role in eye health. Very high levels of DHA are present in the retina, accounting for over half the total fatty acyl groups present in the phospholipids of rod outer segment membranes. DHA turnover in the retina is surprisingly slow and high levels of DHA appear to be maintained despite reductions in dietary intakes of omega-3 fatty acids. In this context, a blood stream "long loop" connect the supply of DHA to the biogenesis of excitable and photoreceptor membranes (Bazan, 1990). In retinal degenerative diseases, a shortage of blood DHA has been demonstrated, and a failure of the "long loop" from the liver is suggested to underlie these changes (Bazan *et al.*, 1986). Notably, the retina of patients with peroxisomal disorders has virtually no DHA (Martinez, 1992) and visual improvement has been obtained in these patients using DHA therapy (Noguer and Martinez, 2009).

Astarita G, Piomelli D OCL 2011;18(4):218-23.

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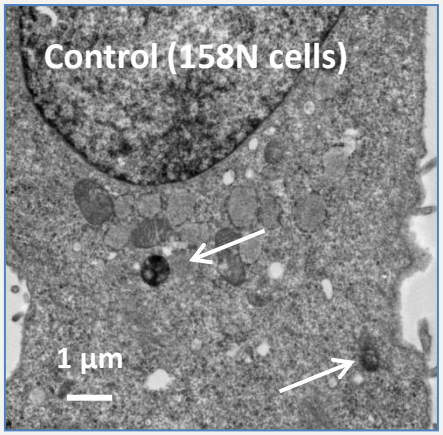
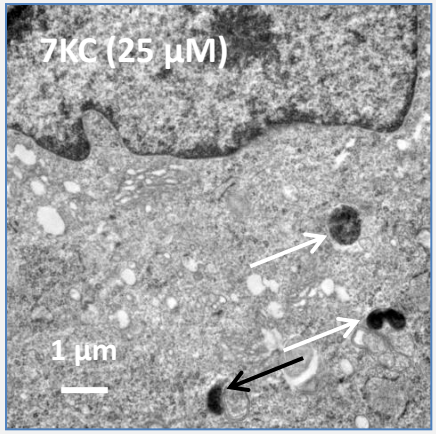
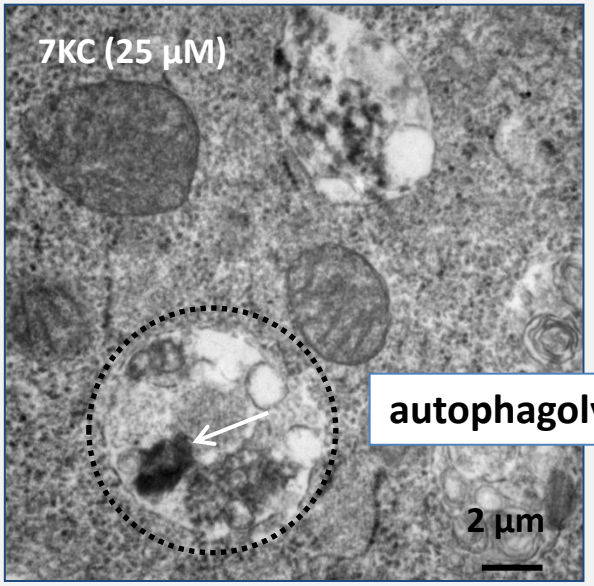
7-Ketocholesterol-Induced Peroxisomal Side Effects







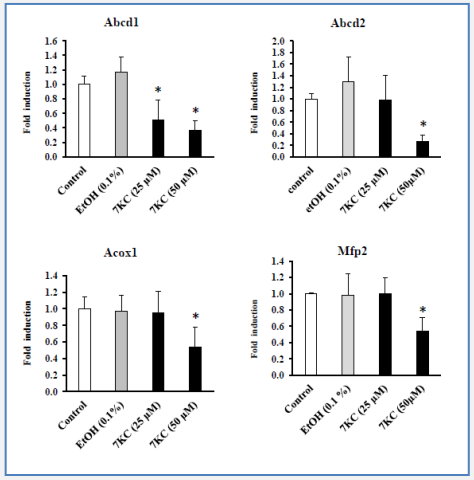
7KC induces:

✓ *Alteration of peroxisomal biogenesis,*

✓ *Peroxisomal degradation (pexophagy)*

✓ *Peroxisomal metabolic dysfunctions*

(affecting peroxisomal β-oxidation)



7KC - induced peroxisomal biogenesis and functional dysfunctions

which could contribute to retinal degeneration

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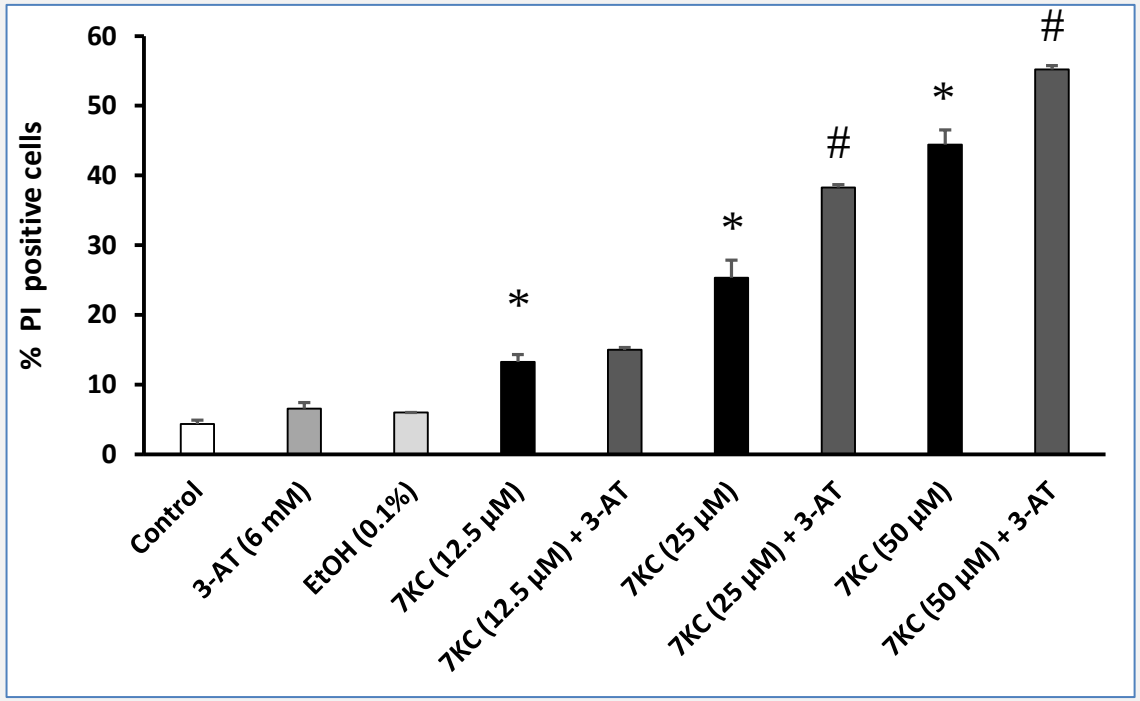
Enhancement of 7-Ketocholesterol-Induced Cytotoxicity By Drugs-Induced Catalase Inhibition

Propidium Iodide
(microglial BV-2 cells)



Enhancement of 7KC-induced cell death in the presence of the catalase inhibitor 3-amino-1, 2, 4-triazol (3AT)

Nury et al., J Steroid Biochem Mol Biol. 2016. doi: 10.1016/j.jsbmb.2016.03.037
Debbabi M, PhD Thesis (2016)

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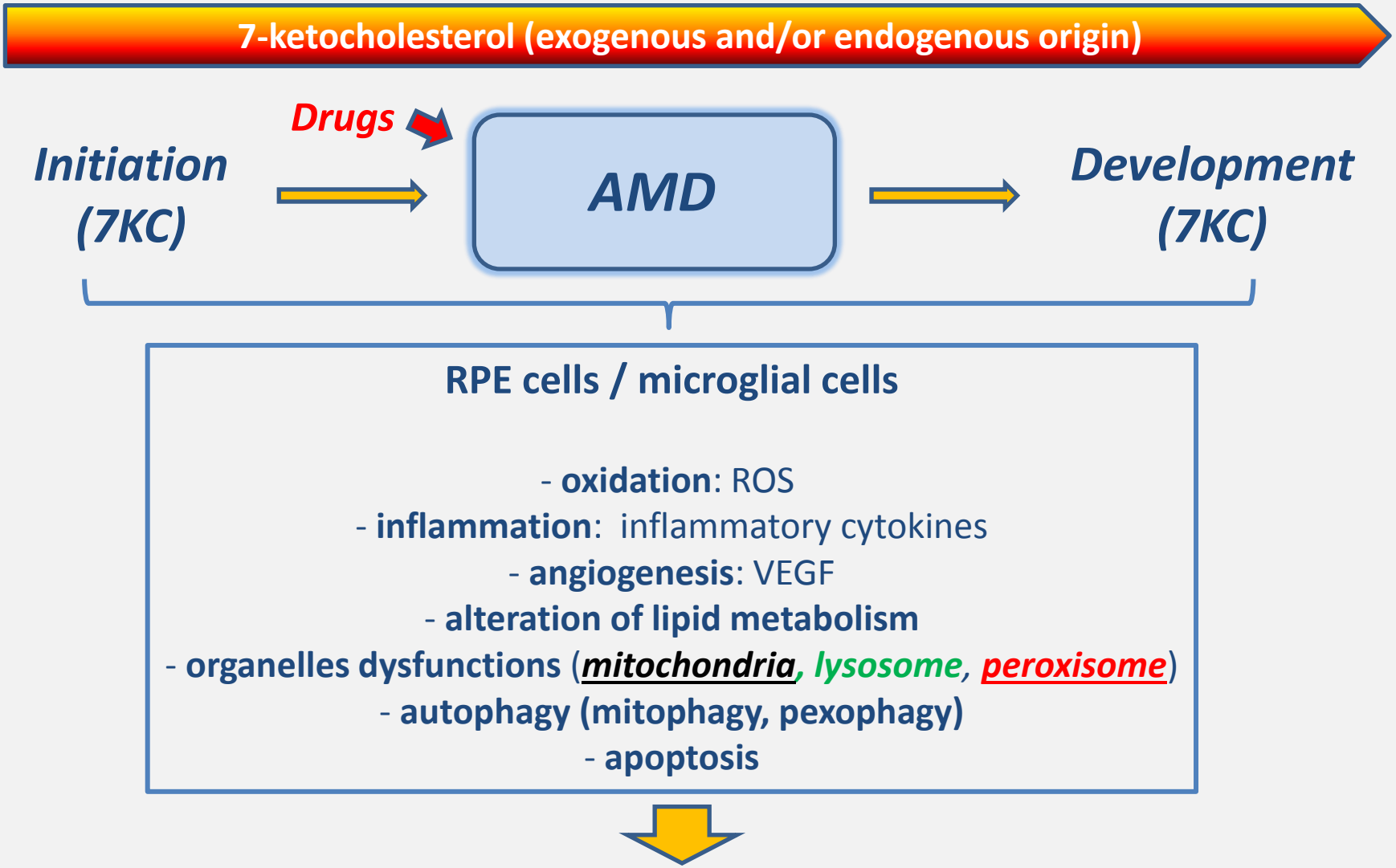
Contribution of 7-Ketocholesterol in the Pathophysiology of AMD



The toxic role of 7-ketocholesterol and existing pathways for its neutralization may point the way to a unified theory that explains the cause of age-related macular degeneration and points towards novel therapeutic interventions

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Prevention of 7-Ketocholesterol-Induced Side Effects In the Context of Age Related Diseases (including AMD)

- ***Antioxidant molecules***

✓ Tocopherols: α-tocopherol, ϒ-tocopherol

✓ Polyphenols: resveratrol, quercetin

As Trolox , which is a strong antioxidant, has no protective effects on 7KC-induced side effects, our data suggest that the anti-oxidative properties of α- / γ-tocopherol are not essential to prevent 7KC-induced side effects, and that the protective effects of these molecules involve other mechanisms.

- ***Non antioxidant molecules***

✓ω3 fatty acids : DHA, oleic acids

✓ organic fatty acids derivatives: Dimethyl fumarate, monomethyl fumarate

- Mixtures of molecules (functional foods)

✓ extra virgin olive oil, argan oil:

rich in tocopherols, ω3 fatty acids, polyphenols, phytosterols

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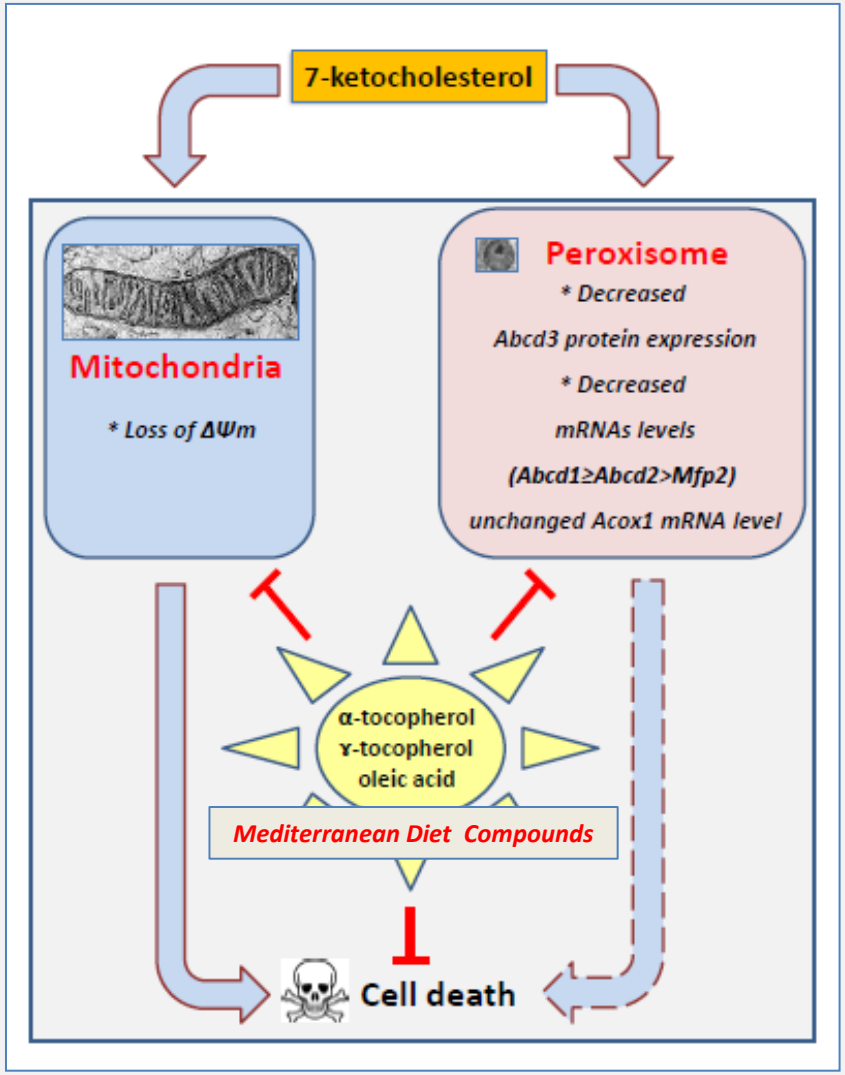
Prevention of Mitochondrial and Peroxisomal Dysfunctions in 7KC-Induced Oxiapoptophagy : Mediterranean Diet Compounds Benefits

Benefits of the Mediterranean diet which is rich in tocopherols, and ω3-fatty acid on oxysterols-induced side effects

Impact of nutrition on age-related diseases and ageing?

Functional foods



Cell death = oxiapoptophagy

OXIdation + APOPTOsis + autoPHAGY

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New Therapies: Regenerative Medicine

Potential strategies for the regulation of the biogenesis of oxysterols formed enzymatically

- **Si-RNAs**

- **Lentivirus**

- **Pluripotents stem cells combined with (CRISPR-cas technology)**

- **3D-bioprinting combined with (iPSCs + CRISPR-cas)**

What about oxysterols resulting from auto-oxidation?

Waddington SN et al. Curr Stem Cell Rep. 2016;2:9-20.

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Innovative Therapies: Medical Bioremedication

Modification of cell metabolism with exogeneous enzymes:
Bacterial enzymes ((cholesterol oxidase)

Microbial Cell Factories

BioMed Central

Review

Open Access

Medical bioremediation of age-related diseases

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Published: 9 April 2009

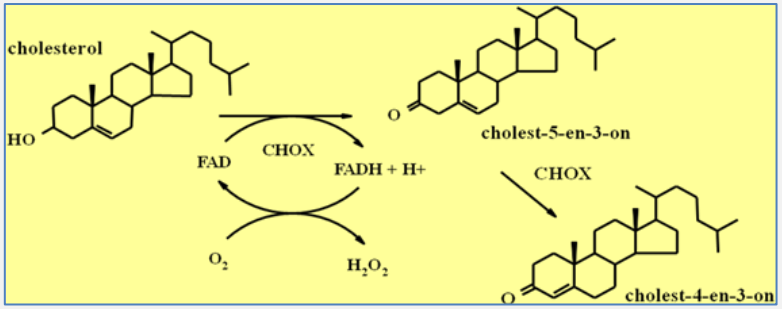
Microbial Cell Factories 2009, **8**:21 doi:10.1186/1475-2859-8-21

Received: 6 January 2009
Accepted: 9 April 2009

This article is available from: http://www.microbialcellfactories.com/content/8/1/21

Abstract

Catabolic insufficiency in humans leads to the gradual accumulation of a number of pathogenic compounds associated with age-related diseases, including atherosclerosis, Alzheimer's disease, and macular degeneration. Removal of these compounds is a widely researched therapeutic option, but the use of antibodies and endogenous human enzymes has failed to produce effective treatments, and may pose risks to cellular homeostasis. Another alternative is "medical bioremediation," the use of microbial enzymes to augment missing catabolic functions. The microbial genetic diversity in most natural environments provides a resource that can be mined for enzymes capable of degrading just about any energy-rich organic compound. This review discusses targets for biodegradation, the identification of candidate microbial enzymes, and enzyme-delivery methods.



COMMUNICATION TO THE EDITOR

BIOTECHNOLOGY AND BIOENGINEERING

Increased Resistance to Oxysterol Cytotoxicity in Fibroblasts Transfected With a Lysosomally Targeted *Chromobacterium* Oxidase

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ABSTRACT: 7-Ketocholesterol (7KC) is a cytotoxic oxysterol that plays a role in many age-related degenerative diseases. 7KC formation and accumulation often occurs in the lysosome, which hinders enzymatic transformations that reduce its toxicity and increase the sensitivity to lysosomal membrane permeabilization. We assayed the potential to mitigate 7KC cytotoxicity and enhance cell viability by overexpressing 7KC-active enzymes in human fibroblasts. One of the enzymes tested, a cholesterol oxidase engineered for lysosomal targeting, significantly increased cell viability in the short term upon treatment with up to 50 μM 7KC relative to controls. These results suggest targeting the lysosome for optimal treatment of oxysterol-mediated cytotoxicity, and support the use of introducing novel catalytic function into the lysosome for therapeutic and research applications.

Biotechnol. Bioeng. 2012;109: 2409–2415.

KEYWORDS: oxysterol; 7-ketocholesterol; lysosome; oxidase

(Brown and Jessup, 1999), age-related macular degeneration (Rodriguez and Larrayoz, 2010), and Alzheimer's disease (Casserly and Topol, 2004). Therefore, the introduction of novel sterol-transforming enzymes into affected cell types may be useful for controlling endogenous 7KC levels, and consequently help reduce the incidence and severity of these diseases (Mathieu et al., 2009).

Sterols, including oxysterols, enter the cell via receptor-mediated endocytosis of low density lipoproteins and traffic to the lysosomes, which are a major site of non-enzymatic oxysterol formation. Consequently, 7KC levels are the highest in the endosomal and lysosomal compartments (Brown et al., 2000). 7KC is known to inhibit sphingomyelinase (Maor et al., 1995) and facilitate the intralysosomal accumulation of both sphingomyelin and cholesterol, possibly leading to foam cell formation. Subsequent free cholesterol loading of lysosomes also promotes de-acidification (Cox et al., 2007), impairs organelle trafficking (Fraldi et al., 2010), and inhibits chaperone mediated autophagy (Kaushik et al., 2006). At micromolar concentrations, 7KC causes lysosomal membrane permeabilization

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Collaborations and Aknowledgements

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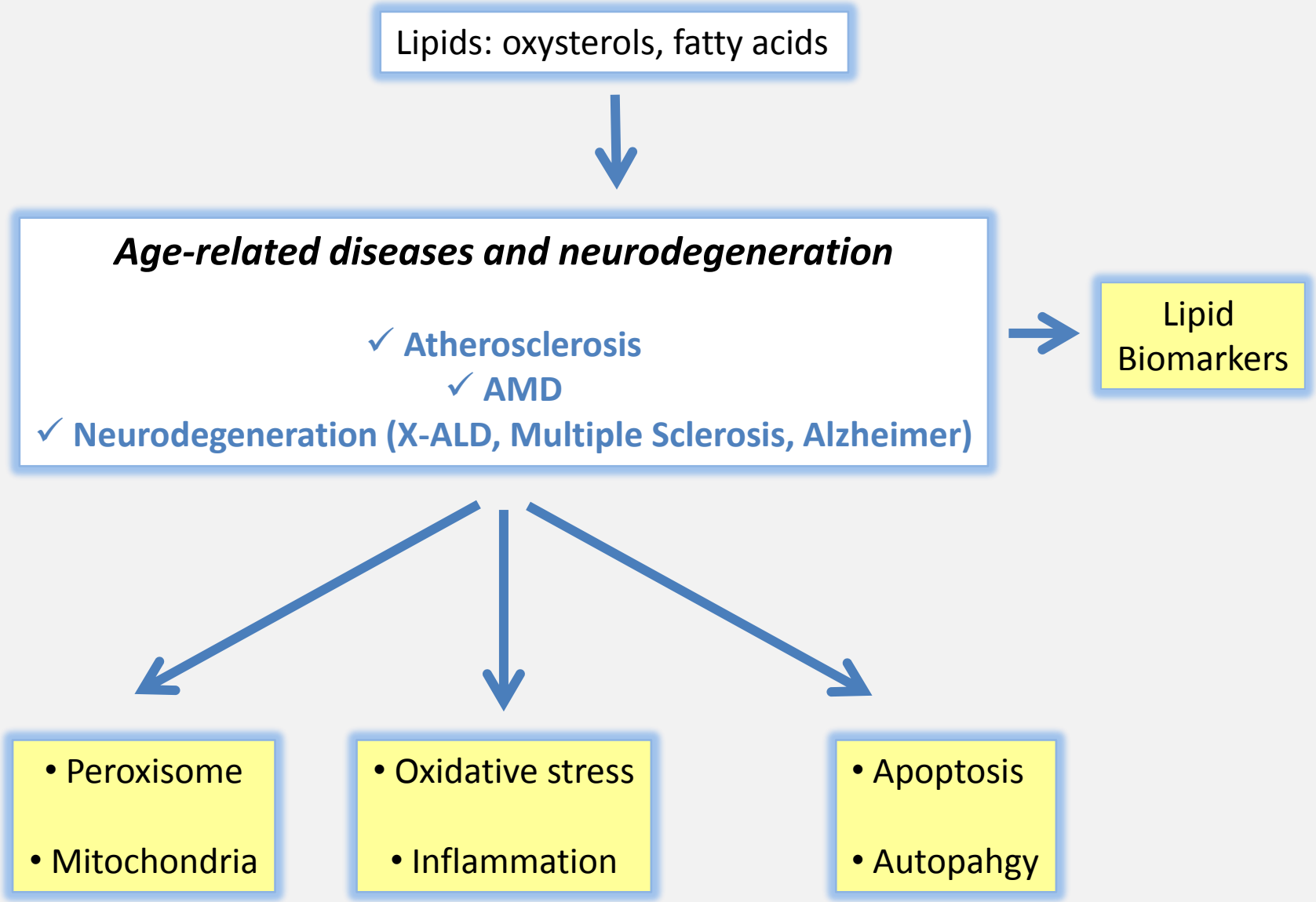

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