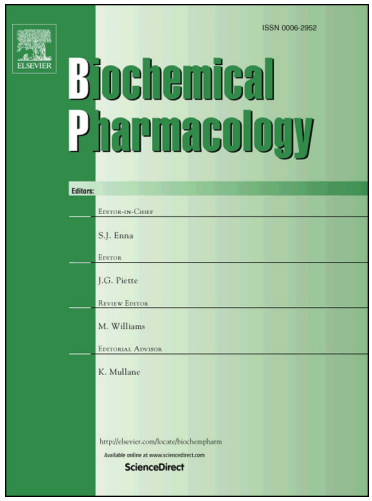


Review

7-ketocholesterol and 7β-hydroxycholesterol: in vitro and animal models used to characterize their activities and to identify molecules preventing their toxicity

Anne Vejux, Dehbia Abed-Vieillard, Khadija Hajji, Amira Zarrouk, John J. Mackrill, Shuhbrima Ghosh, Thomas Nury, Aline Yammine, Mohamed Zaibi, Wafa Mihoubi, Habiba Bouchab, Boubker Nasser, Yaël Grosjean, Gérard Lizard

PII: S0006-2952(19)30347-8
DOI: https://doi.org/10.1016/j.bcp.2019.113648
Reference: BCP 113648

To appear in: *Biochemical Pharmacology*

Received Date: 28 June 2019
Accepted Date: 30 September 2019

Please cite this article as: A. Vejux, D. Abed-Vieillard, K. Hajji, A. Zarrouk, J.J. Mackrill, S. Ghosh, T. Nury, A. Yammine, M. Zaibi, W. Mihoubi, H. Bouchab, B. Nasser, Y. Grosjean, G. Lizard, 7-ketocholesterol and 7β-hydroxycholesterol: in vitro and animal models used to characterize their activities and to identify molecules preventing their toxicity, *Biochemical Pharmacology* (2019), doi: https://doi.org/10.1016/j.bcp.2019.113648

7-ketocholesterol and 7β-hydroxycholesterol: in vitro and animal models used to characterize their activities and to identify molecules preventing their toxicity

Anne Vejux [1*], Dehbia Abed-Vieillard [2], Khadija Hajji [3], Amira Zarrouk [4], John J. Mackrill [5], Shuhbrima Ghosh [6], Thomas Nury [1], Aline Yammine [7], Mohamed Zaibi [8], Wafa Mihoubi [9], Habiba Bouchab [10], Boubker Nasser [10], Yaël Grosjean [2], Gérard Lizard [1*]

1 Université de Bourgogne Franche-Comté / Inserm, Team 'Biochemistry of the Peroxisome, Inflammation and Lipid Metabolism' EA 7270, 21000 Dijon, France

2 Centre des Sciences du Goût et de l'Alimentation, AgroSup Dijon, UMR 6265 CNRS, UMR 1324 INRA, Université Bourgogne Franche-Comté, 21000 Dijon, France

3 University Tunis El Manar, Faculty of Sciences of Tunis, LR18ES03, Laboratory of Neurophysiology, Cellular Physiopathology and Biomolecules Valorisation, Tunis, Tunisia

4 Faculty of Medicine, LR12ES05, Laboratory - NAFS "Nutrition - Functional Food & Vascular Health", Monastir, & University Sousse, Faculty of Medicine, Sousse, Tunisia

5 Department of Physiology, Biosciences Institute, University College Cork, Cork, Ireland

6 Enzyme and Microbial Biochemistry Laboratory, Department of Chemistry, Indian Institute of Technology Delhi, New Delhi, India

7 Bioactive Molecules Research Laboratory, Doctoral School of Sciences and Technologies, Faculty of Sciences, Lebanese University, Beirut 1103, Lebanon

8 Clore Laboratory, University of Buckingham, Hunter Street, Buckingham, United Kingdom

9 Centre de Biotechnologie de Sfax, Lab. Biotechnologie Moléculaire des Eucaryotes, Sfax, Tunisia

10 Laboratory of Biochemistry and Neurosciences, Department of Biology, University Hassan I, 26000 Settat, Morocco

***Correspondence:** Dr Anne Vejux & Dr Gérard Lizard; Faculté des Sciences Gabriel, Laboratoire Bio-PeroxIL, 6, Bd Gabriel, 21000 Dijon, France, electronic address : anne.vejux@u-bourgogne.fr; gerard.lizard@u-bourgogne.fr ;

Tel: 33 3 80 39 62 56 / Fax: 33 3 80 39 62 50

Running title: *In vitro* and *in vivo* models to evaluate the activities of 7-ketocholesterol and 7β-hydroxycholesterol

Founding: Université de Bourgogne / Inserm

Disclosures: Authors have no conflict of interest.

Abbreviations used:

11β–HSD1: 11β-hydroxysteroid-dehydrogenase type I; 11β–HSD2: 11β-hydroxysteroid-dehydrogenase type II; 24S-OHC: 24S-hydroxycholesterol; 3,4,7-THSAP: 3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid; 3,4-DHSAP: 3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid; 3,4,7-THSA: 3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione; 3,4-DHSA: 3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione; 5α, 6α-epox: 5α, 6α- epoxy cholesterol; 5β, 6β-epox: 5β, 6β- epoxy cholesterol; 7-DHC: 7-dehydrocholesterol; 7-KC: 7-ketocholesterol; 7α-OHC: 7α-hydroxycholesterol; 7β-OHC: 7β-hydroxycholesterol; ACAT: Acyl-coenzyme A: cholesterol acyltransferases; ARMD: age-related macular degeneration; ARPE19: human retinal pigment epithelial cells; Aβ: amyloid-beta; BAD: Bcl-2-associated death promoter; CAEC: carotid arterial endothelial cells; CASMC: coronary arterial smooth muscle cells; CH25H: cholesterol 25-hydroxylase; ChEH: cholesterol epoxide hydrolase; cPLA2α: cytosolic phospholipase A2 alpha; DHA: docosahexaenoic acid; DMF: dimethylfumarate; EBI2: Epstein-Barr virus-induced G-protein coupled receptor 2; EGCG: Epigallocatechin 3-gallate; ERK: extracellular signal-regulated kinases; fhRPE: human fetal retinal pigment cells; GPR: G-protein coupled receptor;

HAEC: human aortic endothelial cells; HA-VSMC: human aortic vascular smooth muscle aortic cells; HUVEC: human umbilical vascular endothelial cells; $IP_{3:}$ inositol 1,4,5-trisphosphate; iPSCs: inducible pluripotent stem cells; LCAT: lecithin-cholesterol acyltransferase; LDL: Low Density Lipoprotein; LPS: lipopolysaccharides; LXR: Liver X-receptor; MBP: Myelin Basic Protein; MS: multiple sclerosis; NRF2: nuclear factor erythroid 2–related factor 2; ORPL: oxysterol-binding protein related proteins; PBMC: peripheral blood mononuclear cells; PLP: Proteo Lipid Protein; RAEC: Rat aortic endothelial cells; ROS: reactive oxygen species; RPE: retinal pigment epithelium; RXR: Retinoïd X receptor; SC: Saccharomyces cerevisae; SCFA: Short chain fatty acids; SERCA: sarco-/endo-plasmic reticulum Ca^{2+}-ATPase; SM: smooth muscle; SOAT1: sterol O-acyltransferase 1; SREBP: sterol regulatory element binding protein; SULT: sulfation via sulfotransferase; TP: Tetrahymena pyriformis; VCAM-1: vascular cell adhesion molecule 1; VGCC: L-type voltage-gated Ca^{2+}-channels; X-ALD: X-linked adrenoleukodystrophy;

Abstract

Oxysterols are molecules derived by the oxidation of cholesterol and can be formed either by auto-oxidation, enzymatically or by both processes. Among the oxysterols formed by auto-oxidation, 7-ketocholesterol and 7β-hydroxycholesterol are the main forms generated. These oxysterols, formed endogenously and brought in large quantities by certain foods, have major cytotoxic properties. They are powerful inducers of oxidative stress, inducing dysfunction of organelles (mitochondria, lysosomes and peroxisomes) that can cause cell death. These molecules are often identified in increased amounts in common pathological states such as cardiovascular diseases, certain eye conditions, neurodegenerative disorders and inflammatory bowel diseases. To oppose the cytotoxic effects of these molecules, it is important to know their biological activities and the signaling pathways they affect. Numerous cell models of the vascular wall, eye, brain, and digestive tract have been used. Currently, to counter the cytotoxic effects of 7-ketocholesterol and 7β-hydroxycholesterol, natural molecules and oils, often associated with the Mediterranean diet, as well as synthetic molecules, have proved effective *in vitro*. Bioremediation approaches and the use of functionalized nanoparticles are also promising. At the moment, invertebrate and vertebrate models are mainly used to evaluate the metabolism and the toxicity of 7-ketocholesterol and 7β-hydroxycholesterol. The most frequently used models are mice, rats and rabbits. In order to cope with the difficulty of transferring the results obtained in animals to humans, the development of *in vitro* alternative methods such as organ / body-on-a-chip based on microfluidic technology are hopeful integrative approaches.

Keywords: 7-ketocholesterol, 7β-hydroxycholesterol, cell models, animal models, microfluidic, signaling pathways.

1 – Introduction

At the moment, there are several lines of evidence in numerous cell lines from different species that 7-ketocholesterol (7KC) and 7β-hydroxycholesterol (7β-OHC) induce cytotoxic side effects including rupture of RedOx homeostasis, inflammation and cell death defined as oxiapoptophagy (OXIdative stress + APOPTOsis + autoPHAGY). This is associated with important modifications of the lipid profile and with organelle dysfunction (mitochondria, lysosome, peroxisome) [1]. As increased levels of 7KC and 7β-OHC are associated in major diseases such as age-related diseases (cardiovascular diseases, neurodegeneration, eye disorders, osteoporosis and some cancers) and chronic diseases (bowel diseases) [2, 3], there is a need to better understand the biological activities of these molecules not only *in vitro* but also *in vivo* in order *i*) to identify pharmacological targets, and *ii*) to identify natural and synthetic compounds capable of counteracting 7KC- and 7β-OHC-induced side effects, as well as *iii*) to develop innovative therapeutic strategies (targeted nanotherapies [4], bioremediation [5, 6]). To overcome deficits in animal models whose results are sometimes difficult to transfer to humans, the development of alternative methods ("Lab on a Chip" / "Body on Chip" / organoids), perhaps combined with the use of inducible pluripotent stem cells (iPSCs), are promising integrative approaches.

The present review will discuss the biogenesis and catabolism of 7KC and 7β-OHC; the biological activities of these oxysterols; their signaling pathways associated with oxidative stress, organelle dysfunction, cell death and inflammation; and the natural and synthetic compounds capable of preventing or attenuating their cytotoxic effects. The *in vitro* and *in vivo* models used to demonstrate these effects will be presented. Potentially suitable alternative methods to animal models ("Lab on a Chip" / "Body on Chip" / organoids / iPSCs) allowing to study the biological activities of 7KC and 7β-OHC and to identify molecules capable to modulate their biological activities will also be discussed.

2 - Biogenesis and catabolism of 7-ketocholesterol and 7β-hydroxycholesterol

Sterols are isoprenoid-derived molecules that have essential functions in eukaryotes, and in higher plants. Oxysterols are oxidized derivatives of cholesterol or its precursors post lanosterol [7-9]. They are associated with numerous major pathologies: cardiovascular diseases, neurodegeneration, eye disorders, osteoporosis and some cancers [2, 3, 10]. The most well-

known oxysterols are oxygenated derivatives of cholesterol which are formed by the addition of one or more oxygenated functional groups to 27-carbon cholesterol molecule [11]. Higher plants, algae, most fungi, and vertebrates synthesize sterols whereas insects do not. In vertebrates, cholesterol is the major sterol, whereas a mixture of various sterols, named phytosterols, is present in higher plants with sitosterol [(24R)-24-ethyl cholesterol] usually predominating. The phytosterols, which are structurally similar to cholesterol, can also be oxidized to give oxyphytosterols such as 7-ketositosterol and 7β-hydroxysitosterol [12]. Normocholesterolemic healthy individuals contain a mixture of oxysterols in their peripheral blood, accounting for 1–5% of total cholesterol [13]. Cholesterol oxidation can occur on the hydrocarbon rings (A, B, C and D) of the steroid nucleus or on the side chain of the cholesterol molecule [11]. The oxysterols can be obtained by auto-oxidation, enzymatically or by both processes [11, 14-17]. Oxysterols formed enzymatically are often oxidized on the lateral chain and produced by cytochrome P450 enzymes with the exception of 25-hydroxycholesterol, which is formed by cholesterol 25-hydroxylase (CH25H) [11, 13]. For the latter, there are also arguments in favor of its formation by auto-oxidation of cholesterol [18, 19]. The double bond present on the B ring is the most energetically favorable target of free radical attack and therefore carbon atoms at the positions of 4, 5, 6 and 7 are more susceptible to free radical oxidation [15, 17]. Commonly, B-ring oxidized oxysterols include hydroxylated compounds: 7α-hydroxycholesterol (7α-OHC) and 7β-hydroxycholesterol (7β-OHC); oxysterols with a ketone group [7-ketocholesterol (7-KC, also named 7-oxocholesterol)]; epoxy cholesterols [5β, 6β- epoxy cholesterol (5β, 6β-epox), 5α, 6α-epoxy cholesterol (5α, 6α-epox)] and cholestan-3β, 5α, 6β triol. Among the oxysterols oxidized at C7 [7α-OHC (Systemic name: Cholest-5-en-3β,7α-dio; CAS (Chemical Abstracts Service) number: 566-26-7; lipid map: LMST01010013); 7β-OHC (Systemic name: 5-Cholestene-3β,7β-diol; CAS number: 566-27-8; lipid map: LMST01010047) and 7KC (Systemic name: 7-oxo-Cholest-5-en-3β-ol; CAS number: 566-28-9; lipid map: LMST01010049)] (**Figure 1**). 7α-OHC is formed enzymatically by the enzyme cholesterol 7α-hydroxylase (CYP7A1), and is a precursor of bile acids [20, 21].

The oxysterols 7KC and 7β-OHC can be ingested in certain industrial foods [22-24] and are present in high amounts in atheromatous plaques, oxidized lipoproteins and in the retinal drusen in patients with cardiovascular diseases and age-related macular degeneration (ARMD),

respectively. It has long been considered that these oxysterols were only formed by auto-oxidation of cholesterol [25]. There are also arguments supporting that exogenous 7KC is rapidly metabolized and excreted by the liver, suggesting that dietary 7KC make little or no contribution to atherogenesis [26]. At the moment, several putative mechanisms of cholesterol auto-oxidation are proposed [15, 16, 27, 28]. In cardiovascular diseases and ARMD, 7KC and 7β-OHC can be formed *in situ* under a pro-oxidant environment [14, 29-31]. The proportion of oxysterols in plasma of hypercholesterolemic patients is about 7KC (57%) > 7β /α-OHC (21%) > 5β,6β-cholesterol epoxide (12%) > Triol (10%) while in atherosclerotic plaques it is about 7-KC (55%) > Triol (13%) > 7α /β -OHC (12%) > 5β,6β / 5α,6α -cholesterol epoxide (10%) [32]. Adverse environmental conditions, such as chronic air pollution exposure, have also been reported to favor 7-KC accumulation, foam cell formation, and atherosclerosis in mice [33].

Currently, it is also well established that 7KC can be formed via the CYP7A1 enzyme from 7-dehydrocholesterol (7-DHC), which is the precursor of Vitamin D3 (cholecalciferol), and which is present in high amounts in the plasma of patients with Smith Lemli Opitz syndrome who are deficient in the enzyme 7-dehydrocholesterol reductase (DHCR7) [21, 34]. It has also been demonstrated that the 11β-hydroxysteroid-dehydrogenase type I (11β–HSD1 / gene HSD11B1), which is responsible for the conversion of cortisone in cortisol, is also responsible for the conversion of 7-KC in 7β-OHC [21, 35-37]. In humans, 11β–HSD1 can also catalyse the conversion of 7β-OHC into 7KC [38]. In addition, the 11β-hydroxysteroid-dehydrogenase type II (11β–HSD2 / gene HSD11B2), which is responsible for the conversion of cortisol in cortisone, is also responsible for the conversion of 7β-OHC in 7KC [21, 35, 39]. Of note, the activity and reaction direction of 11β–HSD1 can be altered under conditions of 7-oxysterol excess, and could impact upon the pathophysiology of obesity [40, 41]. How 7KC and 7β-OHC are further metabolized is of great interest since the resulting metabolites may have important biological activities. Oxysterols are present *in vivo* in different forms, namely, the esterified, sulfated, and conjugated forms, as well as free oxysterols [42]. There are now several arguments supporting that 7KC can give rise to numerous metabolites, which probably vary depending on the cell type considered [21, 43]. However, little is known about the catabolism of 7β-OHC. However, for this oxysterol as well as for 7KC, regulation of its activity by sulfation via sulfotransferase 2B1b (SULT 2B1b) cannot be excluded [44]. In addition, potential modifications of the activities of

7KC and 7β-OHC by esterification can also occur. Thus, in human retinal pigment epithelial cells (ARPE19), it has been reported that the esterification of 7KC to fatty acids involves the combined action of cytosolic phospholipase A2 alpha (cPLA2α) and sterol O-acyltransferase (SOAT1) [45]. In addition, Acyl-coenzyme A, a cholesterol acyltransferase (ACAT, also abbreviated as SOATs) which converts cholesterol to cholesteryl esters and which play key roles in the regulation of cellular cholesterol homeostasis, is known to metabolize diverse substrates including both sterols and certain steroids [46]. Oxysterols are substrates of SOAT1 and SOAT2. Of note, in human promonocytic U937 cells, none of the cytotoxic effects observed with 7β-OHC and 7KC were noted with cholesterol, 7β-OHC-3-oleate and 7KC-3-oleate, with the exception of a slight increase in superoxide anion production with 7β-OHC-3-oleate [47]. The enzyme lecithin-cholesterol acyltransferase (LCAT) also permits synthesis of monoesters of 24(S)-hydroxycholesterol [48]. LCAT could also esterify all oxysterols as monoesters (3β-hydroxyl group) and it has been suggested that it could also generate diesters (3β-hydroxyl group and 27-hydroxyl group) in some cases [49]. Specifying the impact of these previously cited enzymes on 7KC and 7β-OHC, in diseases where the levels of these oxysterols are modified, could lead to the identification of new therapeutic targets. It has also been reported on human hepatoma cells (HepG2) that the mitochondrial sterol 27-hydroxylase (CYP27A1) was responsible from the 27-hydroxylated product of 7KC [50].

2 – *In vitro* study of the biological activities of 7-ketocholesterol and 7β-hydroxycholesterol and identification of molecules modulating their activity

The toxicity of certain oxysterols, such as 7KC and 7β-OHC, and the suspected mutagenicity of some of them, such as 5,6-epoxides [51, 52], led to study the toxic and mutagenic properties of these molecules in different cell models. In acidic aqueous solution, 5,6-epoxides can give cholestane-3β,5α,6β-triol (CT) as a single product [53]. Using the Ames test, no mutagenic activity of 7KC, 7β-OHC and 5,6-epoxides was found whereas CT was slightly mutagenic [53, 54]. However, 5,6-epoxides were mutagenic at high concentration on V79 Chinese hamster lung fibroblasts and induced the transformation of murine embryo cells [55, 56]. In addition, no genotoxicity was observed when CHO and Indian Muntjac fibroblastic cells were exposed to 5,6-epoxides [57]. Based on these different studies, it is currently considered unlikely that 5,6-epoxides will be direct carcinogenic [58]. As atherosclerosis is the first pathology in which the

contribution of oxysterols, mainly 7KC and 7β-OHC, was suspected several works were realized in this field [25, 42, 59]. Subsequently, due to several studies suggesting a role of 7KC and / or 7β-OHC in certain ocular diseases (cataract, ARMD), neurodegeneration (Alzheimer's disease in particular) [3], and bowel diseases [2], several types of cells from the eye, brain and digestive tract were used to characterize the cytotoxicity of 7KC and 7β-OHC and to identify molecules capable of preventing their side effects.

2.1 - In vitro models applied to the study of cardiovascular diseases and the identification of cytoprotective compounds in this field

Atherosclerosis, which favors thrombosis, stroke and myocardial infarction, is a slow multifactorial degenerative process involving inflammatory, oxidative and cytotoxic processes. In the development of atherosclerosis, which is a frequent age-related disease [3], major roles are attributed to 7KC and 7β-OHC identified at high levels in the plasma and atheroma plaques of atheromatous patients [60]. In order to determine the biological activities of these molecules, several cellular models have been used especially endothelial cells, smooth muscle cells and monocytic cells.

With regard to cellular models contributing to vessel structure, endothelial cells were used like the human umbilical vascular endothelial cells (HUVEC) [61] and some endothelial cell lines: a) human endothelial cells Eahy926 [62], b) human aortic endothelial cells (HAECs) [63, 64]; c) human endothelial cell line ISO-HAS [65], d) mouse endothelial cells (EOMA cells) [66], e) mouse carotid arterial endothelial cells (CAECs) [67]. The *in vitro* model of arterial relaxation of aortic rings was also used to study the impact of 7-hydroxycholesterols (7KC, 7β-OHC) on endothelial functions [68]. Endothelial cells derived from a primary rat aorta culture (rat aortic endothelial cells (RAEC)) were also used [69].

Rat A7r5 smooth muscle cells and human aortic vascular smooth muscle aortic cells (HA-VSMC) were used to assess the cytotoxic effects of 7-KC and/or 7-βOHC [70, 71]. Smooth muscle cells can also be extracted from aortic tissue of patients [72]. Data obtained on primary cultures from mice (mouse coronary arterial smooth muscle cells (CASMCs) [73] or rabbit [74]) have also been reported.

For inflammatory cells, the most commonly *in vitro* models used are monocytic / macrophage cells THP-1, U937, J774, but also RAW 264.7 and preloaded macrophages (P388D1 cell line) [47, 75-81]. Monocytes were also cultured from human peripheral blood mononuclear cells (PBMCs) [82, 83] or harvested from the mouse peritoneal fluid (peritoneal macrophages) or from mouse aorta (arterial macrophages) [84].

Cardiomyocytes were studied using mouse cardiac cells isolated from HL1-NB mice [85].

These different cell models were not only used to study the impact of 7KC and 7β-OHC on the cells of the vascular wall, but also to identify natural and synthetic molecules capable of preventing the cytotoxicity of 7-KC and 7-βOHC. In these cell models, the following data have been reported: Vitamin E (α-tocopherol) inhibits 7KC-induced oxidative stress, and apoptosis [86, 87]; butyrate, which belongs to a family of gut microbial metabolites, as well as short chain fatty acids (SCFAs) decrease the formation and the activation of Nlrp3 inflammasome induced by 7KC [66]; epigallocatechin 3-gallate (EGCG) inhibits 7KC-induced monocyte–endothelial cell adhesion [65]; indicaxanthin, a bioactive pigment from cactus pear fruit, inhibits 7KC-induced THP-1 cell apoptosis [75]; soy-leaf extract has atheroprotective effects via modulation of Krüppel-Like Factor 2 and adhesion molecules in 7KC-treated HUVECs [88] ; retinoid X receptor α (RXRα) modulator, K-80003, a non-steroidal anti-inflammatory drug, inhibits 7-KC-induced RXRα cytoplasmic translocation [79]; terculic acid, cyclopropene fatty acid, antagonizes 7KC-mediated inflammation [89]; azelnidipine, a calcium channel blocker, inhibits ROS-dependent expression of vascular cell adhesion molecule 1 (VCAM-1) induced by 7KC [90] Aronox, an anthocyanin-rich extract from Aronia melanocarpa E, inhibits apoptosis, ROS generation and the fall of the transmembrane mitochondrial potential induced by 7β-OHC [91]; dimethyl sulfoxide also prevents lysosomal and mitochondrial membrane permeabilization and ROS overproduction induced by 7β-OHC [92].

2.2 - In vitro models applied to the study of eye diseases and the identification of cytoprotective compounds in this field

The eye is composed of different regions, from the cornea to the retina, allowing it to perform its visual function. The retina is the combination of the neurosensory retina and the retinal pigment

epithelium [93]. The neural retina is composed of several cell types, including the glia, whose role is crucial for cholesterol biosynthesis. Cholesterol is the main sterol in the retina. It is present at the cellular level mainly as free cholesterol, but as cholesterol esters in the extracellular environment of the Bruch membrane. The accumulation of cholesterol esters at this level is a marker of retinal aging and decreased retinal function. Subject to dietary influence and local synthesis, cholesterol is also regulated through its interactions with the different cell populations of the retina, and in particular those that involve neuron-glia communication. Age-related macular degeneration (ARMD) is associated with the development of abnormal macromolecular deposits called drusen. These are located in the Bruch membrane [93]. Recent investigations concerning the nature of these deposits, their relationship to inflammatory and immune reactions and the important role of oxidative stress have broadened the hypotheses on the pathophysiological mechanisms leading to ARMD lesions. Many analogies with the mechanisms involved in the genesis of atherosclerosis lesions suggest physiopathological similarities between these two diseases [93]. Currently, several data support the concept that oxysterols, mainly 7KC, are involved in the genesis of ARMD leading to cytotoxic, pro-oxidant and pro-inflammatory activities [14, 94]. In addition, some oxysterols have been identified by gas chromatography in human cataracts obtained after surgery: 7KC, 7β-OHC, 5α, 6α-epoxycholestanol, 20α-hydroxycholesterol, and 25-hydroxycholesterol) [95]. In ARMD, 7KC seems to activate several kinase signaling pathways via multiple transcription factors to induce cytokines and intracellular effectors causing cell death [96].

Among the ocular pathologies studied, several of them concern ARMD and cataracts or vision disorders associated with different pathologies such as Parkinson's disease or Smith Lemli Opitz syndrome [16, 25, 93]. Several *in vitro* models have been used to study the effects of oxysterols on the eye. The most common models are retinal pigment epithelial cells: human ARPE-19 cells [97-100], fetal human retinal pigment epithelial cells (prepared from eyes of human fetuses) [98] or those isolated from ox eyes [101]. Primary cultures of porcine retinal pigment epithelial cells isolated from pigs eyes are also used [102]. Rat retinal precursor cell line R28 has been used less frequently [103]. Microglial retinal cells have also been isolated to study the neuroinflammation phenomena that occur in the outer retina [98, 104]. In addition, 661W photoreceptor cell lines were used [104, 105].

Currently, two molecules were reported to inhibit the toxic effects of 7KC and 7β-OHC on retinal epithelial cells. Sterculic acid, which is a is a cyclopropene fatty acid, antagonizes 7KC-mediated inflammation and inhibits choroidal neovascularization [89]; and resveratrol, a polyphenol belonging to the stilbene class used in the treatment of ARMD attenuate 7KC-induced cell death and prevent 7KC-induced VEGF secretion [106].

2.3 - In vitro models applied to the study of neurodegenerative diseases and the identification of cytoprotective compounds in this field

Since increased levels of 7KC and 7β-OHC were identified in the plasma and/or the cerebrospinal fluid of patients with demyelinating and non-demyelinating neurodegenerative diseases such as multiple sclerosis (MS) [107], X-linked adrenoleukodystrophy (X-ALD) [108], and Niemann-Pick disease [21], the effects of 7KC and 7β-OHC were evaluated on different types of nerve cells (neurons, glial and microglial cells). In these cells, several studies addressed the question of whether these oxysterols can induce organelle dysfunction (mitochondria, lysosome, peroxisome), oxidative stress, metabolic dysfunction, and cell death, which are hallmarks of neurodegeneration.

Thus, undifferentiated and retinoic acid-differentiated human SH-SY5Y neuroblastoma cells were used to study the biological ability of certain oxysterols such as 24S-hydroxycholesterol (24S-OHC). In these cells, 7KC-induced cell death was attenuated by 24S-OHC [109]. Human SK-N-BE neuroblastoma cells were also treated with 7KC at the physiopathological concentration of 1 µM with the aim of comparing the effects of 7KC and 24S-OHC on tau phosphorylation, and to study the abilities to modulate the SIRT1-dependent neuroprotective pathway [110]. SK-N-BE cells were also used to compare the cytotoxicity of 7KC and 7β-OHC. In SK-N-BE cells, 7KC and 7β-OHC induce a loss of mitochondrial activity, an inhibition of cell growth, ROS overproduction, an enhancement of antioxidant enzymes activities, and a slight increase in Ca^{2+} [111]. To determine how 7β-OHC could contribute to the development of Alzheimer's disease, the interaction between 7β-OHC and amyloid-beta (Aβ), whose extracellular accumulation in neuritic plaques is one of the hallmarks of Alzheimer's disease, was studied [112]. It was reported that 7β-OHC enhances the binding of Aβ to these cells by up-regulating the expression and the synthesis of CD36 and β1-integrin receptors [112]. In rat

pheochromocytoma PC12 cells exposed to sub-lethal concentrations of 7β-OHC, a significant increase of cellular glutathione levels and an enhancement of cell tolerance against the subsequent oxidative stress were observed [113]. C6 rat glioma cells were also incubated with 7β-OHC for 24 h with the purpose of evaluating its cytotoxicity and to determine the type of cell death induced by this oxysterol [114]. In these cells, it was demonstrated that 7β-OHC induces a non-apoptotic mode of cell death associated with survival autophagy [114].

Immortalized murine glial cells (oligodendrocytes 158N) and microglial cells (BV-2) were also used to study the cytotoxic effects of 7KC and 7β-OHC in the context of demyelinating and non-demyelinating neurodegenerative diseases. Indeed, these cells are suitable models to analyse oxidative stress, inflammation and cell death (apoptosis and autophagy) and to study the relationships between these different side effects [1, 115-117]. These cells constitute good models to study the impact of 7KC and 7β-OHC on organelle structure, topography, functions and interactions (mitochondria, lysosome and peroxisome) [1]. They also permit to study the part taken by the peroxisome in cell death, as well as to identify natural or synthetic molecules capable of counteracting or attenuating 7KC- and 7β-OHC-induced cytotoxicity.

To precisely define the impact of neuroinflammation in MS, organotypic hippocampal slice cultures were incubated with BV-2 cells without and with lipopolysaccharides (LPS) [118]. Under these conditions, an accumulation of 7KC was observed in brain tissue, and an induction of apoptosis associated with oxidative stress was reported in the BV-2 cells [118]. This phenomenon involves the translocation of NF-κB and the activation of poly(ADP-ribose)polymerase-1 (PARP-1), which is essential for microglial migration and consecutively regulates the expression of the iNOS, CD11a, and ICAM-1. These latter mechanisms are essential for the damaging activity of microglial cells [118]. Use of murine microglial BV-2 cells permitted demonstration of the ability of oleic acid (C18:1 n-9) and docosahexaenoic acid (DHA; C22:6 n-3), two major fatty acids present in the Mediterranean diet, to attenuate 7KC-induced oxiapoptophagy, associated with oxidative stress and with apoptotic and autophagic characteristics [1, 117, 119]. Currently, 158N cells are a widely used model to study the cytotoxicity of 7KC and 7β-OHC, and to determine the effects of these oxysterols on major myelin proteins expressed by these cells (Myelin Basic Protein (MBP), Proteo Lipid Protein (PLP)) [115]. In 158N cells, anti-apoptotic and anti-oxidant capacities of natural or synthetic

compounds and their ability to attenuate or to prevent toxicity of 7KC or 7β-OHC were often evaluated. In 158N cells incubated with 7KC or 7β-OHC, the cytoprotective effect of dimethylfumarate (DMF; Tecfidera) and biotin (vitamin B8), which are used for the treatment of MS, have been shown [120, 121]. Natural products including argan oil, milk thistle seed oil, sea urchin egg oil, *Carpobrotus edulis* ethanol-water extract also strongly attenuate 7KC and/or 7β-OHC-induced cytotoxicity [121-125].

2.3 - In vitro models applied to the study of bowel diseases and the identification of cytoprotective compounds in this field

Oxidative stress is thought to play a key role in the development of intestinal damage in inflammatory bowel disease, because of its primary involvement in intestinal cells aberrant immune and inflammatory responses to dietary antigens and to the commensal bacteria [126, 127].

In this context, human intestinal Caco-2 cells are often used and cultured in the presence of an oxysterols mixture (7KC, 5α,6α epoxycholesterol, 5β,6β-epoxycholesterol, 7α-hydroxycholesterol and 7β-OHC) [127]. In this cell model pro-oxidant and pro-inflammatory effects of the oxysterol mixture were shown [128]. These cytotoxic effects were prevented by wine polyphenols [129, 130], olive oil polyphenols [131] and cocoa bean shells [128].

2.4 - Interest of the yeast model, Saccharomyces cerevisiae and of the protozoa model, Tetrahymena pyriformis, to study 7-ketocholesterol- and 7β-hydroxycholesterol-induced cytotoxicity

The yeast, *Saccharomyces cerevisae* (SC), is an excellent model for studying autophagy [132] . Due to the ability of 7-KC and 7β-OHC to induce oxiapoptophagy [119, 133], the SC model should provide a better understanding of the characteristics of autophagy induced by 7KC and 7β-OHC. In addition, the SC model is a well recognized for studying the peroxisome, which still remains a poorly characterized organelle [134]. The SC model should make it possible to specify the impact of 7-KC and 7β-OHC on the peroxisome in terms of biogenesis and function, peroxisome-mitochondria interaction [135]. The protozoan model *Tetrahymena pyriformis* (TP) is an interesting tool for performing environmental toxicology analyzes [136]. Compared to

commonly used cellular models, it is a reference model to address the impact of molecules on the phagocytosis of nano- or microparticles; on mobility (this protozoan moves thanks to numerous cilia); and on the relationships between organelles (mitochondria, peroxisome) and mobility. The TP model has been successfully used for the identification of cactus (*Opuntia ficus indica*) extracts capable of inhibiting 7KC-induced cell death [137]. These easy-to-implement cellular models are also interesting tools for identifying molecules that modulate the activities of 7-KC and 7β-OHC.

2.5 – 7-ketocholesterol and 7β-hydroxycholesterol signaling pathways based on in vitro studies

The pathways involved in 7KC and 7β-OHC signaling for oxidative stress, and cell death, characterized by apoptotic and autophagic criteria (oxiapoptophagy), and in 7KC- and 7β-OHC-induced inflammation (cytokine secretion, enhancement of adhesion molecule expression) have been primarily determined on the cellular models discussed in the preceding section. It is important to emphasize that these signaling pathways are highly conserved from one cell type to another and seem to be independent of the species considered [25, 87].

2.5.1 - 7-ketocholesterol-induced oxiapoptophagy and inflammation: associated signaling pathways

The schematic signaling pathways presented in **Figure 2** summarize the data obtained with 7KC in cells from different types and species: promonocytic / monocytic human U937 / THP1 cells [25, 47]**;** wild type human mammary tumor MCF-7 cells (caspase-3 deficient), and genetically modified MCF-7 (MCF-7/c3: stably transfected with caspase-3) [138], human retinal pigment epithelial cells (ARPE-19) [97, 139] and rat R28 retinal neurosensory cells [140]; murine oligodendrocytes 158N and murine microglial BV-2 cells [3, 119, 141-145].

In A7r5 rat aortic smooth muscle cells and murine oligodendrocyte 158N cells, 7KC has been shown to accumulate in lipid rafts [141, 146]. In U937 and BV2- cells, and based on artificial membrane models, 7KC interacts with plasma membrane phospholipids leading to important changes in membrane properties [25, 117, 147]. In U937, 158N and BV-2 cells, 7KC induces a mode of cell death defined as oxiapoptophagy [47, 133]. 7KC stimulates an overproduction of ROS: superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [25]. In human aortic smooth muscle cells, 7KC activates the NADPH oxidase (NOX-4) via endoplasmic reticulum stress

involving the IRE-1/JNK/AP-1 signaling pathway which contributes to overproduction of ROS, leading to decreases in ΔΨm and apoptosis [148]. 7KC-induced apoptosis is characterized by an early externalization of phosphatidylserine [149] and by the following events: *(i)* Ca^{2+} influx; *(ii)* activation of calmodulin and calcineurin leading to BAD dephosphorylation and subsequent mitochondrial depolarization; *(iii)* mitochondrial release of cytochrome c, AIF and Endo-G; *(iv)* activation of caspase-2, -3, -7, -8, and -9; *(v)* truncation of BID; *(vi)* lower BCL-2 levels; *(vii)* cleavage of PARP; and *(viii)* of the DNA fragmentation factor (DFF45)/ICAD leading to the activation of caspase activated DNase (CAD) involved in internucleosomal DNA fragmentation [71, 138, 150-152]. In addition, 7KC-induced cell death is associated with an activation of the P2X7 receptor (involved in Na^+/Ca^{2+} influx and K^+ efflux) [97, 143]. In 158N and BV-2 cells, 7KC also triggers an increased level of the voltage-gated K^+ (Kv) channel kv3.1b (involved in K^+ efflux) protein, and an intracellular accumulation of K^+ [143, 144]. The cytoplasmic accumulation of K^+ is positively correlated with increased plasma membrane permeability to PI, ROS overproduction and loss of ΔΨm [143]. In monocytes macrophages (U937, RAW264.7, P388D1) and smooth muscle cells, it has been shown that 7KC inhibits the PDK-1/(Akt/PKB) signaling pathway [86, 153] and triggers the formation of multilamellar cytoplasmic structures named myelin figures [25, 154]. The ability of 7KC to induce also lysosomal modifications [122, 133, 155], formation of monodansyl cadaverine positive structures [156] and activation of LC3-I into LC3-II, also support that 7KC is capable of triggering autophagy. It is suggested that 7KC could induce a protective form of autophagy [73, 157] and it is now considered that myelin figures could be ultrastructural features of reticulophagy. Under treatment with 7KC, peroxisomal dysfunction (decreased peroxisomal mass suggested by decreased expression of ABCD3, altered peroxisomal β-oxidation supported by an accumulation of very long chain fatty acids) has also been reported [158-160]]. Whereas the part taken by the peroxisome in cell death is not well known [161], it has been demonstrated that peroxisomal dysfunctions are able to trigger oxidative stress [162, 163]. As the peroxisome is tightly connected with the mitochondria [164, 165] which plays key roles in 7KC-induced apoptosis [166], the contribution of the peroxisome to 7KC-induced cell death, which is associated with a rupture of the RedOx homeostasia, cannot be excluded [1].

It is also now well established that 7KC is a potent inducer of inflammation. This oxysterol increases cytokine secretion (IL-8, IL-1β) and overexpression of adhesion molecules (VCAM-1,

ICAM-1, E-selectin). However, in U937 and THP-1 cells, the secretion of IL-1β and IL-8, and the expression of VCAM-1, ICAM-1, and E-selectin was lower than with 7β-OHC [61, 167, 168]. Currently, limited data are available on the metabolic pathway(s) contributing to 7KC-induced inflammation. In cultured ARPE-19 cells, 7KC-induced inflammation is mediated mostly through the TLR4 receptor, with some cross-activation of EGFR-related pathways via NF, leading to the activation of NFκB, which is essential in mediating cytokine expression (IL-1β, IL-6 and IL-8) [169]. In addition, the ability of 7KC to induce IL-1β-secretion suggests an activation of the inflammasome. This hypothesis is supported by several experimental studies. Thus, formation and activation of Nlrp3 inflammasomes in bone marrow derived macrophages (BMMs) has been reported in the presence of 7KC [170]. Sublethal concentrations of 7KC in retinal microglia isolated from postnatal C57BL/6J mice resulted in microglial activation and polarization to a pro-inflammatory M1 state, via NLRP3 inflammasome activation [104]. 7KC also efficiently induces inflammasome formation in fetal human RPE (fhRPE), human ARPE-19 cells, primary human brain microglia cells, and human THP-1 monocyte cells [98].

2.5.2 - *7β-hydroxycholesterol-induced oxiapoptophagy and inflammation: associated signaling pathway*

The schematic signaling pathways presented in **Figure 3** summarize the data obtained with 7β-OHC on cells from different types and species: promonocytic / monocytic human U937 / THP1 cells [25, 47]**;** vascular cells (endothelial and smooth muscle cells) [25, 148]**;** wild type human mammary tumor MCF-7 cells (caspase-3 deficient), and genetically modified MCF-7 (MCF-7/c3: stably transfected with caspase-3) [138], human retinal pigment epithelial cells (ARPE-19) [100] / primary porcine retinal epithelial cells; rat C6 glioblastoma cells [171], murine oligodendrocytes 158N and murine microglial BV-2 cells [3, 114, 119, 121, 141, 142]. The different cells used permitted determination of the signaling pathways involved in 7β-OHC-induced cytotoxicity and to define the relationships between ROS overproduction, apoptosis, autophagy and inflammation. Distinctly from 7KC, 7β-OHC, while cytotoxic, does not accumulate in lipid rafts [141]. 7β-OHC is a strong inducer of ROS overproduction and favors the disturbance of redox homeostasis by increasing the formation of lipid peroxidation products (malondialdehyde (MDA), conjugated dienes (CDs)) and of carbonylated proteins (CPs) which can further contribute to cell death [172]. An important impact on the mitochondria was also observed whatever the cells considered. In

human monocytic THP-1, U937 cells, and MCF-7 cells, a down-regulation of Bcl-2 expression was also detected as well as an activation of the pro-apoptotic proteins (Bid, Bax), associated with a release of cytochrome c and an activation of caspase-9, caspase-8, caspase-3 and caspase-7. In U937 cells, 7β-OHC also induced an increase in cytosolic Ca^{2+} concentration, associated with a decrease of Akt activation and a mitochondrial release of various proteins such as cytochrome c, apoptosis-inducing factor (AIF), and endonuclease-G (Endo-G), associated with caspase-3, -7, -8, and -9 activation, Bid cleavage and PARP degradation [25, 138]. In C6 glioblastoma cells, 7β-OHC induces apoptosis through decreased ERK signaling, transient PI3K / Akt activation, loss of GSK3β activation and activation of p38 [171, 172]. In U937 cells, as well as in human retinal pigment epithelial cells (ARPE-19), large myelin figures (evocating reticulophagy) were observed [25, 100]. In ARPE-19 cells, a link between lysosome and cell death was also established [100]. In addition to lysosomal and mitochondrial dysfunctions, in 158N and BV-2 cells, 7β-OHC also induces peroxisomal changes (morphological and topographical changes, reduce number of peroxisome, altered metabolism). In 158N cells, the complex mode of cell death induced by 7β-OHC (oxiapoptophagy) is characterized by a dephosphorylation of PKB / Akt, an activation of GSK3, and by a reduced expression of Bcl-2; altogether these events contribute to mitochondrial depolarization leading to caspase-3 activation, PARP degradation and internucleosomal DNA fragmentation [141]. Moreover, 7β-OHC promotes the conversion of microtubule-associated protein light chain 3 (LC3-I) to LC3-II which is a hallmark criterion of autophagy [119]. The ratio [LC3-II / LC3-I] is also strongly modified by bafilomycin acting on the autophagic flux. Rapamycin, an autophagic inducer, and 3-methyladenine, an autophagic inhibitor, reduce and increase 7β-OHC-induced cell death, respectively, supporting that 7β-OHC induces survival autophagy [114]. Altogether, these data establish that 7β-OHC is a potent inducer of oxiapoptophagy through the concomittent activation of several signaling pathways involved in oxidative stress, apoptosis and autophagy.

There is also evidence that 7β-OHC is a potent inducer of inflammation: it increases cytokine secretion (IL-8, IL-1β) and overexpression of adhesion molecules (VCAM-1, ICAM-1, E-selectin). Inn U937 and THP-1 cells, the secretion of cytokines and the expression of adhesion molecules observed under treatment with 7β-OHC were higher than with 7KC [61, 167, 168]. Currently, no data are available on the relationship between 7β-OHC-induced IL-1β secretion and inflammasome activation. Of note, it has been described in several works that the PKC/ P38/

MEK/ ERK signaling pathway constitutes a link between 7β-OHC-induced apoptosis and inflammation [168, 171, 172].

Comparatively to 7KC, less information are available on the 7β-OHC. It is however well established that 7β-OHC is a more potent inducer of apoptosis than 7KC and is also a more potent inflammatory mediator. Indeed, the percentage of apoptotic cells and the level of cytokine secretion are higher under treatment with 7β-OHC than with 7KC. Although there are common signaling pathways for both molecules, there must also be specific pathways for each of them.

2.5.3 – *Contribution of Ca^{2+} and K^{+} in 7-ketocholesterol and 7β-hydroxycholesterol signaling pathways*

Ca^{2+} is a universal second messenger, participating in the regulation of almost every cellular process, from fertilization to motility, gene expression and death [173]. Consequently, the ability of 7KC or 7β-OHC to modify intracellular Ca^{2+} concentrations in diverse cell-types of the vascular wall, with a range of downstream consequences, have been studied. In HUVECs, incubation with 150 μM 7KC for 2 h elevated resting cytosolic Ca^{2+} levels and enhanced Ca^{2+} responses to histamine [174]. In mouse aortic endothelial cells, micromolar concentrations of 7KC elicited a transient increase in cytoplasmic Ca^{2+} within seconds of addition, via a mechanism partially dependent on Ca^{2+} release from intracellular pools, since it persisted in Ca^{2+}-free extracellular medium. This transitory rise in Ca^{2+} promoted reactive oxygen species (ROS) formation, with subsequent apoptotic cell-death [175]. In contrast, low micromolar concentrations of 7β-OHC promoted survival of HUVECs, increasing the phosphorylation of extracellular signal-regulated kinases (ERKs). These effects are probably mediated by a store-operated Ca^{2+} entry mechanism, since they are blocked by 2-aminoethyl diphenylborinate and by gadolinium ions [176]. In human aortic smooth muscle cells, within a few minutes of addition, micromolar 7β-OHC triggered oscillations in cytoplasmic Ca^{2+} concentrations, followed by depletion of intracellular Ca^{2+} stores sensitive to thapsigargin, an inhibitor of sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps. These changes were associated with activation of ERK1/2 within minutes and with apoptotic cell death over 72 h [177]. In the same cell type, 7KC elicited similar Ca^{2+} oscillations, enhanced activity of the NAD(P)H oxidase Nox-4, increasing ROS production and induced the ER unfolded protein response [148]. In human coronary artery smooth muscle cells, 7KC caused a rise in cytoplasmic Ca^{2+} followed by caspase-3 dependent

apoptosis. Both the ER stress response and caspase-3 activation could be partially inhibited by submicromolar concentrations of nifedipine, an antagonist of L-type voltage-gated Ca^{2+}-channels (VGCCs) [178]. In mouse coronary artery smooth muscle cells, 7KC stimulated rises in cytoplasmic Ca^{2+} that enhanced ROS formation, promoting differentiation via activation of nuclear factor erythroid 2–related factor 2 (NRF2). As revealed in smooth muscle cells from CD38-/- transgenic mice [179], these Ca^{2+} elevations were dependent on CD38, an enzyme that can generate second messengers promoting Ca^{2+} release via ER/SR ryanodine receptors (by cyclic ADP ribose), or from lysosomal Ca^{2+} stores (by nicotinic acid adenine dinucleotide phosphate) [180]. In the A7r5 rat aortic smooth muscle cell-line, incubation with 7KC or 7β-OHC for 24 h elevated resting cytosolic Ca^{2+} concentrations and suppressed responses to bradykinin, or arginine vasopressin, peptide hormones linked to Ca^{2+} mobilization via the phospholipase C-inositol 1,4,5-trisphosphate (IP_3) pathway. In this system, 7β-OHC led to a decrease in the levels of the type 1 IP_3 receptor/Ca^{2+}-release channel protein, in a manner that could be abolished by co-incubation with an inhibitor of the proteasomal pathway [181]. In the THP1 monocyte/macrophage cell-line, 7KC stimulated Ca^{2+} increases that were partially inhibited by micromolar concentrations of the L-type VGCC inhibitors verapamil and nifedipine [152]. These elevations in cytoplasmic Ca^{2+} exerted diverse and opposing effects on cell-death pathways: activating calcineurin to stimulate Bcl-2-associated death promoter (BAD) [152]; causing the release of pro-apoptotic Bim-LC8 from the microtubule-associated dynein motor complex; and activating the tyrosine kinase PYK2, thereby inhibiting apoptosis via the ERK pathway [182]. In this cell-line, 7β-OHC also triggered Ca^{2+} influx dependent on VGCCs, promoted cell survival through the ERK pathway; and stimulated transcription and secretion of interleukin-8 [183]. In differentiated U937 cells, 7-βOHC, but not β-epoxide, caused gradual increases in cytoplasmic Ca^{2+} concentration that lasted for at least 15 minutes and were inhibited by nifedipine, or by removal of extracellular Ca^{2+}. In these cells, nifedipine also reduced cell-death caused by 7β-OHC [184].

The molecular mechanism(s) by which 7KC or 7β-OHC alter cytoplasmic Ca^{2+} levels are unclear [185]. Most of these responses are too rapid to require changes in gene expression, excluding roles for liver X receptor (LXR)- or sterol regulatory element binding protein (SREBP)-dependent oxysterol sensing pathways. Cell-type and congener-specificity indicates that these effects are probably not dependent on biophysical modification of lipid membranes by oxysterols,

instead requiring specific, protein-based receptors. The intracellular oxysterol-binding protein related proteins ORP4L [186], ORP5 and ORP8 [187] modulate Ca^{2+} signaling at membrane contact sites. In radioligand binding assays using heterologous expressed proteins, 7KC displaced 25-hydroxycholesterol bound to ORP4L [188]. In hepatoma cell-lines, ORP8 is required for the cytotoxicity of various oxysterols, including that of 7KC and 7β-OHC, demonstrating that it can act as an effector for these cholesterol oxidation products [189]. To better understand the mechanisms of intracellular Ca^{2+} modulation by 7KC and 7β-OHC, it could be interesting to study the impact of these oxysterols on the inhibition of the catalytic subunit D8D7I of cholesterol epoxide hydrolase (ChEH) [190]. Indeed, 7KC and 7β-OHC are potent inhibitors of ChEH [190], the subunit called 3β-hydroxysterol-Δ^8-Δ^7-isomerase (D8D7I) has been characterized as a Ca^{2+} binding protein [191], and it is not excluded that 7KC could inhibit D8D7I activity [192]. In addition, at least two types of G-protein coupled receptor (GPR) are activated by oxysterols. The repression of the GPR Smoothened by the Sonic hedgehog peptide, thereby removing the inhibitory effect of Patched, increases cytoplasmic Ca^{2+} levels in rat gastric mucosal cells [193]. In NIH 3T3 fibroblasts, 20S-hydroxycholesterol potently activated Smoothened, but 7-βOHC was without detectable effect [194]. A distinct GPR, Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2) or GRP183, was activated by nanomolar levels of 7α, 25-dihydroxycholesterol when heterologous expressed in CHO-K1 cells, causing Ca^{2+} release from intracellular stores [195]. Although 7β-OHC is a very weak agonist of this receptor, both it and 7KC can be converted into more active congeners by the actions of 25-hydroxylase and 11β-hydroxysteroid dehydrogenases [196]. The mechanisms by with 7β-OHC and 7KC can influence Ca^{2+} signaling are summarized in **Figure 4**.

Of note, whereas the cells of the vascular wall display rapid Ca^{2+} responses to oxysterols, such effects were not observed on nerve cells. For example, although both 7KC and 7β-OHC promote oxiapoptophagy in mouse 158N oligodendrocytes, they did not detectably alter cytoplasmic Ca^{2+} concentrations over a 10 minutes time period [141]. These oxysterols did not detectably alter cytoplasmic Ca^{2+} levels in the SK-N-BE neuroblastoma cell-line within 10 minutes of addition, but increased the formation of calcium deposits within 24 hours, as revealed using von Kossa staining [144].

Other mechanisms that potential link 7KC and 7β-OHC to Ca^{2+} signaling have not been completely defined. These mechanisms include P2X7 purinoreceptor cation channels [97], CD38 [179], and the voltage-gated K^+ channel $K_v1.3b$, which modulates membrane potential and thereby VGCC opening [144]. In human retinal cells, 7KC-induced toxicity has been reported to activate the P2X7 receptor which leads to Na^+ and Ca^{2+} influx, and K^+ efflux [97]. Activation of P2X7 receptor triggers the formation of large nonselective membranes pores which results in inflammation through the inflammasome, oxidative stress and, ultimately, cell death especially by apoptosis [97]. In 158N cells, the voltage-gated K^+ (Kv) channels (Kv3.1) designed for high-frequency repetitive firing and expressed by different types of nerve cells in the CNS is affected by 7KC. Increased levels of Kv3.1b protein were shown in 158N cells under treatment with 7KC, and positive correlations between Kv3.1b levels and the intracellular K^+ concentration ($[K^+]_i$) were observed [144]. Under treatment with 7KC, the simultaneous increased of $[K^+]_i$ and of the level of Kv3.1b along with increasing percentage cells with depolarized mitochondria, ROS overproduction and markers of death [144]. This lead to speculation that K^+ retention could contribute to 7KC-induced cytotoxic effects and that enhanced expression of Kv3.1b could occur as a compensatory mechanism, contributing to prevention of 7KC cytotoxicity. This hypothesis is supported by the fact that the blockage of Kv channels with 4-aminopyridine exacerbated 7KC-induced cell dysfunction on 158N murine oligodendrocytes and microglial murine BV-2 cells [143].

3 – Contribution of bacteria for the identification of new strategies to prevent 7-ketocholesterol-induced cytotoxicity: biodegradation of 7-ketocholesterol by bacterial cells and enzymes

Inherent metabolic insufficiency of the human body increases with age, which can lead to the accumulation of pathogenic compounds like 7KC in senile cells, causing deleterious functions and cell death. Alternatively, utilizing exogenous microbial enzymes for targeting cytotoxic compounds enabling subsequent degradation is a promising substitute to endogenous enzymes. Microbes have long been proven to be remarkable in catabolizing toxic xenobiotic compounds like pesticides, solvents and hydrocarbons, which have found application in the remediation of polluted environments [197-199]. In general, the biodegradation process involves microbial use

of the organic compounds as a carbon/energy source, breaking down of complex organic structures, assimilation or release of the degradation products, and subsequent mineralization to carbon dioxide and water. 'Medical bioremediation' as a novel strategy, has been proposed to remediate cytotoxicity of 7KC which involves screening of microbes capable of catabolizing 7KC, identification of relevant enzymes, overproduction of these enzymes, and delivery into targeted organelle of diseased cells [5, 6, 200, 201]. The enzymes could also have application in food productions to remediate 7KC in its dietary sources. In fact, use of such systems against toxic molecules has been reported in amyloid-β degradation by insulin-degrading enzyme [202] and mycoplasma cells [203]; cleavage of bisretinoid lipofuscin (A2E) by horseradish-peroxidase [204]; and hydrolysis of blood cholesterol and triglycerides by *Pseudomonas gessardii* lipase [205].

The earliest studies on 7KC degradation identified several bacterial and actinobacterial strains isolated from soil and activated sludge, namely *Nocardia nova*, *Proteobacterium* Y-134, *Sphingomonas* sp. JEM-1, *Rhodococcus jostii* RHA1 and *Pseudomonas aeruginosa* having the potential to mineralize 7KC, using it as a sole-carbon and energy source. The first four strains were also found to release CO_2 in minimal salt media with 0.1% 7KC [206]. Additional studies by the same group conducted transcriptomic analysis of genes expressed by *Rhodococcus jostii* RHA1 in presence of 7KC and cholesterol revealed 363 differentially expressed in presence of 7KC compared to cholesterol. In fact, 7KC was found to induce a larger number of steroid catabolism genes, mostly belonging to three or four putative clusters. The genes included those responsible for the catabolism of the steroid rings such as *kstD*, *kshA*, *hsaABCDEFG*, or their homologs. The steroid uptake system Mce4 was also found essential for uptake of 7KC into the cell. Other genes identified include *hsaC* coding for a dioxygenase which cleaves the ring A of cholesterol and Cyp125 which initiates side-chain degradation. However, none of these clusters code for a complete 7KC degradation pathway. It seems that cholesterol and 7KC degradation follows a common pathway until the *hsaC* step and the degradation of the side chains occur simultaneously with that of the rings. Several metabolites of the pathway such as 3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid (3,4,7-THSAP), 3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione propionic acid (3,4-DHSAP), 3,4,7-trihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione (3,4,7-THSA) and 3,4-dihydroxy-9,10-seconandrost-1,3,5(10)-triene-9,17-dione (3,4-DHSA) were identified, along with the

involvement of enzymes such as dioxygenase (*hsaC*), 7-keto reductase and dehydratase [207]. Subsequent studies on *Pseudomonas aeruginosa* PseA and *Rhodococcus erythropolis* MTCC 3951 reported respectively 88% and 93% degradation, of an initial 7KC concentration of 1 g/L (1000 ppm), with a high growth of biomass. The extracellular extracts of the bacterial strains were capable of degrading 0.1 g/L (100 ppm) 7KC to a very high extent (65% in case of *P. aeruginosa* and 98% in *R. erythropolis*) within 72 hours, pointing to the involvement of enzymatic systems in the degradation process. Cholesterol oxidase was assayed as the main enzyme responsible for the first reaction pertaining to conversion of 7KC to 4-cholesten-3, 7-dione. In *P. aeruginosa*, cholesta-3, 5-dien-7-one/cholesta-4, 6-dien-3-one were found to be the next intermediate products, while in case of *R. erythropolis*, chol-5-en-3,7-dione and androsta-4-ene-3,7,17-trione were found to be formed downstream in the pathway. Here, the side-chain degradation precedes that of the ring-cleavage of 7KC. Lipase, reductase and dehydrogenase were the other identified enzymes secreted by the microbes in presence of 7KC [208, 209]. More recently, environmental samples such as soil, sea water sediment and manure piles have been explored for the presence of 7KC degrading microbes. *Alcanivorax jadensis* IP4 isolated from sea water sediment, *Streptomyces auratus* IP2 and *Serratia marcescens* IP3 isolated from soil samples and *Thermobifida fusca* IP1 isolated from manure piles proved to be potent strains capable of using 7KC (1 mg/L) as a sole carbon source and to subsequently mineralize it. Out of these, *A. jadensis* IP4, followed by *T. fusca* IP1 were most efficient in degrading 7KC, with 100% degradation of 1 mg/L concentration achieved within 12 days. Intracellular extracts of *A. jadensis* IP4 were able to degrade 65% of same concentration of 7KC within 72 hours [210, 211].

As in the case of storage diseases, bulk of the 7KC absorbed by the cell is localized to lysosomes. Thus, in spite of the reason that several enzymes such as 27-hydroxylase (CYP27A1), 11β-Hydroxysteroid dehydrogenase, cholesterol sulfotransferase (SULT2B1b) and Acyl-CoA cholesterol acyltransferase (ACAT) have been known to act on 7KC, their unavailability in lysozymes, prevents the attenuation of its cytotoxicity. In liver cells, 11beta-hydroxysteroid dehydrogenase type 1 converts 7KC to 7β-OHC, which is then transformed by the hepatic metabolism [212]. A plasmid construct of pEGFP-N3, harboring the *Chromobacterium* DS-1 cholesterol oxidase gene fused with the signal sequence and transmembrane domain of the lysosomal membrane protein LAMP1 (named as pEGFP-COXL1) was found to be localized within the lysosome. Human fibroblast cells transfected with this plasmid were able to withstand

up to 50 µM concentration of 7KC, compared to the control, proving the cytoprotective effect of the enzyme [213]. The role of cholesterol oxidase in 7KC biotransformation was further proved in another application study, where the enzyme immobilized on magnetic Iron (II, III) oxide nanoparticles was able to convert cholesterol and 7KC to 4-cholesten-3-one and 4-cholesten-3, 7-dione respectively, which could be used as steroid precursors or anti-obesity drugs [214]. An interesting investigation reports the ability of growing and resting cells of *Lactobacillus casei* ATCC334 in removing oxysterols from solution, probably through binding to the cell surface or by membrane incorporation. The resting cells were most efficient in removal (37 to 61%) of oxysterols such as 7KC followed by 7α/7β-OHC, cholestanetriol, 5,6β/5,6α-epoxycholesterol and 25-hydroxycholesterol respectively. Being probiotics, *L. casei* may find application in inhibiting the intestinal absorption of harmful oxysterols [215].

Thus, microbial biodegradation and consequent mining of therapeutic enzymes may provide a promising route to remediate oxysterol-mediated cytotoxicity, especially that of 7KC and 7β-OHC.

4 – Animal models and analysis of the biological activities of 7-ketocholesterol and 7β-hydroxycholesterol

Currently, the biological activities of 7KC and 7β-OHC have mainly been evaluated in animal models developed to study atherosclerosis. In this context, numerous animal models are available. At the opposite extreme, only a few animal models are described to determine the incidence of these oxysterols in eye, neurodegenerative and bowel diseases.

4.1 – Mouse transgenic models

To address the incidence of oxysterols in different pathologies, murine transgenic animal models have been developed when the relevant oxysterols considered are enzymatically produced. This is mainly the case for oxysterols oxidized on the lateral chain, which do not include 7KC and 7β-OHC. Indeed, the oxysterols oxidized on the lateral chain interact with the Liver X Receptors (LXRα/β) [216]. LXR deficient transgenic mice have contributed to demonstrate the importance of LXR in the fertility and development of prostate cancer) [217-219]. LXRβ is also involved in

amyloidogenesis associated with neurodegeneration in Alzheimer's disease [220]. On the other hand, CYP46A1 is mainly expressed in the central nervous system neurons and allows the production of 24S-hydroxycholesterol (24S-OHC) [221]. The use of CYP46A1-deficient mice permitted to demonstrate the importance of this enzyme and 24S-OHC in major neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease [222-225]. As for the enzyme CYP27A1 which makes it possible to produce 27-hydroxycholesterol (27-OHC) from cholesterol essentially at the peripheral level, the use of CYP47A1 deficient mice has demonstrated the importance of this enzyme and of 27-OHC in cancer development (breast cancer, melanoma) [226, 227].

Unlike oxysterols oxidized on the lateral chain such as 24S-OHC and 27-OHC, 7KC and 7β-OHC are essentially formed by auto-oxidation of cholesterol in different tissues (vascular wall, retina, brain) under the influence of an environmental oxidative stress [25]. They also accumulate in the vascular wall, and this process is favored by a diet rich in oxysterols even if these latter are metabolized by the liver [25].

ApoE deficient mice were used to determine the vascular effects of 7β-OHC [228]. CX3C chemokine receptor 1 ($CX3CR1^{GFP/+}$) mice were also successfully used to demonstrate that retinal microglia have a prominent chemotropism to 7KC and internalize 7KC. Sub-lethal concentrations of 7KC resulted in microglial activation and polarization to a pro-inflammatory M1 state via NLRP3 inflammasome activation. In addition, microglia exposed to 7KC reduced expression of neurotrophic growth factors but increased expression of angiogenic factors, transitioning to a neurotoxic and pro-angiogenic phenotype [104]. Since the enzyme 11β-HSD11B1 has been shown to convert 7KC to 7β-OHC, mouse models deficient for this enzyme, that also converts cortisone to cortisol, have been developed to study atherosclerosis and metabolic syndrome [36, 37, 229-231]. In the context of obesity (with or without metabolic syndrome, and type 2 diabetes), the role of 11β-HSD11B1 and 11β-HSD11B2 in ob/ob mice is a subject of new research [232, 233]. Mice deficient in the enzyme 7-dehydrocholesterol reductase used, as a model of Smith-Lemli-Opitz syndrome, are also available to address the conversion of 7-dehydrocholesterol to 7KC in this disease [234]. In addition, ApoE-deficient mice are of interest in clarifying the role of 7KC and 7β-OHC in atherogenesis [84].

4.2 – Non transgenic invertebrate and vertebrate animal models

Due to the identification of 7KC and 7β-OHC in large quantities in different types of foods, the *in vivo* effects of these molecules have been studied in animal models, in order to determine their effects on diseases associated with poor eating habits, such as cardiovascular diseases. To this end, several models (pigeon, mouse, rat, hamster, rabbit and pig) were used (**Figure 5**). Among these models, some of them were also employed to study the impact of 7KC in aged related diseases, and in this context the *Caenorhabditis elegans* model was recently developed.

4.2.1. Caenorhabditis elegans

With the use of the nematode *Caenorhabditis elegans* as model organism, the toxicity of 7KC was investigated [235]. The effects of 7KC on life span, on reproduction, thermotolerance, germline apoptosis, and ROS generation resulting from *C. elegans* exposure to 7KC were investigated at concentrations ranging from 0 to 200 μg/mL. In these conditions, 7KC reduced reproductive capacity, shortened the life span in a concentration-dependent manner, and impaired thermotolerance of the adult nematode. 7KC also induced germline apoptotic cell death and increased ROS generation. It is suggested that the model *C. elegans* could be suitable for assessment of the bioactivity of 7KC in aging.

4.2.2. Pigeon

The pigeon model has been one of the first to examine the effects of tobacco smoking and of a diet enriched in cholesterol on the development of atherosclerosis [236, 237]. The toxicity of oxysterols was also studied in experiments performed on White Carneau pigeons. When the pigeons were feed with a diet supplemented with 0.05% pure cholesterol (control group); 0.05% pure cholesterol plus cholestane-3*β*,5*α*,6*β*- triol (which can be present in powered milk in quantity as high as 7KC [238], total aortic cholesterol, aortic cholesterol ester, and the ratio (aortic cholesterol ester / aortic cholesterol) were similar among pigeons from both groups, whereas an accumulation of calcium in the aortas of pigeons fed with cholesterol plus cholestane-3*β*,5*α*,6*β*- triol was observed [239].

4.2.3. Golden Syrian hamster

Syrian hamster is a widely used experimental pharmacological model to identify natural and synthetic anti-atherosclerotic drugs [199, 240-244]. In addition, the effect of dietary oxysterols on

coronary atherosclerosis was also studied in golden Syrian hamsters fed for 3 months with three different diets: a normolipidaemic diet containing corn oil plus fish oil (group low L); a hyperlipidaemic diet composed of the normolipidaemic diet supplemented with cholesterol (group High L); a third diet, similar to the hyperlipidaemic diet, in which cholesterol was replaced by a mixture of oxysterols: 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, 7α-hydroxycholesterol, 7β-OHC, 7KC and trace amounts of 7-hydroperoxycholesterols (group High L + OS) [245]. Feeding the high-lipid diet (group High L) increased the plasma level of 7β-OHC, 7KC and cholestanetriol. The presence of oxysterols in the diet (group High L+OS) further increased the concentrations of 7β-OHC and 7KC in the plasma. 7KC was increased in myocardial lipids of groups (High L) and (High L+OS). However, as evidenced by myocardial Ca^{2+}, acyl-CoA cholesterol acyl transferase (ACAT) activity and coronary reactivity to sodium nitroprusside, severe atherosclerosis did not develop during the 3-month enriched lipid diet.

4.2.4. Mouse

Wild type mice treated with oxysterols, which are oxidized on the side chain or steroid nucleus, are used to mimic inflammatory bowel diseases [2]. In mice, 7KC and 7β-OHC are often incorporated into a mixture of oxysterols (7KC, 5α,6α-epoxicholesterol, 5β,6β-epoxicholesterol, 7α-hydroxycholesterol and 7β-OHC) to mimic food leading to inflammation of the intestinal wall to identify cytoprotective molecules; in the mouse, this oxysterol mixture induces TLR-2 and TLR4 over-expression and activation together with cytokine induction [128].

Anti-tumor properties of 7KC have also been described in mice [246] as well as anti-tumor activity of 7β-OHC against Krebs II ascitic carcinoma transplanted on mice [247]. Anti-tumor activity of the water-soluble monophosphoric acid diesters of 7β-OHC on mastocytoma P815 in the mouse model has also been reported [248]. The pioneering work realized by Bischoff *et al.* concerning the cytotoxic and anti-tumor activity of some oxysterols, including 7β-OHC, lead to the suggestion that some oxysterols could constitute potent anti-tumoral molecules [249]. There are now several lines of evidence that 7KC and 7β-OHC have major impacts on the metabolism of cancer cells [250], supporting the concept that chemotherapies targeting metabolism offer promising perspectives for cancer treatment.

4.2.5. Rat

The rat model has been used to study the metabolism of 7KC [26, 50, 251], to identify molecules capable of preventing cardiovascular injuries [252] and to determine the impact of some nutrients and aliments on the biogenesis of oxysterols formed by auto-oxidation including 7KC and 7β-OHC [253-255]. In addition, using 7KC-containing implants inserted into the anterior chamber of the rat eye, it has been demonstrated that the cytokinic inflammation induced by 7KC mostly occurs through the TLR4 receptor [169]. A significant increase in vascular endothelial growth factor (VEGF) was also observed in the rat eye with 7KC-containing implants by fluorescent immunolabeling and by immunoblot of the aqueous humor [256]. To define the role of 7KC in Smith-Lemli-Opitz syndrome, intra-vitreal injection of 7KC into a normal rat eye rapidly induced panretinal degeneration [257]. Strong impacts of 7KC and 7β-OHC were also observed when these compounds were injected into the rat prefrontal cortex, based on the analysis of RNA extracted from this brain region at 24 hours post-injection [258]. Microarray analyses identified 1365 genes, whose expression were affected by these two oxysterols: down-regulated genes outnumbered up-regulated genes. Pathway analysis showed that down-regulated genes had roles in carbohydrate metabolism, cell signaling and nucleic acid metabolism; and that the majority of these encode G-protein coupled receptors (GPRs) involved in the synaptic function [258]. On tumors induced by C6 cells in the rat brain cortex, it has also been reported that the intra-tumoral injection of liposomes containing 7β-OHC ether or ester inhibited tumor growth [259].

4.2.6. Rabbit

The rabbit is an animal model used for several years for the study of atherosclerosis [260, 261]. One of the oldest studies to address the *in vivo* toxicity of 7KC was conducted in the rabbit in 1949 by Cox and Spencer [262]. It was also established in the rabbit that 7KC inhibits cholesterol uptake by the arterial wall, and that this oxysterol can bind to the lipoproteins [263, 264]. In 1980, experimental evidence of the toxicity of 7KC were described in the rabbit: it was shown that the rate constant determining tissue uptake of 7KC was higher than tissue efflux, and suggested that the red cells and peripheral tissues act as a reservoir for the oxygenated sterol [265]. The rabbit is also a source of vascular cells and pioneering works supporting cytotoxic effects of 7KC and 7β-OHC were performed on cultured rabbit aortic smooth muscle cells [266]. In addition, *ex-vivo* experiments on rabbit aortic segments demonstrated that 7KC and 7β-OHC were able to prevent arterial relaxation [267].

4.2.7. Pig

The domestic pig and mini-pig are conventional models of study in pharmacology, imaging and cardiovascular surgery [74, 268]. The pig is often a source of vascular wall and ocular cells that have made it possible to characterize the effects of different oxysterols, such as 7KC and 7β-OHC. In smooth muscle cells of porcine aorta, it has been shown that the cytotoxicity of oxidized Low Density Lipoprotein (LDL) was associated with 7KC and 7β-OHC [102, 269]. The use of pig in the mechanical ventilation of the lungs made it also possible to establish that 7KC was probably an important player in pulmonary inflammation associated with respiratory support [270].

4.2.8 Monkey

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the conversion of cortisone to cortisol and controls a key pathway in the regulation of stress [271]. 11β-HSD1 also converts 7KC to 7β-OHC; these two oxysterols are at increased levels in the brain of Alzheimer's patients [110, 272]. F18-radiolabeled ligands have been developed to determine the topographical expression of 11β-HSD1 in the brain of rhesus monkey *(Macaca mulatta*) [273]. The authors had previously developed [^{11}C] AS2471907 PET radiotracer for imaging 11β-HSD1 in the brain, but their syntheses were not reliable, so they developed new probes and optimized the synthesis. Pharmacokinetic analyses and verification of the binding specificity of the new probes were performed in monkeys. The developed probes have a heterogeneous distribution, binding specificity and longer half-life than the previous developed probes as well as easier production, making them efficient and suitable PET radiotracer for 11β-HSD1 brain imaging.

5 - Alternatives to animal models for the study of 7-ketocholesterol- and 7β-hydroxycholesterol: organoids and microfluidic associated technologies

There are now evidence that whatever the relevance of models used to evaluate the biological activities of natural or synthetic compounds, it is difficult to anticipate their biological activities in humans. So, there is a need to develop new models including several parameters and mimicking the complexity of an organ or of the whole organism. These approaches constitute an important biotechnological challenge to prevent and cure major diseases (such as cardiovascular

diseases and neurodegeneration), where no efficient treatments are available. These new cell culture technologies include organoids, as well as organ-on-a-chip and multi-organ microphysiological system models, also named body on-a-chip, based on a microfluidic technology [274].

- ✓ Organoids mimicking the respiratory system, bowel or brain have already been made and can be produced from various sources such as primary cells [275]**,** pluripotent stem cells [276, 277], embryonic [278] or adult stem cells [279]**,** and patient-derived induced pluripotent stem cells [276, 280]**.** Although several studies have been carried out using established cell lines or primary cells, the use of stem cells is increasing because of the tremendous potential to model various disease models or biological systems.
- ✓ Organ-on-a-chip strategies are based on microfluidic cell systems to model physiological functions of tissues, or organs. To this end different types of stem cells can be used. Currently, the focus is not to rebuild a whole living organ, but to mimic minimal functional units that recapitulate tissue and organ level functions. Precise control of stem cell differentiation in the microfluidic microenvironment makes tissue engineering and organ-on-a-chip developments highly promising [281]. To date, a number of proof-of-concept, organ-on-a-chip systems using cells differentiated from stem cells have been described [282, 283]**.** Patient-derived and genetically engineered iPSCs with tissue engineering to elucidate the pathophysiology underlying the cardiovascular diseases have been combined through 'heart-on chip' for modeling the mitochondrial cardiopathy of Barth syndrome [284]**.** These microfluidic organ-on-a-chip models can recapitulate important organ-level functions, multicellular microarchitecture, and environment dynamics. Therefore, the engineered novel heart- and vasculature-on-a-chip systems could contribute to the development of suitable high-throughput platforms for drug development and disease modeling of major cardiovascular diseases [285]. Furthermore, organ-on-a-chip platforms have also the potential to strongly impact and improve the drug screening process for neurodegenerative diseases. Patient-derived neurons from different regions of the brain can be directly grown and differentiated on a brain-on-a-chip device in where the disease development, progression and pharmacological treatments can be studied and monitored in real time for Alzheimer's and Parkinson's diseases [286-288]**.**
- ✓ Personalized multi-organ microphysiological system models, body on-a-chip, based on

patient-derived iPSCs represent also a promising approach to elucidate physiopathology and therapies [282, 289]. The technology of multi-organs can mimic complex biological processes involving organ-organ interaction, system homeostasis and pharmacokinetics [290, 291].

These organoids and on chip models based on microfluidic technologies could be very useful to study 7KC- and 7β-OHC-induced biological activities on different organs and to identify new molecules and new strategies capable to prevent their side effects.

6 – Conclusion

The potential implications of 7KC and 7β-OHC in many common and disabling diseases (cardiovascular diseases, cataract, ARMD, neurodegenerative diseases, inflammatory bowel diseases) are well documented. However, the demonstration of direct or indirect involvement of these molecules in these diseases still requires significant work on cells, animal models and / or alternatives to animal models. These different approaches will make it possible to better understand the biological activities of 7KC and 7β-OHC, and to identify natural or synthetic molecules, or mixtures of molecules, capable of preventing their deleterious effects in fatal and/or strongly debilitating diseases, with important societal impacts.

Acknowledgments

This article/publication is based upon work from COST Action NutRedOx-CA16112 supported by COST (European Cooperation in Science and Technology). Some authors are also active members of the European Network for Oxysterols Research (ENOR: https://www.oxysterols.net): Anne Vejux, Amira Zarrouk, Shubhrima Ghosh, Thomas Nury, John J Mackrill and Gérard Lizard).

Conflict of interest

The authors have no conflict of interest to declare.

Figure Legends

Figure 1: Oxysterols oxidized at C7. Oxysterols oxidized at C7 (7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol (also named 7-oxocholesterol)) are formed either by auto-oxidation of cholesterol or enzymatically: 7α-hydroxycholesterol is formed via the enzyme CYP7A1; 7β-hydroxycholesterol and 7-ketocholesterol are formed by auto-oxidation. 7-ketocholesterol can be converted in 7β-hydroxycholesterol via the enzyme 11β-HSD1 (the enzyme works less efficiently to convert 7β-hydroxycholesterol in 7-ketocholesterol) and 7β-hydroxycholesterol can be converted in 7-ketocholesterol via the enzyme 11β-HSD2 [41, 292]**.**

Figure 2: Signaling pathways associated with 7-ketocholesterol-induced oxiapoptophagy and inflammation. The schematic signaling pathways associated with 7KC-induced oxiapoptophagy and inflammation are obtained from cells of different types and of different species. 7KC, which accumulates in lipid rafts and triggers phosphatidylserine externalization, modulates the activity of Ca^{2+}, K^+ and Na^+/K^+ channels such as P2X7 and Kv3.1 receptors. This favours an intracellular accumulation of Ca^{2+} and K^+. 7KC also activates endoplasmic reticulum stress and oxidative stress (ROS overproduction) which contributes to organelle dyfunctions (mitochondria, peroxisome, lysosome). In addition, the ability of 7KC to inhibit the PI3-K/PKB/Akt signaling pathway participates to the loss of transmembrane mitochondrial potential ($\Delta\Psi m$). Altogether, these different effects induce autophagy and apoptosis. The simultaneous induction of oxidative stress, apoptosis and autophagy is defined as oxiapoptophagy. 7KC is also a pro-inflammatory molecule which triggers inflammation via the activation of TLR4 receptor.

Figure 3: Signaling pathways associated with 7β-hydroxycholesterol-induced oxiapoptophagy and inflammation. The schematic signaling pathways associated with 7β-OHC-induced oxiapoptophagy and inflammation are obtained from cells of different types and of different species. 7β-OHC, which does not accumulates in lipid rafts, favors phophatidylserine externalization. It also favors an intracellular accumulation of Ca^{2+}, and inhibits the PI3-

K/PKB/Akt signalling pathway; this later participates to the loss of transmembrane mitochondrial potential (ΔΨm). In addition, 7β-OHC is also a potent inducer of oxidative stress (ROS oversproduction; enhanced levels of conjugated dienes (CDs), malondialdehyde (MDA) and carbonylated proteins (CPs)) wich contributes to organelle dysfunctions (mitochondria, peroxisome, lysosome) and to the induction of cell death defined as oxiapoptophagy. As 7β-OHC and 7KC are two potent inducers of oxiapoptophagy some signaling pathways are similar. However, as 7β-OHC is a stronger inducer of apoptosis and inflammation than 7KC, this suggests some differences between these two oxysterols. In addition, the activation of the PKC/ P38/ MEK/ ERK signaling pathway constitutes a link between 7β-OHC-induced apotosis and 7β-OHC-induced inflammation allowing thus to rely oxiapoptophagy and inflammation.

Figure 4 Mechanisms by which 7-ketocholesterol and 7β-hydroxycholesterol can modulate Ca^{2+} signal transduction in mammalian cells. Both oxysterols (7KC, 7β-OHC) can be metabolized to 7α, 25-hydroxycholesterol, a potent agonist of the G-protein coupled receptor EBI-2/GPR183 that mobilizes Ca^{2+} from the endoplasmic reticulum (ER) by gating inositol 1,4,5-trisphosphate receptors (IP_3R). The enzyme CD38 is activated by 7KC or 7β-OHC via an undetermined mechanism, generating the second messengers cyclic ADP ribose (cADPr), stimulating Ca^{2+} release from the ER or smooth reticulum via ryanodine receptor (RyR) channels, and nicotinic acid adenine dinucleotide phosphate (NAADP), that mobilizes Ca^{2+} from the lysosome via transient receptor potential mucolipin (TRPML) and two-pore (TPC) channels. Both 7KC and 7β-OHC also promote Ca^{2+} influx by activating multiple families of plasmalemmal cation channels: voltage-gated calcium channels (VGCC); store-operated calcium channels (SOCE); transient receptor potential (TRP) channels; and the purinoceptor 2X7 (P2X7). By increasing the levels and function of potassium channels such as K_v1.3b, oxysterols alter membrane potential, thereby modifying the gating of VGCCs.

Figure 5: Animal models used to evaluate the biological activities of 7-ketocholesterol and 7β-hydroxycholesterol. Among these models transgenic and wild type mice have been widely used. The other animal models mainly concern wild type animals.

References:

[1] T. Nury, R. Sghaier, A. Zarrouk, F. Menetrier, T. Uzun, V. Leoni, C. Caccia, W. Meddeb, A. Namsi, K. Sassi, W. Mihoubi, J.M. Riedinger, M. Cherkaoui-Malki, T. Moreau, A. Vejux, G. Lizard, Induction of peroxisomal changes in oligodendrocytes treated with 7-ketocholesterol: Attenuation by alpha-tocopherol, Biochimie 153 (2018) 181-202.
[2] G. Poli, F. Biasi, G. Leonarduzzi, Oxysterols in the pathogenesis of major chronic diseases, Redox Biol 1 (2013) 125-30.
[3] A. Zarrouk, A. Vejux, J. Mackrill, Y. O'Callaghan, M. Hammami, N. O'Brien, G. Lizard, Involvement of oxysterols in age-related diseases and ageing processes, Ageing Res Rev 18 (2014) 148-62.
[4] N.R.S. Sibuyi, M. Meyer, M.O. Onani, A. Skepu, A.M. Madiehe, Vascular targeted nanotherapeutic approach for obesity treatment, Int J Nanomedicine 13 (2018) 7915-7929.
[5] J.M. Mathieu, J. Schloendorn, B.E. Rittmann, P.J. Alvarez, Medical bioremediation of age-related diseases, Microb Cell Fact 8 (2009) 21.
[6] J. Schloendorn, T. Webb, K. Kemmish, M. Hamalainen, D. Jackemeyer, L. Jiang, J. Mathieu, J. Rebo, J. Sankman, L. Sherman, L. Tontson, A. Qureshi, P. Alvarez, B. Rittmann, Medical bioremediation: a concept moving toward reality, Rejuvenation Res 12(6) (2009) 411-9.
[7] L.L. Smith, Review of progress in sterol oxidations: 1987-1995, Lipids 31(5) (1996) 453-87.
[8] W.J. Griffiths, Y. Wang, An update on oxysterol biochemistry: New discoveries in lipidomics, Biochem Biophys Res Commun 504(3) (2018) 617-622.
[9] G.J. Schroepfer, Jr., Oxysterols: modulators of cholesterol metabolism and other processes, Physiol Rev 80(1) (2000) 361-554.
[10] S. Silvente-Poirot, F. Dalenc, M. Poirot, The Effects of Cholesterol-Derived Oncometabolites on Nuclear Receptor Function in Cancer, Cancer Res 78(17) (2018) 4803-4808.
[11] V. Mutemberezi, O. Guillemot-Legris, G.G. Muccioli, Oxysterols: From cholesterol metabolites to key mediators, Prog Lipid Res 64 (2016) 152-169.
[12] F. Guardiola, Dutta, P.C., Codony, R., Savage, G.P., Cholesterol and phytosterol oxidation products: analysis, occurrence, and biological effects, 2002.
[13] D. Lembo, V. Cagno, A. Civra, G. Poli, Oxysterols: An emerging class of broad spectrum antiviral effectors, Mol Aspects Med 49 (2016) 23-30.
[14] I.R. Rodriguez, I.M. Larrayoz, Cholesterol oxidation in the retina: implications of 7KCh formation in chronic inflammation and age-related macular degeneration, J Lipid Res 51(10) (2010) 2847-62.
[15] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, Chem Phys Lipids 164(6) (2011) 457-68.
[16] A. Vejux, M. Samadi, G. Lizard, Contribution of cholesterol and oxysterols in the physiopathology of cataract: implication for the development of pharmacological treatments, J Ophthalmol 2011 (2011) 471947.
[17] C. Zerbinati, L. Iuliano, Cholesterol and related sterols autoxidation, Free Radic Biol Med 111 (2017) 151-155.
[18] Z.N. Weber D, Vetter D, Hoffman R, Fedorova M, Electrochemical oxidation of cholesterol: An easy way to generate numerous oxysterols in short reaction times, Eur J Life Science & Technology 118 (2016) 325-331.
[19] M. Brzeska, K. Szymczyk, A. Szterk, Current Knowledge about Oxysterols: A Review, J Food Sci 81(10) (2016) R2299-R2308.

[20] W.J. Griffiths, J. Abdel-Khalik, P.J. Crick, E. Yutuc, Y. Wang, New methods for analysis of oxysterols and related compounds by LC-MS, J Steroid Biochem Mol Biol 162 (2016) 4-26.
[21] W.J. Griffiths, E. Yutuc, J. Abdel-Khalik, P.J. Crick, T. Hearn, A. Dickson, B.W. Bigger, T. Hoi-Yee Wu, A. Goenka, A. Ghosh, S.A. Jones, D.F. Covey, D.S. Ory, Y. Wang, Metabolism of Non-Enzymatically Derived Oxysterols: Clues from sterol metabolic disorders, Free Radic Biol Med (2019).
[22] D. Derewiaka, E. Molinska nee Sosinska, Cholesterol transformations during heat treatment, Food Chem 171 (2015) 233-40.
[23] M. Gonzalez-Larena, G. Garcia-Llatas, G. Clemente, R. Barbera, M.J. Lagarda, Plant sterol oxides in functional beverages: influence of matrix and storage, Food Chem 173 (2015) 881-9.
[24] B. Barriuso, D. Ansorena, I. Astiasaran, Oxysterols formation: A review of a multifactorial process, J Steroid Biochem Mol Biol 169 (2017) 39-45.
[25] A. Vejux, G. Lizard, Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis, Mol Aspects Med 30(3) (2009) 153-70.
[26] M.A. Lyons, S. Samman, L. Gatto, A.J. Brown, Rapid hepatic metabolism of 7-ketocholesterol in vivo: implications for dietary oxysterols, J Lipid Res 40(10) (1999) 1846-57.
[27] J. Terao, Cholesterol hydroperoxides and their degradation mechanism, Subcell Biochem 77 (2014) 83-91.
[28] Z.A. Zielinski, D.A. Pratt, Cholesterol Autoxidation Revisited: Debunking the Dogma Associated with the Most Vilified of Lipids, J Am Chem Soc 138(22) (2016) 6932-5.
[29] J. Vaya, The association between biomarkers in the blood and carotid plaque composition-focusing on oxidized lipids, oxysterols and plaque status, Biochem Pharmacol 86(1) (2013) 15-8.
[30] S. Gargiulo, G. Testa, P. Gamba, E. Staurenghi, G. Poli, G. Leonarduzzi, Oxysterols and 4-hydroxy-2-nonenal contribute to atherosclerotic plaque destabilization, Free Radic Biol Med 111 (2017) 140-150.
[31] I.R. Rodriguez, S.J. Fliesler, Photodamage generates 7-keto- and 7-hydroxycholesterol in the rat retina via a free radical-mediated mechanism, Photochem Photobiol 85(5) (2009) 1116-25.
[32] C. Helmschrodt, S. Becker, J. Schroter, M. Hecht, G. Aust, J. Thiery, U. Ceglarek, Fast LC-MS/MS analysis of free oxysterols derived from reactive oxygen species in human plasma and carotid plaque, Clin Chim Acta 425 (2013) 3-8.
[33] X. Rao, J. Zhong, A. Maiseyeu, B. Gopalakrishnan, F.A. Villamena, L.C. Chen, J.R. Harkema, Q. Sun, S. Rajagopalan, CD36-dependent 7-ketocholesterol accumulation in macrophages mediates progression of atherosclerosis in response to chronic air pollution exposure, Circ Res 115(9) (2014) 770-780.
[34] R. Shinkyo, L. Xu, K.A. Tallman, Q. Cheng, N.A. Porter, F.P. Guengerich, Conversion of 7-dehydrocholesterol to 7-ketocholesterol is catalyzed by human cytochrome P450 7A1 and occurs by direct oxidation without an epoxide intermediate, J Biol Chem 286(38) (2011) 33021-8.
[35] R.H. Stimson, J. Andersson, R. Andrew, D.N. Redhead, F. Karpe, P.C. Hayes, T. Olsson, B.R. Walker, Cortisol release from adipose tissue by 11beta-hydroxysteroid dehydrogenase type 1 in humans, Diabetes 58(1) (2009) 46-53.
[36] T. Mitic, R. Andrew, B.R. Walker, P.W. Hadoke, 11beta-Hydroxysteroid dehydrogenase type 1 contributes to the regulation of 7-oxysterol levels in the arterial wall through the inter-conversion of 7-ketocholesterol and 7beta-hydroxycholesterol, Biochimie 95(3) (2013) 548-55.
[37] T. Mitic, S. Shave, N. Semjonous, I. McNae, D.F. Cobice, G.G. Lavery, S.P. Webster, P.W. Hadoke, B.R. Walker, R. Andrew, 11beta-Hydroxysteroid dehydrogenase type 1 contributes to the balance between 7-keto- and 7-hydroxy-oxysterols in vivo, Biochem Pharmacol 86(1) (2013) 146-53.
[38] H. Larsson, Y. Bottiger, L. Iuliano, U. Diczfalusy, In vivo interconversion of 7beta-hydroxycholesterol and 7-ketocholesterol, potential surrogate markers for oxidative stress, Free Radic Biol Med 43(5) (2007) 695-701.

[39] D.R. Raleigh, N. Sever, P.K. Choksi, M.A. Sigg, K.M. Hines, B.M. Thompson, D. Elnatan, P. Jaishankar, P. Bisignano, F.R. Garcia-Gonzalo, A.L. Krup, M. Eberl, E.F.X. Byrne, C. Siebold, S.Y. Wong, A.R. Renslo, M. Grabe, J.G. McDonald, L. Xu, P.A. Beachy, J.F. Reiter, Cilia-Associated Oxysterols Activate Smoothened, Mol Cell 72(2) (2018) 316-327 e5.
[40] M. Wamil, R. Andrew, K.E. Chapman, J. Street, N.M. Morton, J.R. Seckl, 7-oxysterols modulate glucocorticoid activity in adipocytes through competition for 11beta-hydroxysteroid dehydrogenase type, Endocrinology 149(12) (2008) 5909-18.
[41] K. Chapman, M. Holmes, J. Seckl, 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action, Physiol Rev 93(3) (2013) 1139-206.
[42] A.J. Brown, W. Jessup, Oxysterols and atherosclerosis, Atherosclerosis 142(1) (1999) 1-28.
[43] Y. Wang, W.J. Griffiths, Unravelling new pathways of sterol metabolism: lessons learned from in-born errors and cancer, Curr Opin Clin Nutr Metab Care 21(2) (2018) 90-96.
[44] H. Fuda, N.B. Javitt, K. Mitamura, S. Ikegawa, C.A. Strott, Oxysterols are substrates for cholesterol sulfotransferase, J Lipid Res 48(6) (2007) 1343-52.
[45] J.W. Lee, J.D. Huang, I.R. Rodriguez, Extra-hepatic metabolism of 7-ketocholesterol occurs by esterification to fatty acids via cPLA2alpha and SOAT1 followed by selective efflux to HDL, Biochim Biophys Acta 1851(5) (2015) 605-19.
[46] M.A. Rogers, J. Liu, B.L. Song, B.L. Li, C.C. Chang, T.Y. Chang, Acyl-CoA:cholesterol acyltransferases (ACATs/SOATs): Enzymes with multiple sterols as substrates and as activators, J Steroid Biochem Mol Biol 151 (2015) 102-7.
[47] S. Monier, M. Samadi, C. Prunet, M. Denance, A. Laubriet, A. Athias, A. Berthier, E. Steinmetz, G. Jurgens, A. Negre-Salvayre, G. Bessede, S. Lemaire-Ewing, D. Neel, P. Gambert, G. Lizard, Impairment of the cytotoxic and oxidative activities of 7 beta-hydroxycholesterol and 7-ketocholesterol by esterification with oleate, Biochem Biophys Res Commun 303(3) (2003) 814-24.
[48] V. La Marca, M.S. Spagnuolo, L. Cigliano, D. Marasco, P. Abrescia, The enzyme lecithin-cholesterol acyltransferase esterifies cerebrosterol and limits the toxic effect of this oxysterol on SH-SY5Y cells, J Neurochem 130(1) (2014) 97-108.
[49] S.E. Szedlacsek, E. Wasowicz, S.A. Hulea, H.I. Nishida, F.A. Kummerow, T. Nishida, Esterification of oxysterols by human plasma lecithin-cholesterol acyltransferase, J Biol Chem 270(20) (1995) 11812-9.
[50] M.A. Lyons, A.J. Brown, Metabolism of an oxysterol, 7-ketocholesterol, by sterol 27-hydroxylase in HepG2 cells, Lipids 36(7) (2001) 701-11.
[51] A. Sevanian, A.R. Peterson, The cytotoxic and mutagenic properties of cholesterol oxidation products, Food Chem Toxicol 24(10-11) (1986) 1103-10.
[52] P.M. Eckl, N. Bresgen, Genotoxicity of lipid oxidation compounds, Free Radic Biol Med 111 (2017) 244-252.
[53] L.L. Smith, V.B. Smart, N. Made Gowda, Mutagenic sterol hydroperoxides, Mutat Res 161(1) (1986) 39-48.
[54] Y.W. Cheng, J.J. Kang, Y.L. Shih, Y.L. Lo, C.F. Wang, Cholesterol-3-beta, 5-alpha, 6-beta-triol induced genotoxicity through reactive oxygen species formation, Food Chem Toxicol 43(4) (2005) 617-22.
[55] A.R. Peterson, H. Peterson, C.P. Spears, J.E. Trosko, A. Sevanian, Mutagenic characterization of cholesterol epoxides in Chinese hamster V79 cells, Mutat Res 203(5) (1988) 355-66.
[56] M.I. Kelsey, R.J. Pienta, Transformation of hamster embryo cells by cholesterol-alpha-epoxide and lithocholic acid, Cancer Lett 6(3) (1979) 143-9.
[57] J.A. Woods, N.M. O'Brien, Investigation of the potential genotoxicity of cholesterol oxidation products in two mammalian fibroblast cell lines, Nutr Cancer 31(3) (1998) 192-8.
[58] M. Poirot, S. Silvente-Poirot, Cholesterol-5,6-epoxides: chemistry, biochemistry, metabolic fate and cancer, Biochimie 95(3) (2013) 622-31.

[59] A.A. Kandutsch, H.W. Chen, Consequences of blocked sterol synthesis in cultured cells. DNA synthesis and membrane composition, J Biol Chem 252(2) (1977) 409-15.
[60] G. Leonarduzzi, B. Sottero, G. Poli, Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review), J Nutr Biochem 13(12) (2002) 700-710.
[61] S. Lemaire, G. Lizard, S. Monier, C. Miguet, S. Gueldry, F. Volot, P. Gambert, D. Neel, Different patterns of IL-1beta secretion, adhesion molecule expression and apoptosis induction in human endothelial cells treated with 7alpha-, 7beta-hydroxycholesterol, or 7-ketocholesterol, FEBS Lett 440(3) (1998) 434-9.
[62] M.C. Chang, Y.J. Chen, E.J. Liou, W.Y. Tseng, C.P. Chan, H.J. Lin, W.C. Liao, Y.C. Chang, P.Y. Jeng, J.H. Jeng, 7-Ketocholesterol induces ATM/ATR, Chk1/Chk2, PI3K/Akt signalings, cytotoxicity and IL-8 production in endothelial cells, Oncotarget 7(46) (2016) 74473-74483.
[63] X. Fu, X. Huang, P. Li, W. Chen, M. Xia, 7-Ketocholesterol inhibits isocitrate dehydrogenase 2 expression and impairs endothelial function via microRNA-144, Free Radic Biol Med 71 (2014) 1-15.
[64] C. He, H. Zhu, W. Zhang, I. Okon, Q. Wang, H. Li, Y.Z. Le, Z. Xie, 7-Ketocholesterol induces autophagy in vascular smooth muscle cells through Nox4 and Atg4B, Am J Pathol 183(2) (2013) 626-37.
[65] K. Yamagata, N. Tanaka, K. Suzuki, Epigallocatechin 3-gallate inhibits 7-ketocholesterol-induced monocyte-endothelial cell adhesion, Microvasc Res 88 (2013) 25-31.
[66] X. Yuan, L. Wang, O.M. Bhat, H. Lohner, P.L. Li, Differential effects of short chain fatty acids on endothelial Nlrp3 inflammasome activation and neointima formation: Antioxidant action of butyrate, Redox Biol 16 (2018) 21-31.
[67] S. Koka, M. Xia, Y. Chen, O.M. Bhat, X. Yuan, K.M. Boini, P.L. Li, Endothelial NLRP3 inflammasome activation and arterial neointima formation associated with acid sphingomyelinase during hypercholesterolemia, Redox Biol 13 (2017) 336-344.
[68] F. Luchetti, B. Canonico, E. Cesarini, M. Betti, L. Galluzzi, L. Galli, J. Tippins, C. Zerbinati, S. Papa, L. Iuliano, 7-Ketocholesterol and 5,6-secosterol induce human endothelial cell dysfunction by differential mechanisms, Steroids 99(Pt B) (2015) 204-11.
[69] G.R. Romeo, A. Kazlauskas, Oxysterol and diabetes activate STAT3 and control endothelial expression of profilin-1 via OSBP1, J Biol Chem 283(15) (2008) 9595-605.
[70] Y. Son, W. Chun, Y.T. Ahn, K. Kim, C.W. Lee, J.M. Kim, C. Lee, W.G. An, 7-Ketocholesterol induces the reduction of KCNMB1 in atherosclerotic blood vessels, Biochem Biophys Res Commun 457(3) (2015) 324-7.
[71] G. Lizard, S. Monier, C. Cordelet, L. Gesquiere, V. Deckert, S. Gueldry, L. Lagrost, P. Gambert, Characterization and comparison of the mode of cell death, apoptosis versus necrosis, induced by 7beta-hydroxycholesterol and 7-ketocholesterol in the cells of the vascular wall, Arterioscler Thromb Vasc Biol 19(5) (1999) 1190-200.
[72] Z. Adiguzel, N. Arda, O. Kacar, M. Serhatli, S. Gezer Tas, A.T. Baykal, K. Baysal, C. Acilan, Evaluation of apoptotic molecular pathways for smooth muscle cells isolated from thoracic aortic aneurysms in response to oxidized sterols, Mol Biol Rep 41(12) (2014) 7875-84.
[73] X. Li, M. Xu, A.L. Pitzer, M. Xia, K.M. Boini, P.L. Li, Y. Zhang, Control of autophagy maturation by acid sphingomyelinase in mouse coronary arterial smooth muscle cells: protective role in atherosclerosis, J Mol Med (Berl) 92(5) (2014) 473-85.
[74] J. Yin, X. Chaufour, C. McLachlan, M. McGuire, G. White, N. King, B. Hambly, Apoptosis of vascular smooth muscle cells induced by cholesterol and its oxides in vitro and in vivo, Atherosclerosis 148(2) (2000) 365-74.
[75] L. Tesoriere, A. Attanzio, M. Allegra, C. Gentile, M.A. Livrea, Phytochemical indicaxanthin suppresses 7-ketocholesterol-induced THP-1 cell apoptosis by preventing cytosolic Ca(2+) increase and oxidative stress, Br J Nutr 110(2) (2013) 230-40.

[76] R.T. Iborra, A. Machado-Lima, G. Castilho, V.S. Nunes, D.S. Abdalla, E.R. Nakandakare, M. Passarelli, Advanced glycation in macrophages induces intracellular accumulation of 7-ketocholesterol and total sterols by decreasing the expression of ABCA-1 and ABCG-1, Lipids Health Dis 10 (2011) 172.
[77] D.A. Larsson, S. Baird, J.D. Nyhalah, X.M. Yuan, W. Li, Oxysterol mixtures, in atheroma-relevant proportions, display synergistic and proapoptotic effects, Free Radic Biol Med 41(6) (2006) 902-10.
[78] G. Leonarduzzi, B. Vizio, B. Sottero, V. Verde, P. Gamba, C. Mascia, E. Chiarpotto, G. Poli, F. Biasi, Early involvement of ROS overproduction in apoptosis induced by 7-ketocholesterol, Antioxid Redox Signal 8(3-4) (2006) 375-80.
[79] L. Shen, Z. Sun, P. Nie, R. Yuan, Z. Cai, C. Wu, L. Hu, S. Jin, H. Zhou, X. Zhang, B. He, Sulindac-derived retinoid X receptor-alpha modulator attenuates atherosclerotic plaque progression and destabilization in ApoE(-/-) mice, Br J Pharmacol (2019).
[80] A. Vejux, E. Kahn, F. Menetrier, T. Montange, J. Lherminier, J.M. Riedinger, G. Lizard, Cytotoxic oxysterols induce caspase-independent myelin figure formation and caspase-dependent polar lipid accumulation, Histochem Cell Biol 127(6) (2007) 609-24.
[81] L. Gesquiere, N. Loreau, D. Blache, Impaired cellular cholesterol efflux by oxysterol-enriched high density lipoproteins, Free Radic Biol Med 23(4) (1997) 541-7.
[82] M. Zhang, H. Zhu, Y. Ding, Z. Liu, Z. Cai, M.H. Zou, AMP-activated protein kinase alpha1 promotes atherogenesis by increasing monocyte-to-macrophage differentiation, J Biol Chem 292(19) (2017) 7888-7903.
[83] B. Buttari, E. Profumo, L. Segoni, D. D'Arcangelo, S. Rossi, F. Facchiano, L. Saso, R. Businaro, L. Iuliano, R. Rigano, Resveratrol counteracts inflammation in human M1 and M2 macrophages upon challenge with 7-oxo-cholesterol: potential therapeutic implications in atherosclerosis, Oxid Med Cell Longev 2014 (2014) 257543.
[84] M. Rosenblat, M. Aviram, Oxysterol-induced activation of macrophage NADPH-oxidase enhances cell-mediated oxidation of LDL in the atherosclerotic apolipoprotein E deficient mouse: inhibitory role for vitamin E, Atherosclerosis 160(1) (2002) 69-80.
[85] E. Kahn, M. Baarine, S. Pelloux, J.M. Riedinger, F. Frouin, Y. Tourneur, G. Lizard, Iron nanoparticles increase 7-ketocholesterol-induced cell death, inflammation, and oxidation on murine cardiac HL1-NB cells, Int J Nanomedicine 5 (2010) 185-95.
[86] A. Vejux, S. Guyot, T. Montange, J.M. Riedinger, E. Kahn, G. Lizard, Phospholipidosis and down-regulation of the PI3-K/PDK-1/Akt signalling pathway are vitamin E inhibitable events associated with 7-ketocholesterol-induced apoptosis, J Nutr Biochem 20(1) (2009) 45-61.
[87] F. Brahmi, A. Vejux, R. Sghaier, A. Zarrouk, T. Nury, W. Meddeb, L. Rezig, A. Namsi, K. Sassi, A. Yammine, I. Badreddine, D. Vervandier-Fasseur, K. Madani, L. Boulekbache-Makhlouf, B. Nasser, G. Lizard, Prevention of 7-ketocholesterol-induced side effects by natural compounds, Crit Rev Food Sci Nutr (2018) 1-20.
[88] J.M. Han, H. Li, M.H. Cho, S.H. Baek, C.H. Lee, H.Y. Park, T.S. Jeong, Soy-Leaf Extract Exerts Atheroprotective Effects via Modulation of Kruppel-Like Factor 2 and Adhesion Molecules, Int J Mol Sci 18(2) (2017).
[89] J.D. Huang, J. Amaral, J.W. Lee, I.M. Larrayoz, I.R. Rodriguez, Sterculic acid antagonizes 7-ketocholesterol-mediated inflammation and inhibits choroidal neovascularization, Biochim Biophys Acta 1821(4) (2012) 637-46.
[90] Y. Naito, M. Shimozawa, H. Manabe, N. Nakabe, K. Katada, S. Kokura, N. Yoshida, H. Ichikawa, T. Kon, T. Yoshikawa, Azelnidipine, a new calcium channel blocker, inhibits endothelial inflammatory response by reducing intracellular levels of reactive oxygen species, Eur J Pharmacol 546(1-3) (2006) 11-8.

[91] D. Zapolska-Downar, G. Nowicka, G. Sygitowicz, M. Jarosz, Anthocyanin-rich Aronox extract from Aronia melanocarpa E protects against 7beta-hydroxycholesterol-induced apoptosis of endothelial cells, Ann Nutr Metab 53(3-4) (2008) 283-94.
[92] A. Laskar, X.M. Yuan, W. Li, Dimethyl sulfoxide prevents 7beta-hydroxycholesterol-induced apoptosis by preserving lysosomes and mitochondria, J Cardiovasc Pharmacol 56(3) (2010) 263-7.
[93] L. Malvitte, T. Montange, C. Joffre, A. Vejux, C. Maiza, A. Bron, C. Creuzot-Garcher, G. Lizard, [Analogies between atherosclerosis and age-related maculopathy: expected roles of oxysterols], J Fr Ophtalmol 29(5) (2006) 570-8.
[94] N.B. Javitt, J.C. Javitt, The retinal oxysterol pathway: a unifying hypothesis for the cause of age-related macular degeneration, Curr Opin Ophthalmol 20(3) (2009) 151-7.
[95] H. Girao, M.C. Mota, J. Ramalho, P. Pereira, Cholesterol oxides accumulate in human cataracts, Exp Eye Res 66(5) (1998) 645-52.
[96] A. Pariente, R. Pelaez, A. Perez-Sala, I.M. Larrayoz, Inflammatory and cell death mechanisms induced by 7-ketocholesterol in the retina. Implications for age-related macular degeneration, Exp Eye Res 187 (2019) 107746.
[97] E. Olivier, M. Dutot, A. Regazzetti, T. Leguillier, D. Dargere, N. Auzeil, O. Laprevote, P. Rat, P2X7-pannexin-1 and amyloid beta-induced oxysterol input in human retinal cell: Role in age-related macular degeneration?, Biochimie 127 (2016) 70-8.
[98] G. Shi, S. Chen, W.S. Wandu, O. Ogbeifun, L.F. Nugent, A. Maminishkis, S.J. Hinshaw, I.R. Rodriguez, I. Gery, Inflammasomes Induced by 7-Ketocholesterol and Other Stimuli in RPE and in Bone Marrow-Derived Cells Differ Markedly in Their Production of IL-1beta and IL-18, Invest Ophthalmol Vis Sci 56(3) (2015) 1658-64.
[99] B. Dasari, J.R. Prasanthi, C. Meiers, B.B. Singh, O. Ghribi, Differential effects of the estrogen receptor agonist estradiol on toxicity induced by enzymatically-derived or autoxidation-derived oxysterols in human ARPE-19 cells, Curr Eye Res 38(11) (2013) 1159-71.
[100] L. Malvitte, T. Montange, A. Vejux, C. Joffre, A. Bron, C. Creuzot-Garcher, G. Lizard, Activation of a caspase-3-independent mode of cell death associated with lysosomal destabilization in cultured human retinal pigment epithelial cells (ARPE-19) exposed to 7beta-hydroxycholesterol, Curr Eye Res 33(9) (2008) 769-81.
[101] G.Y. Heo, I. Bederman, N. Mast, W.L. Liao, I.V. Turko, I.A. Pikuleva, Conversion of 7-ketocholesterol to oxysterol metabolites by recombinant CYP27A1 and retinal pigment epithelial cells, J Lipid Res 52(6) (2011) 1117-27.
[102] C. Joffre, L. Leclere, B. Buteau, L. Martine, S. Cabaret, L. Malvitte, N. Acar, G. Lizard, A. Bron, C. Creuzot-Garcher, L. Bretillon, Oxysterols induced inflammation and oxidation in primary porcine retinal pigment epithelial cells, Curr Eye Res 32(3) (2007) 271-80.
[103] J.M. Ong, A.M. Aoki, G.M. Seigel, I. Sacerio, R. Castellon, A.B. Nesburn, M.C. Kenney, Oxysterol-induced toxicity in R28 and ARPE-19 cells, Neurochem Res 28(6) (2003) 883-91.
[104] M. Indaram, W. Ma, L. Zhao, R.N. Fariss, I.R. Rodriguez, W.T. Wong, 7-Ketocholesterol increases retinal microglial migration, activation, and angiogenicity: a potential pathogenic mechanism underlying age-related macular degeneration, Sci Rep 5 (2015) 9144.
[105] E. Tan, X.Q. Ding, A. Saadi, N. Agarwal, M.I. Naash, M.R. Al-Ubaidi, Expression of cone-photoreceptor-specific antigens in a cell line derived from retinal tumors in transgenic mice, Invest Ophthalmol Vis Sci 45(3) (2004) 764-8.
[106] B. Dugas, S. Charbonnier, M. Baarine, K. Ragot, D. Delmas, F. Menetrier, J. Lherminier, L. Malvitte, T. Khalfaoui, A. Bron, C. Creuzot-Garcher, N. Latruffe, G. Lizard, Effects of oxysterols on cell viability, inflammatory cytokines, VEGF, and reactive oxygen species production on human retinal cells: cytoprotective effects and prevention of VEGF secretion by resveratrol, Eur J Nutr 49(7) (2010) 435-46.

[107] S. Mukhopadhyay, K. Fellows, R.W. Browne, P. Khare, S. Krishnan Radhakrishnan, J. Hagemeier, B. Weinstock-Guttman, R. Zivadinov, M. Ramanathan, Interdependence of oxysterols with cholesterol profiles in multiple sclerosis, Mult Scler 23(6) (2017) 792-801.
[108] V. Leoni, D. Lutjohann, T. Masterman, Levels of 7-oxocholesterol in cerebrospinal fluid are more than one thousand times lower than reported in multiple sclerosis, J Lipid Res 46(2) (2005) 191-5.
[109] A. Okabe, Y. Urano, S. Itoh, N. Suda, R. Kotani, Y. Nishimura, Y. Saito, N. Noguchi, Adaptive responses induced by 24S-hydroxycholesterol through liver X receptor pathway reduce 7-ketocholesterol-caused neuronal cell death, Redox Biol 2 (2013) 28-35.
[110] G. Testa, E. Staurenghi, S. Giannelli, S. Gargiulo, M. Guglielmotto, M. Tabaton, E. Tamagno, P. Gamba, G. Leonarduzzi, A silver lining for 24-hydroxycholesterol in Alzheimer's disease: The involvement of the neuroprotective enzyme sirtuin 1, Redox Biol 17 (2018) 423-431.
[111] A. Zarrouk, T. Nury, M. Samadi, Y. O'Callaghan, M. Hammami, N.M. O'Brien, G. Lizard, J.J. Mackrill, Effects of cholesterol oxides on cell death induction and calcium increase in human neuronal cells (SK-N-BE) and evaluation of the protective effects of docosahexaenoic acid (DHA; C22:6 n-3), Steroids 99(Pt B) (2015) 238-47.
[112] P. Gamba, G. Leonarduzzi, E. Tamagno, M. Guglielmotto, G. Testa, B. Sottero, S. Gargiulo, F. Biasi, A. Mauro, J. Vina, G. Poli, Interaction between 24-hydroxycholesterol, oxidative stress, and amyloid-beta in amplifying neuronal damage in Alzheimer's disease: three partners in crime, Aging Cell 10(3) (2011) 403-17.
[113] Z.H. Chen, Y. Yoshida, Y. Saito, A. Sekine, N. Noguchi, E. Niki, Induction of adaptive response and enhancement of PC12 cell tolerance by 7-hydroxycholesterol and 15-deoxy-delta(12,14)-prostaglandin J2 through up-regulation of cellular glutathione via different mechanisms, J Biol Chem 281(20) (2006) 14440-5.
[114] K. Sassi, T. Nury, A. Zarrouk, R. Sghaier, A. Khalafi-Nezhad, A. Vejux, M. Samadi, F.B. Aissa-Fennira, G. Lizard, Induction of a non-apoptotic mode of cell death associated with autophagic characteristics with steroidal maleic anhydrides and 7beta-hydroxycholesterol on glioma cells, J Steroid Biochem Mol Biol 191 (2019) 105371.
[115] M. Baarine, K. Ragot, E.C. Genin, H. El Hajj, D. Trompier, P. Andreoletti, M.S. Ghandour, F. Menetrier, M. Cherkaoui-Malki, S. Savary, G. Lizard, Peroxisomal and mitochondrial status of two murine oligodendrocytic cell lines (158N, 158JP): potential models for the study of peroxisomal disorders associated with dysmyelination processes, J Neurochem 111(1) (2009) 119-31.
[116] M. Debbabi, T. Nury, I. Helali, E.M. Karym, F. Geillon, C. Gondcaille, D. Trompier, A. Najid, S. Terreau, M. Bezine, A. Zarrouk, A. Vejux, P. Andreoletti, M. Cherkaoui-Malki, S. Savary, G. Lizard, Flow Cytometric Analysis of the Expression Pattern of Peroxisomal Proteins, Abcd1, Abcd2, and Abcd3 in BV-2 Murine Microglial Cells, Methods Mol Biol 1595 (2017) 257-265.
[117] M. Debbabi, A. Zarrouk, M. Bezine, W. Meddeb, T. Nury, A. Badreddine, E.M. Karym, R. Sghaier, L. Bretillon, S. Guyot, M. Samadi, M. Cherkaoui-Malki, B. Nasser, M. Mejri, S. Ben-Hammou, M. Hammami, G. Lizard, 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, Chem Phys Lipids 207(Pt B) (2017) 151-170.
[118] A. Diestel, O. Aktas, D. Hackel, I. Hake, S. Meier, C.S. Raine, R. Nitsch, F. Zipp, O. Ullrich, Activation of microglial poly(ADP-ribose)-polymerase-1 by cholesterol breakdown products during neuroinflammation: a link between demyelination and neuronal damage, J Exp Med 198(11) (2003) 1729-40.
[119] T. Nury, A. Zarrouk, J.J. Mackrill, M. Samadi, P. Durand, J.M. Riedinger, M. Doria, A. Vejux, E. Limagne, D. Delmas, M. Prost, T. Moreau, M. Hammami, R. Delage-Mourroux, N.M. O'Brien, G. Lizard, Induction of oxiapoptophagy on 158N murine oligodendrocytes treated by 7-ketocholesterol-, 7beta-

hydroxycholesterol-, or 24(S)-hydroxycholesterol: Protective effects of alpha-tocopherol and docosahexaenoic acid (DHA; C22:6 n-3), Steroids 99(Pt B) (2015) 194-203.
[120] A. Zarrouk, T. Nury, E.M. Karym, A. Vejux, R. Sghaier, C. Gondcaille, P. Andreoletti, D. Trompier, S. Savary, M. Cherkaoui-Malki, M. Debbabi, A. Fromont, J.M. Riedinger, T. Moreau, G. Lizard, Attenuation of 7-ketocholesterol-induced overproduction of reactive oxygen species, apoptosis, and autophagy by dimethyl fumarate on 158N murine oligodendrocytes, J Steroid Biochem Mol Biol 169 (2017) 29-38.
[121] R. Sghaier, A. Zarrouk, T. Nury, I. Badreddine, N. O'Brien, J.J. Mackrill, A. Vejux, M. Samadi, B. Nasser, C. Caccia, V. Leoni, T. Moreau, M. Cherkaoui-Malki, A. Salhedine Masmoudi, G. Lizard, Biotin attenuation of oxidative stress, mitochondrial dysfunction, lipid metabolism alteration and 7beta-hydroxycholesterol-induced cell death in 158N murine oligodendrocytes, Free Radic Res 53(5) (2019) 535-561.
[122] A. Badreddine, A. Zarrouk, E.M. Karym, M. Debbabi, T. Nury, W. Meddeb, R. Sghaier, M. Bezine, A. Vejux, L. Martine, S. Gregoire, L. Bretillon, E. Prost-Camus, P. Durand, M. Prost, T. Moreau, M. Cherkaoui-Malki, B. Nasser, G. Lizard, Argan Oil-Mediated Attenuation of Organelle Dysfunction, Oxidative Stress and Cell Death Induced by 7-Ketocholesterol in Murine Oligodendrocytes 158N, Int J Mol Sci 18(10) (2017).
[123] W. Meddeb, L. Rezig, A. Zarrouk, T. Nury, A. Vejux, M. Prost, L. Bretillon, M. Mejri, G. Lizard, Cytoprotective Activities of Milk Thistle Seed Oil Used in Traditional Tunisian Medicine on 7-Ketocholesterol and 24S-Hydroxycholesterol-Induced Toxicity on 158N Murine Oligodendrocytes, Antioxidants (Basel) 7(7) (2018).
[124] A. Zarrouk, M.A. Smach, J. Hafsa, R. Sghaier, H. Majdoub, M. Hammami, B. Charfeddine, Effects of Carpobrotus edulis Extract on Oxidative Stress and 158N Oligodendrocyte Death, Biomed Environ Sci 32(4) (2019) 291-299.
[125] A. Zarrouk, Y. Ben Salem, J. Hafsa, R. Sghaier, B. Charfeddine, K. Limem, M. Hammami, H. Majdoub, 7beta-hydroxycholesterol-induced cell death, oxidative stress, and fatty acid metabolism dysfunctions attenuated with sea urchin egg oil, Biochimie 153 (2018) 210-219.
[126] F. Biasi, G. Leonarduzzi, P.I. Oteiza, G. Poli, Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets, Antioxid Redox Signal 19(14) (2013) 1711-47.
[127] B. Sottero, D. Rossin, G. Poli, F. Biasi, Lipid Oxidation Products in the Pathogenesis of Inflammation-related Gut Diseases, Curr Med Chem 25(11) (2018) 1311-1326.
[128] D. Rossin, L. Barbosa-Pereira, N. Iaia, G. Testa, B. Sottero, G. Poli, G. Zeppa, F. Biasi, A Dietary Mixture of Oxysterols Induces In Vitro Intestinal Inflammation through TLR2/4 Activation: The Protective Effect of Cocoa Bean Shells, Antioxidants (Basel) 8(6) (2019).
[129] F. Biasi, T. Guina, M. Maina, B. Cabboi, M. Deiana, C.I. Tuberoso, S. Calfapietra, E. Chiarpotto, B. Sottero, P. Gamba, S. Gargiulo, V. Brunetto, G. Testa, M.A. Dessi, G. Poli, G. Leonarduzzi, Phenolic compounds present in Sardinian wine extracts protect against the production of inflammatory cytokines induced by oxysterols in CaCo-2 human enterocyte-like cells, Biochem Pharmacol 86(1) (2013) 138-45.
[130] T. Guina, M. Deiana, S. Calfapietra, B. Cabboi, M. Maina, C.I. Tuberoso, G. Leonarduzzi, P. Gamba, S. Gargiulo, G. Testa, G. Poli, F. Biasi, The role of p38 MAPK in the induction of intestinal inflammation by dietary oxysterols: modulation by wine phenolics, Food Funct 6(4) (2015) 1218-28.
[131] G. Serra, A. Incani, G. Serreli, L. Porru, M.P. Melis, C.I.G. Tuberoso, D. Rossin, F. Biasi, M. Deiana, Olive oil polyphenols reduce oxysterols -induced redox imbalance and pro-inflammatory response in intestinal cells, Redox Biol 17 (2018) 348-354.
[132] D.J. Klionsky, K. Abdelmohsen, A. Abe, M.J. Abedin, H. Abeliovich, A. Acevedo Arozena, H. Adachi, C.M. Adams, P.D. Adams, K. Adeli, P.J. Adhihetty, S.G. Adler, G. Agam, R. Agarwal, M.K. Aghi, M. Agnello, P. Agostinis, P.V. Aguilar, J. Aguirre-Ghiso, E.M. Airoldi, S. Ait-Si-Ali, T. Akematsu, E.T. Akporiaye, M. Al-Rubeai, G.M. Albaiceta, C. Albanese, D. Albani, M.L. Albert, J. Aldudo, H. Algul, M. Alirezaei, I. Alloza, A. Almasan, M. Almonte-Beceril, E.S. Alnemri, C. Alonso, N. Altan-Bonnet, D.C. Altieri, S. Alvarez, L. Alvarez-

Erviti, S. Alves, G. Amadoro, A. Amano, C. Amantini, S. Ambrosio, I. Amelio, A.O. Amer, M. Amessou, A. Amon, Z. An, F.A. Anania, S.U. Andersen, U.P. Andley, C.K. Andreadi, N. Andrieu-Abadie, A. Anel, D.K. Ann, S. Anoopkumar-Dukie, M. Antonioli, H. Aoki, N. Apostolova, S. Aquila, K. Aquilano, K. Araki, E. Arama, A. Aranda, J. Araya, A. Arcaro, E. Arias, H. Arimoto, A.R. Ariosa, J.L. Armstrong, T. Arnould, I. Arsov, K. Asanuma, V. Askanas, E. Asselin, R. Atarashi, S.S. Atherton, J.D. Atkin, L.D. Attardi, P. Auberger, G. Auburger, L. Aurelian, R. Autelli, L. Avagliano, M.L. Avantaggiati, L. Avrahami, S. Awale, N. Azad, T. Bachetti, J.M. Backer, D.H. Bae, J.S. Bae, O.N. Bae, S.H. Bae, E.H. Baehrecke, S.H. Baek, S. Baghdiguian, A. Bagniewska-Zadworna, H. Bai, J. Bai, X.Y. Bai, Y. Bailly, K.N. Balaji, W. Balduini, A. Ballabio, R. Balzan, R. Banerjee, G. Banhegyi, H. Bao, B. Barbeau, M.D. Barrachina, E. Barreiro, B. Bartel, A. Bartolome, D.C. Bassham, M.T. Bassi, R.C. Bast, Jr., A. Basu, M.T. Batista, H. Batoko, M. Battino, K. Bauckman, B.L. Baumgarner, K.U. Bayer, R. Beale, J.F. Beaulieu, G.R. Beck, Jr., C. Becker, J.D. Beckham, P.A. Bedard, P.J. Bednarski, T.J. Begley, C. Behl, C. Behrends, G.M. Behrens, K.E. Behrns, E. Bejarano, A. Belaid, F. Belleudi, G. Benard, G. Berchem, D. Bergamaschi, M. Bergami, B. Berkhout, L. Berliocchi, A. Bernard, M. Bernard, F. Bernassola, A. Bertolotti, A.S. Bess, S. Besteiro, S. Bettuzzi, S. Bhalla, S. Bhattacharyya, S.K. Bhutia, C. Biagosch, M.W. Bianchi, M. Biard-Piechaczyk, V. Billes, C. Bincoletto, B. Bingol, S.W. Bird, M. Bitoun, I. Bjedov, C. Blackstone, L. Blanc, G.A. Blanco, H.K. Blomhoff, E. Boada-Romero, S. Bockler, M. Boes, K. Boesze-Battaglia, L.H. Boise, A. Bolino, A. Boman, P. Bonaldo, M. Bordi, J. Bosch, L.M. Botana, J. Botti, G. Bou, M. Bouche, M. Bouchecareilh, M.J. Boucher, M.E. Boulton, S.G. Bouret, P. Boya, M. Boyer-Guittaut, P.V. Bozhkov, N. Brady, V.M. Braga, C. Brancolini, G.H. Braus, J.M. Bravo-San Pedro, L.A. Brennan, E.H. Bresnick, P. Brest, D. Bridges, M.A. Bringer, M. Brini, G.C. Brito, B. Brodin, P.S. Brookes, E.J. Brown, K. Brown, H.E. Broxmeyer, A. Bruhat, P.C. Brum, J.H. Brumell, N. Brunetti-Pierri, R.J. Bryson-Richardson, S. Buch, A.M. Buchan, H. Budak, D.V. Bulavin, S.J. Bultman, G. Bultynck, V. Bumbasirevic, Y. Burelle, R.E. Burke, M. Burmeister, P. Butikofer, L. Caberlotto, K. Cadwell, M. Cahova, D. Cai, J. Cai, Q. Cai, S. Calatayud, N. Camougrand, M. Campanella, G.R. Campbell, M. Campbell, S. Campello, R. Candau, I. Caniggia, L. Cantoni, L. Cao, A.B. Caplan, M. Caraglia, C. Cardinali, S.M. Cardoso, J.S. Carew, L.A. Carleton, C.R. Carlin, S. Carloni, S.R. Carlsson, D. Carmona-Gutierrez, L.A. Carneiro, O. Carnevali, S. Carra, A. Carrier, B. Carroll, C. Casas, J. Casas, G. Cassinelli, P. Castets, S. Castro-Obregon, G. Cavallini, I. Ceccherini, F. Cecconi, A.I. Cederbaum, V. Cena, S. Cenci, C. Cerella, D. Cervia, S. Cetrullo, H. Chaachouay, H.J. Chae, A.S. Chagin, C.Y. Chai, G. Chakrabarti, G. Chamilos, E.Y. Chan, M.T. Chan, D. Chandra, P. Chandra, C.P. Chang, R.C. Chang, T.Y. Chang, J.C. Chatham, S. Chatterjee, S. Chauhan, Y. Che, M.E. Cheetham, R. Cheluvappa, C.J. Chen, G. Chen, G.C. Chen, G. Chen, H. Chen, J.W. Chen, J.K. Chen, M. Chen, M. Chen, P. Chen, Q. Chen, Q. Chen, S.D. Chen, S. Chen, S.S. Chen, W. Chen, W.J. Chen, W.Q. Chen, W. Chen, X. Chen, Y.H. Chen, Y.G. Chen, Y. Chen, Y. Chen, Y. Chen, Y.J. Chen, Y.Q. Chen, Y. Chen, Z. Chen, Z. Chen, A. Cheng, C.H. Cheng, H. Cheng, H. Cheong, S. Cherry, J. Chesney, C.H. Cheung, E. Chevet, H.C. Chi, S.G. Chi, F. Chiacchiera, H.L. Chiang, R. Chiarelli, M. Chiariello, M. Chieppa, L.S. Chin, M. Chiong, G.N. Chiu, D.H. Cho, S.G. Cho, W.C. Cho, Y.Y. Cho, Y.S. Cho, A.M. Choi, E.J. Choi, E.K. Choi, J. Choi, M.E. Choi, S.I. Choi, T.F. Chou, S. Chouaib, D. Choubey, V. Choubey, K.C. Chow, K. Chowdhury, C.T. Chu, T.H. Chuang, T. Chun, H. Chung, T. Chung, Y.L. Chung, Y.J. Chwae, V. Cianfanelli, R. Ciarcia, I.A. Ciechomska, M.R. Ciriolo, M. Cirone, S. Claerhout, M.J. Clague, J. Claria, P.G. Clarke, R. Clarke, E. Clementi, C. Cleyrat, M. Cnop, E.M. Coccia, T. Cocco, P. Codogno, J. Coers, E.E. Cohen, D. Colecchia, L. Coletto, N.S. Coll, E. Colucci-Guyon, S. Comincini, M. Condello, K.L. Cook, G.H. Coombs, C.D. Cooper, J.M. Cooper, I. Coppens, M.T. Corasaniti, M. Corazzari, R. Corbalan, E. Corcelle-Termeau, M.D. Cordero, C. Corral-Ramos, O. Corti, A. Cossarizza, P. Costelli, S. Costes, S.L. Cotman, A. Coto-Montes, S. Cottet, E. Couve, L.R. Covey, L.A. Cowart, J.S. Cox, F.P. Coxon, C.B. Coyne, M.S. Cragg, R.J. Craven, T. Crepaldi, J.L. Crespo, A. Criollo, V. Crippa, M.T. Cruz, A.M. Cuervo, J.M. Cuezva, T. Cui, P.R. Cutillas, M.J. Czaja, M.F. Czyzyk-Krzeska, R.K. Dagda, U. Dahmen, C. Dai, W. Dai, Y. Dai, K.N. Dalby, L. Dalla Valle, G. Dalmasso, M. D'Amelio, M. Damme, A. Darfeuille-Michaud, C. Dargemont, V.M. Darley-Usmar, S. Dasarathy, B. Dasgupta, S. Dash, C.R. Dass, H.M. Davey, L.M. Davids, D. Davila, R.J. Davis, T.M. Dawson, V.L. Dawson, P. Daza, J. de Belleroche, P. de Figueiredo, R.C. de

Figueiredo, J. de la Fuente, L. De Martino, A. De Matteis, G.R. De Meyer, A. De Milito, M. De Santi, W. de Souza, V. De Tata, D. De Zio, J. Debnath, R. Dechant, J.P. Decuypere, S. Deegan, B. Dehay, B. Del Bello, D.P. Del Re, R. Delage-Mourroux, L.M. Delbridge, L. Deldicque, E. Delorme-Axford, Y. Deng, J. Dengjel, M. Denizot, P. Dent, C.J. Der, V. Deretic, B. Derrien, E. Deutsch, T.P. Devarenne, R.J. Devenish, S. Di Bartolomeo, N. Di Daniele, F. Di Domenico, A. Di Nardo, S. Di Paola, A. Di Pietro, L. Di Renzo, A. DiAntonio, G. Diaz-Araya, I. Diaz-Laviada, M.T. Diaz-Meco, J. Diaz-Nido, C.A. Dickey, R.C. Dickson, M. Diederich, P. Digard, I. Dikic, S.P. Dinesh-Kumar, C. Ding, W.X. Ding, Z. Ding, L. Dini, J.H. Distler, A. Diwan, M. Djavaheri-Mergny, K. Dmytruk, R.C. Dobson, V. Doetsch, K. Dokladny, S. Dokudovskaya, M. Donadelli, X.C. Dong, X. Dong, Z. Dong, T.M. Donohue, Jr., K.S. Doran, G. D'Orazi, G.W. Dorn, 2nd, V. Dosenko, S. Dridi, L. Drucker, J. Du, L.L. Du, L. Du, A. du Toit, P. Dua, L. Duan, P. Duann, V.K. Dubey, M.R. Duchen, M.A. Duchosal, H. Duez, I. Dugail, V.I. Dumit, M.C. Duncan, E.A. Dunlop, W.A. Dunn, Jr., N. Dupont, L. Dupuis, R.V. Duran, T.M. Durcan, S. Duvezin-Caubet, U. Duvvuri, V. Eapen, D. Ebrahimi-Fakhari, A. Echard, L. Eckhart, C.L. Edelstein, A.L. Edinger, L. Eichinger, T. Eisenberg, A. Eisenberg-Lerner, N.T. Eissa, W.S. El-Deiry, V. El-Khoury, Z. Elazar, H. Eldar-Finkelman, C.J. Elliott, E. Emanuele, U. Emmenegger, N. Engedal, A.M. Engelbrecht, S. Engelender, J.M. Enserink, R. Erdmann, J. Erenpreisa, R. Eri, J.L. Eriksen, A. Erman, R. Escalante, E.L. Eskelinen, L. Espert, L. Esteban-Martinez, T.J. Evans, M. Fabri, G. Fabrias, C. Fabrizi, A. Facchiano, N.J. Faergeman, A. Faggioni, W.D. Fairlie, C. Fan, D. Fan, J. Fan, S. Fang, M. Fanto, A. Fanzani, T. Farkas, M. Faure, F.B. Favier, H. Fearnhead, M. Federici, E. Fei, T.C. Felizardo, H. Feng, Y. Feng, Y. Feng, T.A. Ferguson, A.F. Fernandez, M.G. Fernandez-Barrena, J.C. Fernandez-Checa, A. Fernandez-Lopez, M.E. Fernandez-Zapico, O. Feron, E. Ferraro, C.V. Ferreira-Halder, L. Fesus, R. Feuer, F.C. Fiesel, E.C. Filippi-Chiela, G. Filomeni, G.M. Fimia, J.H. Fingert, S. Finkbeiner, T. Finkel, F. Fiorito, P.B. Fisher, M. Flajolet, F. Flamigni, O. Florey, S. Florio, R.A. Floto, M. Folini, C. Follo, E.A. Fon, F. Fornai, F. Fortunato, A. Fraldi, R. Franco, A. Francois, A. Francois, L.B. Frankel, I.D. Fraser, N. Frey, D.G. Freyssenet, C. Frezza, S.L. Friedman, D.E. Frigo, D. Fu, J.M. Fuentes, J. Fueyo, Y. Fujitani, Y. Fujiwara, M. Fujiya, M. Fukuda, S. Fulda, C. Fusco, B. Gabryel, M. Gaestel, P. Gailly, M. Gajewska, S. Galadari, G. Galili, I. Galindo, M.F. Galindo, G. Galliciotti, L. Galluzzi, L. Galluzzi, V. Galy, N. Gammoh, S. Gandy, A.K. Ganesan, S. Ganesan, I.G. Ganley, M. Gannage, F.B. Gao, F. Gao, J.X. Gao, L. Garcia Nannig, E. Garcia Vescovi, M. Garcia-Macia, C. Garcia-Ruiz, A.D. Garg, P.K. Garg, R. Gargini, N.C. Gassen, D. Gatica, E. Gatti, J. Gavard, E. Gavathiotis, L. Ge, P. Ge, S. Ge, P.W. Gean, V. Gelmetti, A.A. Genazzani, J. Geng, P. Genschik, L. Gerner, J.E. Gestwicki, D.A. Gewirtz, S. Ghavami, E. Ghigo, D. Ghosh, A.M. Giammarioli, F. Giampieri, C. Giampietri, A. Giatromanolaki, D.J. Gibbings, L. Gibellini, S.B. Gibson, V. Ginet, A. Giordano, F. Giorgini, E. Giovannetti, S.E. Girardin, S. Gispert, S. Giuliano, C.L. Gladson, A. Glavic, M. Gleave, N. Godefroy, R.M. Gogal, Jr., K. Gokulan, G.H. Goldman, D. Goletti, M.S. Goligorsky, A.V. Gomes, L.C. Gomes, H. Gomez, C. Gomez-Manzano, R. Gomez-Sanchez, D.A. Goncalves, E. Goncu, Q. Gong, C. Gongora, C.B. Gonzalez, P. Gonzalez-Alegre, P. Gonzalez-Cabo, R.A. Gonzalez-Polo, I.S. Goping, C. Gorbea, N.V. Gorbunov, D.R. Goring, A.M. Gorman, S.M. Gorski, S. Goruppi, S. Goto-Yamada, C. Gotor, R.A. Gottlieb, I. Gozes, D. Gozuacik, Y. Graba, M. Graef, G.E. Granato, G.D. Grant, S. Grant, G.L. Gravina, D.R. Green, A. Greenhough, M.T. Greenwood, B. Grimaldi, F. Gros, C. Grose, J.F. Groulx, F. Gruber, P. Grumati, T. Grune, J.L. Guan, K.L. Guan, B. Guerra, C. Guillen, K. Gulshan, J. Gunst, C. Guo, L. Guo, M. Guo, W. Guo, X.G. Guo, A.A. Gust, A.B. Gustafsson, E. Gutierrez, M.G. Gutierrez, H.S. Gwak, A. Haas, J.E. Haber, S. Hadano, M. Hagedorn, D.R. Hahn, A.J. Halayko, A. Hamacher-Brady, K. Hamada, A. Hamai, A. Hamann, M. Hamasaki, I. Hamer, Q. Hamid, E.M. Hammond, F. Han, W. Han, J.T. Handa, J.A. Hanover, M. Hansen, M. Harada, L. Harhaji-Trajkovic, J.W. Harper, A.H. Harrath, A.L. Harris, J. Harris, U. Hasler, P. Hasselblatt, K. Hasui, R.G. Hawley, T.S. Hawley, C. He, C.Y. He, F. He, G. He, R.R. He, X.H. He, Y.W. He, Y.Y. He, J.K. Heath, M.J. Hebert, R.A. Heinzen, G.V. Helgason, M. Hensel, E.P. Henske, C. Her, P.K. Herman, A. Hernandez, C. Hernandez, S. Hernandez-Tiedra, C. Hetz, P.R. Hiesinger, K. Higaki, S. Hilfiker, B.G. Hill, J.A. Hill, W.D. Hill, K. Hino, D. Hofius, P. Hofman, G.U. Hoglinger, J. Hohfeld, M.K. Holz, Y. Hong, D.A. Hood, J.J. Hoozemans, T. Hoppe, C. Hsu, C.Y. Hsu, L.C. Hsu, D. Hu, G. Hu, H.M. Hu, H. Hu, M.C. Hu, Y.C. Hu, Z.W. Hu, F. Hua, Y. Hua, C. Huang, H.L.

Huang, K.H. Huang, K.Y. Huang, S. Huang, S. Huang, W.P. Huang, Y.R. Huang, Y. Huang, Y. Huang, T.B. Huber, P. Huebbe, W.K. Huh, J.J. Hulmi, G.M. Hur, J.H. Hurley, Z. Husak, S.N. Hussain, S. Hussain, J.J. Hwang, S. Hwang, T.I. Hwang, A. Ichihara, Y. Imai, C. Imbriano, M. Inomata, T. Into, V. Iovane, J.L. Iovanna, R.V. Iozzo, N.Y. Ip, J.E. Irazoqui, P. Iribarren, Y. Isaka, A.J. Isakovic, H. Ischiropoulos, J.S. Isenberg, M. Ishaq, H. Ishida, I. Ishii, J.E. Ishmael, C. Isidoro, K. Isobe, E. Isono, S. Issazadeh-Navikas, K. Itahana, E. Itakura, A.I. Ivanov, A.K. Iyer, J.M. Izquierdo, Y. Izumi, V. Izzo, M. Jaattela, N. Jaber, D.J. Jackson, W.T. Jackson, T.G. Jacob, T.S. Jacques, C. Jagannath, A. Jain, N.R. Jana, B.K. Jang, A. Jani, B. Janji, P.R. Jannig, P.J. Jansson, S. Jean, M. Jendrach, J.H. Jeon, N. Jessen, E.B. Jeung, K. Jia, L. Jia, H. Jiang, H. Jiang, L. Jiang, T. Jiang, X. Jiang, X. Jiang, X. Jiang, Y. Jiang, Y. Jiang, A. Jimenez, C. Jin, H. Jin, L. Jin, M. Jin, S. Jin, U.K. Jinwal, E.K. Jo, T. Johansen, D.E. Johnson, G.V. Johnson, J.D. Johnson, E. Jonasch, C. Jones, L.A. Joosten, J. Jordan, A.M. Joseph, B. Joseph, A.M. Joubert, D. Ju, J. Ju, H.F. Juan, K. Juenemann, G. Juhasz, H.S. Jung, J.U. Jung, Y.K. Jung, H. Jungbluth, M.J. Justice, B. Jutten, N.O. Kaakoush, K. Kaarniranta, A. Kaasik, T. Kabuta, B. Kaeffer, K. Kagedal, A. Kahana, S. Kajimura, O. Kakhlon, M. Kalia, D.V. Kalvakolanu, Y. Kamada, K. Kambas, V.O. Kaminskyy, H.H. Kampinga, M. Kandouz, C. Kang, R. Kang, T.C. Kang, T. Kanki, T.D. Kanneganti, H. Kanno, A.G. Kanthasamy, M. Kantorow, M. Kaparakis-Liaskos, O. Kapuy, V. Karantza, M.R. Karim, P. Karmakar, A. Kaser, S. Kaushik, T. Kawula, A.M. Kaynar, P.Y. Ke, Z.J. Ke, J.H. Kehrl, K.E. Keller, J.K. Kemper, A.K. Kenworthy, O. Kepp, A. Kern, S. Kesari, D. Kessel, R. Ketteler, C. Kettelhut Ido, B. Khambu, M.M. Khan, V.K. Khandelwal, S. Khare, J.G. Kiang, A.A. Kiger, A. Kihara, A.L. Kim, C.H. Kim, D.R. Kim, D.H. Kim, E.K. Kim, H.Y. Kim, H.R. Kim, J.S. Kim, J.H. Kim, J.C. Kim, J.H. Kim, K.W. Kim, M.D. Kim, M.M. Kim, P.K. Kim, S.W. Kim, S.Y. Kim, Y.S. Kim, Y. Kim, A. Kimchi, A.C. Kimmelman, T. Kimura, J.S. King, K. Kirkegaard, V. Kirkin, L.A. Kirshenbaum, S. Kishi, Y. Kitajima, K. Kitamoto, Y. Kitaoka, K. Kitazato, R.A. Kley, W.T. Klimecki, M. Klinkenberg, J. Klucken, H. Knaevelsrud, E. Knecht, L. Knuppertz, J.L. Ko, S. Kobayashi, J.C. Koch, C. Koechlin-Ramonatxo, U. Koenig, Y.H. Koh, K. Kohler, S.D. Kohlwein, M. Koike, M. Komatsu, E. Kominami, D. Kong, H.J. Kong, E.G. Konstantakou, B.T. Kopp, T. Korcsmaros, L. Korhonen, V.I. Korolchuk, N.V. Koshkina, Y. Kou, M.I. Koukourakis, C. Koumenis, A.L. Kovacs, T. Kovacs, W.J. Kovacs, D. Koya, C. Kraft, D. Krainc, H. Kramer, T. Kravic-Stevovic, W. Krek, C. Kretz-Remy, R. Krick, M. Krishnamurthy, J. Kriston-Vizi, G. Kroemer, M.C. Kruer, R. Kruger, N.T. Ktistakis, K. Kuchitsu, C. Kuhn, A.P. Kumar, A. Kumar, A. Kumar, D. Kumar, D. Kumar, R. Kumar, S. Kumar, M. Kundu, H.J. Kung, A. Kuno, S.H. Kuo, J. Kuret, T. Kurz, T. Kwok, T.K. Kwon, Y.T. Kwon, I. Kyrmizi, A.R. La Spada, F. Lafont, T. Lahm, A. Lakkaraju, T. Lam, T. Lamark, S. Lancel, T.H. Landowski, D.J. Lane, J.D. Lane, C. Lanzi, P. Lapaquette, L.R. Lapierre, J. Laporte, J. Laukkarinen, G.W. Laurie, S. Lavandero, L. Lavie, M.J. LaVoie, B.Y. Law, H.K. Law, K.B. Law, R. Layfield, P.A. Lazo, L. Le Cam, K.G. Le Roch, H. Le Stunff, V. Leardkamolkarn, M. Lecuit, B.H. Lee, C.H. Lee, E.F. Lee, G.M. Lee, H.J. Lee, H. Lee, J.K. Lee, J. Lee, J.H. Lee, J.H. Lee, M. Lee, M.S. Lee, P.J. Lee, S.W. Lee, S.J. Lee, S.J. Lee, S.Y. Lee, S.H. Lee, S.S. Lee, S.J. Lee, S. Lee, Y.R. Lee, Y.J. Lee, Y.H. Lee, C. Leeuwenburgh, S. Lefort, R. Legouis, J. Lei, Q.Y. Lei, D.A. Leib, G. Leibowitz, I. Lekli, S.D. Lemaire, J.J. Lemasters, M.K. Lemberg, A. Lemoine, S. Leng, G. Lenz, P. Lenzi, L.O. Lerman, D. Lettieri Barbato, J.I. Leu, H.Y. Leung, B. Levine, P.A. Lewis, F. Lezoualc'h, C. Li, F. Li, F.J. Li, J. Li, K. Li, L. Li, M. Li, M. Li, Q. Li, R. Li, S. Li, W. Li, W. Li, X. Li, Y. Li, J. Lian, C. Liang, Q. Liang, Y. Liao, J. Liberal, P.P. Liberski, P. Lie, A.P. Lieberman, H.J. Lim, K.L. Lim, K. Lim, R.T. Lima, C.S. Lin, C.F. Lin, F. Lin, F. Lin, F.C. Lin, K. Lin, K.H. Lin, P.H. Lin, T. Lin, W.W. Lin, Y.S. Lin, Y. Lin, R. Linden, D. Lindholm, L.M. Lindqvist, P. Lingor, A. Linkermann, L.A. Liotta, M.M. Lipinski, V.A. Lira, M.P. Lisanti, P.B. Liton, B. Liu, C. Liu, C.F. Liu, F. Liu, H.J. Liu, J. Liu, J.J. Liu, J.L. Liu, K. Liu, L. Liu, L. Liu, Q. Liu, R.Y. Liu, S. Liu, S. Liu, W. Liu, X.D. Liu, X. Liu, X.H. Liu, X. Liu, X. Liu, X. Liu, Y. Liu, Y. Liu, Z. Liu, Z. Liu, J.P. Liuzzi, G. Lizard, M. Ljujic, I.J. Lodhi, S.E. Logue, B.L. Lokeshwar, Y.C. Long, S. Lonial, B. Loos, C. Lopez-Otin, C. Lopez-Vicario, M. Lorente, P.L. Lorenzi, P. Lorincz, M. Los, M.T. Lotze, P.E. Lovat, B. Lu, B. Lu, J. Lu, Q. Lu, S.M. Lu, S. Lu, Y. Lu, F. Luciano, S. Luckhart, J.M. Lucocq, P. Ludovico, A. Lugea, N.W. Lukacs, J.J. Lum, A.H. Lund, H. Luo, J. Luo, S. Luo, C. Luparello, T. Lyons, J. Ma, Y. Ma, Y. Ma, Z. Ma, J. Machado, G.M. Machado-Santelli, F. Macian, G.C. MacIntosh, J.P. MacKeigan, K.F. Macleod, J.D. MacMicking, L.A. MacMillan-Crow, F. Madeo, M. Madesh, J. Madrigal-Matute, A. Maeda, T. Maeda, G. Maegawa, E. Maellaro, H. Maes, M.

Magarinos, K. Maiese, T.K. Maiti, L. Maiuri, M.C. Maiuri, C.G. Maki, R. Malli, W. Malorni, A. Maloyan, F. Mami-Chouaib, N. Man, J.D. Mancias, E.M. Mandelkow, M.A. Mandell, A.A. Manfredi, S.N. Manie, C. Manzoni, K. Mao, Z. Mao, Z.W. Mao, P. Marambaud, A.M. Marconi, Z. Marelja, G. Marfe, M. Margeta, E. Margittai, M. Mari, F.V. Mariani, C. Marin, S. Marinelli, G. Marino, I. Markovic, R. Marquez, A.M. Martelli, S. Martens, K.R. Martin, S.J. Martin, S. Martin, M.A. Martin-Acebes, P. Martin-Sanz, C. Martinand-Mari, W. Martinet, J. Martinez, N. Martinez-Lopez, U. Martinez-Outschoorn, M. Martinez-Velazquez, M. Martinez-Vicente, W.K. Martins, H. Mashima, J.A. Mastrianni, G. Matarese, P. Matarrese, R. Mateo, S. Matoba, N. Matsumoto, T. Matsushita, A. Matsuura, T. Matsuzawa, M.P. Mattson, S. Matus, N. Maugeri, C. Mauvezin, A. Mayer, D. Maysinger, G.D. Mazzolini, M.K. McBrayer, K. McCall, C. McCormick, G.M. McInerney, S.C. McIver, S. McKenna, J.J. McMahon, I.A. McNeish, F. Mechta-Grigoriou, J.P. Medema, D.L. Medina, K. Megyeri, M. Mehrpour, J.L. Mehta, Y. Mei, U.C. Meier, A.J. Meijer, A. Melendez, G. Melino, S. Melino, E.J. de Melo, M.A. Mena, M.D. Meneghini, J.A. Menendez, R. Menezes, L. Meng, L.H. Meng, S. Meng, R. Menghini, A.S. Menko, R.F. Menna-Barreto, M.B. Menon, M.A. Meraz-Rios, G. Merla, L. Merlini, A.M. Merlot, A. Meryk, S. Meschini, J.N. Meyer, M.T. Mi, C.Y. Miao, L. Micale, S. Michaeli, C. Michiels, A.R. Migliaccio, A.S. Mihailidou, D. Mijaljica, K. Mikoshiba, E. Milan, L. Miller-Fleming, G.B. Mills, I.G. Mills, G. Minakaki, B.A. Minassian, X.F. Ming, F. Minibayeva, E.A. Minina, J.D. Mintern, S. Minucci, A. Miranda-Vizuete, C.H. Mitchell, S. Miyamoto, K. Miyazawa, N. Mizushima, K. Mnich, B. Mograbi, S. Mohseni, L.F. Moita, M. Molinari, M. Molinari, A.B. Moller, B. Mollereau, F. Mollinedo, M. Mongillo, M.M. Monick, S. Montagnaro, C. Montell, D.J. Moore, M.N. Moore, R. Mora-Rodriguez, P.I. Moreira, E. Morel, M.B. Morelli, S. Moreno, M.J. Morgan, A. Moris, Y. Moriyasu, J.L. Morrison, L.A. Morrison, E. Morselli, J. Moscat, P.L. Moseley, S. Mostowy, E. Motori, D. Mottet, J.C. Mottram, C.E. Moussa, V.E. Mpakou, H. Mukhtar, J.M. Mulcahy Levy, S. Muller, R. Munoz-Moreno, C. Munoz-Pinedo, C. Munz, M.E. Murphy, J.T. Murray, A. Murthy, I.U. Mysorekar, I.R. Nabi, M. Nabissi, G.A. Nader, Y. Nagahara, Y. Nagai, K. Nagata, A. Nagelkerke, P. Nagy, S.R. Naidu, S. Nair, H. Nakano, H. Nakatogawa, M. Nanjundan, G. Napolitano, N.I. Naqvi, R. Nardacci, D.P. Narendra, M. Narita, A.C. Nascimbeni, R. Natarajan, L.C. Navegantes, S.T. Nawrocki, T.Y. Nazarko, V.Y. Nazarko, T. Neill, L.M. Neri, M.G. Netea, R.T. Netea-Maier, B.M. Neves, P.A. Ney, I.P. Nezis, H.T. Nguyen, H.P. Nguyen, A.S. Nicot, H. Nilsen, P. Nilsson, M. Nishimura, I. Nishino, M. Niso-Santano, H. Niu, R.A. Nixon, V.C. Njar, T. Noda, A.A. Noegel, E.M. Nolte, E. Norberg, K.K. Norga, S.K. Noureini, S. Notomi, L. Notterpek, K. Nowikovsky, N. Nukina, T. Nurnberger, V.B. O'Donnell, T. O'Donovan, P.J. O'Dwyer, I. Oehme, C.L. Oeste, M. Ogawa, B. Ogretmen, Y. Ogura, Y.J. Oh, M. Ohmuraya, T. Ohshima, R. Ojha, K. Okamoto, T. Okazaki, F.J. Oliver, K. Ollinger, S. Olsson, D.P. Orban, P. Ordonez, I. Orhon, L. Orosz, E.J. O'Rourke, H. Orozco, A.L. Ortega, E. Ortona, L.D. Osellame, J. Oshima, S. Oshima, H.D. Osiewacz, T. Otomo, K. Otsu, J.H. Ou, T.F. Outeiro, D.Y. Ouyang, H. Ouyang, M. Overholtzer, M.A. Ozbun, P.H. Ozdinler, B. Ozpolat, C. Pacelli, P. Paganetti, G. Page, G. Pages, U. Pagnini, B. Pajak, S.C. Pak, K. Pakos-Zebrucka, N. Pakpour, Z. Palkova, F. Palladino, K. Pallauf, N. Pallet, M. Palmieri, S.R. Paludan, C. Palumbo, S. Palumbo, O. Pampliega, H. Pan, W. Pan, T. Panaretakis, A. Pandey, A. Pantazopoulou, Z. Papackova, D.L. Papademetrio, I. Papassideri, A. Papini, N. Parajuli, J. Pardo, V.V. Parekh, G. Parenti, J.I. Park, J. Park, O.K. Park, R. Parker, R. Parlato, J.B. Parys, K.R. Parzych, J.M. Pasquet, B. Pasquier, K.B. Pasumarthi, D. Patschan, C. Patterson, S. Pattingre, S. Pattison, A. Pause, H. Pavenstadt, F. Pavone, Z. Pedrozo, F.J. Pena, M.A. Penalva, M. Pende, J. Peng, F. Penna, J.M. Penninger, A. Pensalfini, S. Pepe, G.J. Pereira, P.C. Pereira, V. Perez-de la Cruz, M.E. Perez-Perez, D. Perez-Rodriguez, D. Perez-Sala, C. Perier, A. Perl, D.H. Perlmutter, I. Perrotta, S. Pervaiz, M. Pesonen, J.E. Pessin, G.J. Peters, M. Petersen, I. Petrache, B.J. Petrof, G. Petrovski, J.M. Phang, M. Piacentini, M. Pierdominici, P. Pierre, V. Pierrefite-Carle, F. Pietrocola, F.X. Pimentel-Muinos, M. Pinar, B. Pineda, R. Pinkas-Kramarski, M. Pinti, P. Pinton, B. Piperdi, J.M. Piret, L.C. Platanias, H.W. Platta, E.D. Plowey, S. Poggeler, M. Poirot, P. Polcic, A. Poletti, A.H. Poon, H. Popelka, B. Popova, I. Poprawa, S.M. Poulose, J. Poulton, S.K. Powers, T. Powers, M. Pozuelo-Rubio, K. Prak, R. Prange, M. Prescott, M. Priault, S. Prince, R.L. Proia, T. Proikas-Cezanne, H. Prokisch, V.J. Promponas, K. Przyklenk, R. Puertollano, S. Pugazhenthi, L. Puglielli, A. Pujol, J. Puyal, D.

Pyeon, X. Qi, W.B. Qian, Z.H. Qin, Y. Qiu, Z. Qu, J. Quadrilatero, F. Quinn, N. Raben, H. Rabinowich, F. Radogna, M.J. Ragusa, M. Rahmani, K. Raina, S. Ramanadham, R. Ramesh, A. Rami, S. Randall-Demllo, F. Randow, H. Rao, V.A. Rao, B.B. Rasmussen, T.M. Rasse, E.A. Ratovitski, P.E. Rautou, S.K. Ray, B. Razani, B.H. Reed, F. Reggiori, M. Rehm, A.S. Reichert, T. Rein, D.J. Reiner, E. Reits, J. Ren, X. Ren, M. Renna, J.E. Reusch, J.L. Revuelta, L. Reyes, A.R. Rezaie, R.I. Richards, D.R. Richardson, C. Richetta, M.A. Riehle, B.H. Rihn, Y. Rikihisa, B.E. Riley, G. Rimbach, M.R. Rippo, K. Ritis, F. Rizzi, E. Rizzo, P.J. Roach, J. Robbins, M. Roberge, G. Roca, M.C. Roccheri, S. Rocha, C.M. Rodrigues, C.I. Rodriguez, S.R. de Cordoba, N. Rodriguez-Muela, J. Roelofs, V.V. Rogov, T.T. Rohn, B. Rohrer, D. Romanelli, L. Romani, P.S. Romano, M.I. Roncero, J.L. Rosa, A. Rosello, K.V. Rosen, P. Rosenstiel, M. Rost-Roszkowska, K.A. Roth, G. Roue, M. Rouis, K.M. Rouschop, D.T. Ruan, D. Ruano, D.C. Rubinsztein, E.B. Rucker, 3rd, A. Rudich, E. Rudolf, R. Rudolf, M.A. Ruegg, C. Ruiz-Roldan, A.A. Ruparelia, P. Rusmini, D.W. Russ, G.L. Russo, G. Russo, R. Russo, T.E. Rusten, V. Ryabovol, K.M. Ryan, S.W. Ryter, D.M. Sabatini, M. Sacher, C. Sachse, M.N. Sack, J. Sadoshima, P. Saftig, R. Sagi-Eisenberg, S. Sahni, P. Saikumar, T. Saito, T. Saitoh, K. Sakakura, M. Sakoh-Nakatogawa, Y. Sakuraba, M. Salazar-Roa, P. Salomoni, A.K. Saluja, P.M. Salvaterra, R. Salvioli, A. Samali, A.M. Sanchez, J.A. Sanchez-Alcazar, R. Sanchez-Prieto, M. Sandri, M.A. Sanjuan, S. Santaguida, L. Santambrogio, G. Santoni, C.N. Dos Santos, S. Saran, M. Sardiello, G. Sargent, P. Sarkar, S. Sarkar, M.R. Sarrias, M.M. Sarwal, C. Sasakawa, M. Sasaki, M. Sass, K. Sato, M. Sato, J. Satriano, N. Savaraj, S. Saveljeva, L. Schaefer, U.E. Schaible, M. Scharl, H.M. Schatzl, R. Schekman, W. Scheper, A. Schiavi, H.M. Schipper, H. Schmeisser, J. Schmidt, I. Schmitz, B.E. Schneider, E.M. Schneider, J.L. Schneider, E.A. Schon, M.J. Schonenberger, A.H. Schonthal, D.F. Schorderet, B. Schroder, S. Schuck, R.J. Schulze, M. Schwarten, T.L. Schwarz, S. Sciarretta, K. Scotto, A.I. Scovassi, R.A. Screaton, M. Screen, H. Seca, S. Sedej, L. Segatori, N. Segev, P.O. Seglen, J.M. Segui-Simarro, J. Segura-Aguilar, E. Seki, C. Sell, I. Seiliez, C.F. Semenkovich, G.L. Semenza, U. Sen, A.L. Serra, A. Serrano-Puebla, H. Sesaki, T. Setoguchi, C. Settembre, J.J. Shacka, A.N. Shajahan-Haq, I.M. Shapiro, S. Sharma, H. She, C.K. Shen, C.C. Shen, H.M. Shen, S. Shen, W. Shen, R. Sheng, X. Sheng, Z.H. Sheng, T.G. Shepherd, J. Shi, Q. Shi, Q. Shi, Y. Shi, S. Shibutani, K. Shibuya, Y. Shidoji, J.J. Shieh, C.M. Shih, Y. Shimada, S. Shimizu, D.W. Shin, M.L. Shinohara, M. Shintani, T. Shintani, T. Shioi, K. Shirabe, R. Shiri-Sverdlov, O. Shirihai, G.C. Shore, C.W. Shu, D. Shukla, A.A. Sibirny, V. Sica, C.J. Sigurdson, E.M. Sigurdsson, P.S. Sijwali, B. Sikorska, W.A. Silveira, S. Silvente-Poirot, G.A. Silverman, J. Simak, T. Simmet, A.K. Simon, H.U. Simon, C. Simone, M. Simons, A. Simonsen, R. Singh, S.V. Singh, S.K. Singh, D. Sinha, S. Sinha, F.A. Sinicrope, A. Sirko, K. Sirohi, B.J. Sishi, A. Sittler, P.M. Siu, E. Sivridis, A. Skwarska, R. Slack, I. Slaninova, N. Slavov, S.S. Smaili, K.S. Smalley, D.R. Smith, S.J. Soenen, S.A. Soleimanpour, A. Solhaug, K. Somasundaram, J.H. Son, A. Sonawane, C. Song, F. Song, H.K. Song, J.X. Song, W. Song, K.Y. Soo, A.K. Sood, T.W. Soong, V. Soontornniyomkij, M. Sorice, F. Sotgia, D.R. Soto-Pantoja, A. Sotthibundhu, M.J. Sousa, H.P. Spaink, P.N. Span, A. Spang, J.D. Sparks, P.G. Speck, S.A. Spector, C.D. Spies, W. Springer, D.S. Clair, A. Stacchiotti, B. Staels, M.T. Stang, D.T. Starczynowski, P. Starokadomskyy, C. Steegborn, J.W. Steele, L. Stefanis, J. Steffan, C.M. Stellrecht, H. Stenmark, T.M. Stepkowski, S.T. Stern, C. Stevens, B.R. Stockwell, V. Stoka, Z. Storchova, B. Stork, V. Stratoulias, D.J. Stravopodis, P. Strnad, A.M. Strohecker, A.L. Strom, P. Stromhaug, J. Stulik, Y.X. Su, Z. Su, C.S. Subauste, S. Subramaniam, C.M. Sue, S.W. Suh, X. Sui, S. Sukseree, D. Sulzer, F.L. Sun, J. Sun, J. Sun, S.Y. Sun, Y. Sun, Y. Sun, Y. Sun, V. Sundaramoorthy, J. Sung, H. Suzuki, K. Suzuki, N. Suzuki, T. Suzuki, Y.J. Suzuki, M.S. Swanson, C. Swanton, K. Sward, G. Swarup, S.T. Sweeney, P.W. Sylvester, Z. Szatmari, E. Szegezdi, P.W. Szlosarek, H. Taegtmeyer, M. Tafani, E. Taillebourg, S.W. Tait, K. Takacs-Vellai, Y. Takahashi, S. Takats, G. Takemura, N. Takigawa, N.J. Talbot, E. Tamagno, J. Tamburini, C.P. Tan, L. Tan, M.L. Tan, M. Tan, Y.J. Tan, K. Tanaka, M. Tanaka, D. Tang, D. Tang, G. Tang, I. Tanida, K. Tanji, B.A. Tannous, J.A. Tapia, I. Tasset-Cuevas, M. Tatar, I. Tavassoly, N. Tavernarakis, A. Taylor, G.S. Taylor, G.A. Taylor, J.P. Taylor, M.J. Taylor, E.V. Tchetina, A.R. Tee, F. Teixeira-Clerc, S. Telang, T. Tencomnao, B.B. Teng, R.J. Teng, F. Terro, G. Tettamanti, A.L. Theiss, A.E. Theron, K.J. Thomas, M.P. Thome, P.G. Thomes, A. Thorburn, J. Thorner, T. Thum, M. Thumm, T.L. Thurston, L. Tian, A. Till, J.P. Ting, V.I. Titorenko, L. Toker, S. Toldo, S.A. Tooze, I. Topisirovic, M.L.

Torgersen, L. Torosantucci, A. Torriglia, M.R. Torrisi, C. Tournier, R. Towns, V. Trajkovic, L.H. Travassos, G. Triola, D.N. Tripathi, D. Trisciuoglio, R. Troncoso, I.P. Trougakos, A.C. Truttmann, K.J. Tsai, M.P. Tschan, Y.H. Tseng, T. Tsukuba, A. Tsung, A.S. Tsvetkov, S. Tu, H.Y. Tuan, M. Tucci, D.A. Tumbarello, B. Turk, V. Turk, R.F. Turner, A.A. Tveita, S.C. Tyagi, M. Ubukata, Y. Uchiyama, A. Udelnow, T. Ueno, M. Umekawa, R. Umemiya-Shirafuji, B.R. Underwood, C. Ungermann, R.P. Ureshino, R. Ushioda, V.N. Uversky, N.L. Uzcategui, T. Vaccari, M.I. Vaccaro, L. Vachova, H. Vakifahmetoglu-Norberg, R. Valdor, E.M. Valente, F. Vallette, A.M. Valverde, G. Van den Berghe, L. Van Den Bosch, G.R. van den Brink, F.G. van der Goot, I.J. van der Klei, L.J. van der Laan, W.G. van Doorn, M. van Egmond, K.L. van Golen, L. Van Kaer, M. van Lookeren Campagne, P. Vandenabeele, W. Vandenberghe, I. Vanhorebeek, I. Varela-Nieto, M.H. Vasconcelos, R. Vasko, D.G. Vavvas, I. Vega-Naredo, G. Velasco, A.D. Velentzas, P.D. Velentzas, T. Vellai, E. Vellenga, M.H. Vendelbo, K. Venkatachalam, N. Ventura, S. Ventura, P.S. Veras, M. Verdier, B.G. Vertessy, A. Viale, M. Vidal, H.L. Vieira, R.D. Vierstra, N. Vigneswaran, N. Vij, M. Vila, M. Villar, V.H. Villar, J. Villarroya, C. Vindis, G. Viola, M.T. Viscomi, G. Vitale, D.T. Vogl, O.V. Voitsekhovskaja, C. von Haefen, K. von Schwarzenberg, D.E. Voth, V. Vouret-Craviari, K. Vuori, J.M. Vyas, C. Waeber, C.L. Walker, M.J. Walker, J. Walter, L. Wan, X. Wan, B. Wang, C. Wang, C.Y. Wang, C. Wang, C. Wang, C. Wang, D. Wang, F. Wang, F. Wang, G. Wang, H.J. Wang, H. Wang, H.G. Wang, H. Wang, H.D. Wang, J. Wang, J. Wang, M. Wang, M.Q. Wang, P.Y. Wang, P. Wang, R.C. Wang, S. Wang, T.F. Wang, X. Wang, X.J. Wang, X.W. Wang, X. Wang, X. Wang, Y. Wang, Y. Wang, Y. Wang, Y.J. Wang, Y. Wang, Y. Wang, Y.T. Wang, Y. Wang, Z.N. Wang, P. Wappner, C. Ward, D.M. Ward, G. Warnes, H. Watada, Y. Watanabe, K. Watase, T.E. Weaver, C.D. Weekes, J. Wei, T. Weide, C.C. Weihl, G. Weindl, S.N. Weis, L. Wen, X. Wen, Y. Wen, B. Westermann, C.M. Weyand, A.R. White, E. White, J.L. Whitton, A.J. Whitworth, J. Wiels, F. Wild, M.E. Wildenberg, T. Wileman, D.S. Wilkinson, S. Wilkinson, D. Willbold, C. Williams, K. Williams, P.R. Williamson, K.F. Winklhofer, S.S. Witkin, S.E. Wohlgemuth, T. Wollert, E.J. Wolvetang, E. Wong, G.W. Wong, R.W. Wong, V.K. Wong, E.A. Woodcock, K.L. Wright, C. Wu, D. Wu, G.S. Wu, J. Wu, J. Wu, M. Wu, M. Wu, S. Wu, W.K. Wu, Y. Wu, Z. Wu, C.P. Xavier, R.J. Xavier, G.X. Xia, T. Xia, W. Xia, Y. Xia, H. Xiao, J. Xiao, S. Xiao, W. Xiao, C.M. Xie, Z. Xie, Z. Xie, M. Xilouri, Y. Xiong, C. Xu, C. Xu, F. Xu, H. Xu, H. Xu, J. Xu, J. Xu, J. Xu, L. Xu, X. Xu, Y. Xu, Y. Xu, Z.X. Xu, Z. Xu, Y. Xue, T. Yamada, A. Yamamoto, K. Yamanaka, S. Yamashina, S. Yamashiro, B. Yan, B. Yan, X. Yan, Z. Yan, Y. Yanagi, D.S. Yang, J.M. Yang, L. Yang, M. Yang, P.M. Yang, P. Yang, Q. Yang, W. Yang, W.Y. Yang, X. Yang, Y. Yang, Y. Yang, Z. Yang, Z. Yang, M.C. Yao, P.J. Yao, X. Yao, Z. Yao, Z. Yao, L.S. Yasui, M. Ye, B. Yedvobnick, B. Yeganeh, E.S. Yeh, P.L. Yeyati, F. Yi, L. Yi, X.M. Yin, C.K. Yip, Y.M. Yoo, Y.H. Yoo, S.Y. Yoon, K. Yoshida, T. Yoshimori, K.H. Young, H. Yu, J.J. Yu, J.T. Yu, J. Yu, L. Yu, W.H. Yu, X.F. Yu, Z. Yu, J. Yuan, Z.M. Yuan, B.Y. Yue, J. Yue, Z. Yue, D.N. Zacks, E. Zacksenhaus, N. Zaffaroni, T. Zaglia, Z. Zakeri, V. Zecchini, J. Zeng, M. Zeng, Q. Zeng, A.S. Zervos, D.D. Zhang, F. Zhang, G. Zhang, G.C. Zhang, H. Zhang, H. Zhang, H. Zhang, H. Zhang, J. Zhang, J. Zhang, J. Zhang, J. Zhang, J.P. Zhang, L. Zhang, L. Zhang, L. Zhang, L. Zhang, M.Y. Zhang, X. Zhang, X.D. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, Y. Zhang, M. Zhao, W.L. Zhao, X. Zhao, Y.G. Zhao, Y. Zhao, Y. Zhao, Y.X. Zhao, Z. Zhao, Z.J. Zhao, D. Zheng, X.L. Zheng, X. Zheng, B. Zhivotovsky, Q. Zhong, G.Z. Zhou, G. Zhou, H. Zhou, S.F. Zhou, X.J. Zhou, H. Zhu, H. Zhu, W.G. Zhu, W. Zhu, X.F. Zhu, Y. Zhu, S.M. Zhuang, X. Zhuang, E. Ziparo, C.E. Zois, T. Zoladek, W.X. Zong, A. Zorzano, S.M. Zughaier, Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition), Autophagy 12(1) (2016) 1-222.

[133] T. Nury, A. Zarrouk, A. Vejux, M. Doria, J.M. Riedinger, R. Delage-Mourroux, G. Lizard, Induction of oxiapoptophagy, a mixed mode of cell death associated with oxidative stress, apoptosis and autophagy, on 7-ketocholesterol-treated 158N murine oligodendrocytes: impairment by alpha-tocopherol, Biochem Biophys Res Commun 446(3) (2014) 714-9.

[134] J. Wolf, W. Schliebs, R. Erdmann, Peroxisomes as dynamic organelles: peroxisomal matrix protein import, FEBS J 277(16) (2010) 3268-78.

[135] P. Dakik, V.I. Titorenko, Communications between Mitochondria, the Nucleus, Vacuoles, Peroxisomes, the Endoplasmic Reticulum, the Plasma Membrane, Lipid Droplets, and the Cytosol during Yeast Chronological Aging, Front Genet 7 (2016) 177.
[136] M.P. Sauvant, D. Pepin, E. Piccinni, Tetrahymena pyriformis: a tool for toxicological studies. A review, Chemosphere 38(7) (1999) 1631-69.
[137] E.M. Karym, Impacts d'antioxydants naturels sur la neurodégénérescence induite par des acides gras à très longue chaîne : aspects biochimiques et métaboliques., PhD Thesis, Univ. Bourgogne Franche-Comté, Dijon, France & Univ. Hassan 1er, Settat, Morocco, 2016.
[138] C. Prunet, S. Lemaire-Ewing, F. Menetrier, D. Neel, G. Lizard, Activation of caspase-3-dependent and -independent pathways during 7-ketocholesterol- and 7beta-hydroxycholesterol-induced cell death: a morphological and biochemical study, J Biochem Mol Toxicol 19(5) (2005) 311-26.
[139] S. Luthra, B. Fardin, J. Dong, D. Hertzog, S. Kamjoo, S. Gebremariam, V. Butani, R. Narayanan, J.K. Mungcal, B.D. Kuppermann, M.C. Kenney, Activation of caspase-8 and caspase-12 pathways by 7-ketocholesterol in human retinal pigment epithelial cells, Invest Ophthalmol Vis Sci 47(12) (2006) 5569-75.
[140] A. Neekhra, S. Luthra, M. Chwa, G. Seigel, A.L. Gramajo, B.D. Kuppermann, M.C. Kenney, Caspase-8, -12, and -3 activation by 7-ketocholesterol in retinal neurosensory cells, Invest Ophthalmol Vis Sci 48(3) (2007) 1362-7.
[141] K. Ragot, J.J. Mackrill, A. Zarrouk, T. Nury, V. Aires, A. Jacquin, A. Athias, J.P. Pais de Barros, A. Vejux, J.M. Riedinger, D. Delmas, G. Lizard, Absence of correlation between oxysterol accumulation in lipid raft microdomains, calcium increase, and apoptosis induction on 158N murine oligodendrocytes, Biochem Pharmacol 86(1) (2013) 67-79.
[142] T. Nury, A. Zarrouk, K. Ragot, M. Debbabi, J.M. Riedinger, A. Vejux, P. Aubourg, G. Lizard, 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 Steroid Biochem Mol Biol 169 (2017) 123-136.
[143] M. Bezine, M. Debbabi, T. Nury, R. Ben-Khalifa, M. Samadi, M. Cherkaoui-Malki, A. Vejux, Q. Raas, J. de Seze, T. Moreau, M. El-Ayeb, G. Lizard, Evidence of K(+) homeostasis disruption in cellular dysfunction triggered by 7-ketocholesterol, 24S-hydroxycholesterol, and tetracosanoic acid (C24:0) in 158N murine oligodendrocytes, Chem Phys Lipids 207(Pt B) (2017) 135-150.
[144] M. Bezine, S. Maatoug, R. Ben Khalifa, M. Debbabi, A. Zarrouk, Y. Wang, W.J. Griffiths, T. Nury, M. Samadi, A. Vejux, J. de Seze, T. Moreau, R. Kharrat, M. El Ayeb, G. Lizard, Modulation of Kv3.1b potassium channel level and intracellular potassium concentration in 158N murine oligodendrocytes and BV-2 murine microglial cells treated with 7-ketocholesterol, 24S-hydroxycholesterol or tetracosanoic acid (C24:0), Biochimie 153 (2018) 56-69.
[145] M. Bezine, Implication du canal potassium Kv3.1 dans la lipotoxicité du 7-cétocholestérol, 24S-hydroxycholestérol et de l'acide tétracosanoïque sur des cellules nerveuses 158N et BV-2 : Etude des relations entre Kv3.1, homéostasie potassique et métabolisme peroxysomal dans la maladie d'Alzheimer, PhD Thesis, Univ. Bourgogne Franche-Comté & Univ. Tunis El Manar, Tunis, Tunisia (2017).
[146] M.C. Royer, S. Lemaire-Ewing, C. Desrumaux, S. Monier, J.P. Pais de Barros, A. Athias, D. Neel, L. Lagrost, 7-ketocholesterol incorporation into sphingolipid/cholesterol-enriched (lipid raft) domains is impaired by vitamin E: a specific role for alpha-tocopherol with consequences on cell death, J Biol Chem 284(23) (2009) 15826-34.
[147] V.M. Olkkonen, R. Hynynen, Interactions of oxysterols with membranes and proteins, Mol Aspects Med 30(3) (2009) 123-33.
[148] E. Pedruzzi, C. Guichard, V. Ollivier, F. Driss, M. Fay, C. Prunet, J.C. Marie, C. Pouzet, M. Samadi, C. Elbim, Y. O'Dowd, M. Bens, A. Vandewalle, M.A. Gougerot-Pocidalo, G. Lizard, E. Ogier-Denis, NAD(P)H

oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells, Mol Cell Biol 24(24) (2004) 10703-17.
[149] E. Kahn, G. Lizard, F. Frouin, J.C. Bernengo, C. Souchier, G. Bessede, O. Clement, H. Siitari, P. Gambert, G. Frija, A. Todd-Pokropek, Confocal analysis of phosphatidylserine externalization with the use of biotinylated annexin V revealed with streptavidin-FITC, -europium, -phycoerythrin or -Texas Red in oxysterol-treated apoptotic cells, Anal Quant Cytol Histol 23(1) (2001) 47-55.
[150] C. Miguet, S. Monier, A. Bettaieb, A. Athias, G. Bessede, A. Laubriet, S. Lemaire, D. Neel, P. Gambert, G. Lizard, Ceramide generation occurring during 7beta-hydroxycholesterol- and 7-ketocholesterol-induced apoptosis is caspase independent and is not required to trigger cell death, Cell Death Differ 8(1) (2001) 83-99.
[151] C.S. Lee, W.J. Park, E.S. Han, H. Bang, Differential modulation of 7-ketocholesterol toxicity against PC12 cells by calmodulin antagonists and Ca2+ channel blockers, Neurochem Res 32(1) (2007) 87-98.
[152] A. Berthier, S. Lemaire-Ewing, C. Prunet, S. Monier, A. Athias, G. Bessede, J.P. Pais de Barros, A. Laubriet, P. Gambert, G. Lizard, D. Neel, Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis, Cell Death Differ 11(8) (2004) 897-905.
[153] A.E. Rusinol, D. Thewke, J. Liu, N. Freeman, S.R. Panini, M.S. Sinensky, AKT/protein kinase B regulation of BCL family members during oxysterol-induced apoptosis, J Biol Chem 279(2) (2004) 1392-9.
[154] W. Martinet, M. De Bie, D.M. Schrijvers, G.R. De Meyer, A.G. Herman, M.M. Kockx, 7-ketocholesterol induces protein ubiquitination, myelin figure formation, and light chain 3 processing in vascular smooth muscle cells, Arterioscler Thromb Vasc Biol 24(12) (2004) 2296-301.
[155] S. Lemaire-Ewing, C. Prunet, T. Montange, A. Vejux, A. Berthier, G. Bessede, L. Corcos, P. Gambert, D. Neel, G. Lizard, Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols, Cell Biol Toxicol 21(2) (2005) 97-114.
[156] C. Miguet-Alfonsi, C. Prunet, S. Monier, G. Bessede, S. Lemaire-Ewing, A. Berthier, F. Menetrier, D. Neel, P. Gambert, G. Lizard, Analysis of oxidative processes and of myelin figures formation before and after the loss of mitochondrial transmembrane potential during 7beta-hydroxycholesterol and 7-ketocholesterol-induced apoptosis: comparison with various pro-apoptotic chemicals, Biochem Pharmacol 64(3) (2002) 527-41.
[157] Y. Zhang, M. Xu, M. Xia, X. Li, K.M. Boini, M. Wang, E. Gulbins, P.H. Ratz, P.L. Li, Defective autophagosome trafficking contributes to impaired autophagic flux in coronary arterial myocytes lacking CD38 gene, Cardiovasc Res 102(1) (2014) 68-78.
[158] A. Badreddine, A. Zarrouk, E.M. Karym, M. Debbabi, T. Nury, W. Meddeb, R. Sghaier, M. Bezine, A. Vejux, L. Martine, Argan Oil-Mediated Attenuation of Organelle Dysfunction, Oxidative Stress and Cell Death Induced by 7-Ketocholesterol in Murine Oligodendrocytes 158N, International journal of molecular sciences 18(10) (2017) 2220.
[159] M. Debbabi, T. Nury, A. Zarrouk, N. Mekahli, M. Bezine, R. Sghaier, S. Grégoire, L. Martine, P. Durand, E. Camus, Protective effects of α-tocopherol, γ-tocopherol and oleic acid, three compounds of olive oils, and no effect of trolox, on 7-ketocholesterol-induced mitochondrial and peroxisomal dysfunction in microglial BV-2 cells, International journal of molecular sciences 17(12) (2016) 1973.
[160] T. Nury, A. Zarrouk, K. Ragot, M. Debbabi, J.-M. Riedinger, A. Vejux, P. Aubourg, G. Lizard, 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, The Journal of steroid biochemistry and molecular biology 169 (2017) 123-136.
[161] S. Manivannan, C.Q. Scheckhuber, M. Veenhuis, I.J. van der Klei, The impact of peroxisomes on cellular aging and death, Front Oncol 2 (2012) 50.
[162] M. Baarine, P. Andreoletti, A. Athias, T. Nury, A. Zarrouk, K. Ragot, A. Vejux, J.M. Riedinger, Z. Kattan, G. Bessede, D. Trompier, S. Savary, M. Cherkaoui-Malki, G. Lizard, Evidence of oxidative stress in

very long chain fatty acid--treated oligodendrocytes and potentialization of ROS production using RNA interference-directed knockdown of ABCD1 and ACOX1 peroxisomal proteins, Neuroscience 213 (2012) 1-18.
[163] M. Fransen, C. Lismont, Redox Signaling from and to Peroxisomes: Progress, Challenges, and Prospects, Antioxid Redox Signal 30(1) (2019) 95-112.
[164] M. Fransen, C. Lismont, P. Walton, The Peroxisome-Mitochondria Connection: How and Why?, Int J Mol Sci 18(6) (2017).
[165] M. Schrader, S. Grille, H.D. Fahimi, M. Islinger, Peroxisome interactions and cross-talk with other subcellular compartments in animal cells, Subcell Biochem 69 (2013) 1-22.
[166] V. Leoni, T. Nury, A. Vejux, A. Zarrouk, C. Caccia, M. Debbabi, A. Fromont, R. Sghaier, T. Moreau, G. Lizard, Mitochondrial dysfunctions in 7-ketocholesterol-treated 158N oligodendrocytes without or with alpha-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 Steroid Biochem Mol Biol 169 (2017) 96-110.
[167] G. Lizard, S. Lemaire, S. Monier, S. Gueldry, D. Neel, P. Gambert, Induction of apoptosis and of interleukin-1beta secretion by 7beta-hydroxycholesterol and 7-ketocholesterol: partial inhibition by Bcl-2 overexpression, FEBS Lett 419(2-3) (1997) 276-80.
[168] C. Prunet, T. Montange, A. Vejux, A. Laubriet, J.F. Rohmer, J.M. Riedinger, A. Athias, S. Lemaire-Ewing, D. Neel, J.M. Petit, E. Steinmetz, R. Brenot, P. Gambert, G. Lizard, Multiplexed flow cytometric analyses of pro- and anti-inflammatory cytokines in the culture media of oxysterol-treated human monocytic cells and in the sera of atherosclerotic patients, Cytometry A 69(5) (2006) 359-73.
[169] J.D. Huang, J. Amaral, J.W. Lee, I.R. Rodriguez, 7-Ketocholesterol-induced inflammation signals mostly through the TLR4 receptor both in vitro and in vivo, PLoS One 9(7) (2014) e100985.
[170] X. Li, Y. Zhang, M. Xia, E. Gulbins, K.M. Boini, P.L. Li, Activation of Nlrp3 inflammasomes enhances macrophage lipid-deposition and migration: implication of a novel role of inflammasome in atherogenesis, PLoS One 9(1) (2014) e87552.
[171] L. Clarion, M. Schindler, J. de Weille, K. Lolmede, A. Laroche-Clary, E. Uro-Coste, J. Robert, M. Mersel, N. Bakalara, 7beta-Hydroxycholesterol-induced energy stress leads to sequential opposing signaling responses and to death of C6 glioblastoma cells, Biochem Pharmacol 83(1) (2012) 37-46.
[172] R. Sghaier, T. Nury, V. Leoni, C. Caccia, J.P. Pais De Barros, A. Cherif, A. Vejux, T. Moreau, K. Limem, M. Samadi, J.J. Mackrill, A.S. Masmoudi, G. Lizard, A. Zarrouk, Dimethyl fumarate and monomethyl fumarate attenuate oxidative stress and mitochondrial alterations leading to oxiapoptophagy in 158N murine oligodendrocytes treated with 7beta-hydroxycholesterol, J Steroid Biochem Mol Biol 194 (2019) 105432.
[173] D.E. Clapham, Calcium signaling, Cell 131(6) (2007) 1047-58.
[174] E. Millanvoye-Van Brussel, G. Topal, A. Brunet, T. Do Pham, V. Deckert, F. Rendu, M. David-Dufilho, Lysophosphatidylcholine and 7-oxocholesterol modulate Ca2+ signals and inhibit the phosphorylation of endothelial NO synthase and cytosolic phospholipase A2, Biochem J 380(Pt 2) (2004) 533-9.
[175] L. Zhou, M. Shi, Z. Guo, W. Brisbon, R. Hoover, H. Yang, Different cytotoxic injuries induced by lysophosphatidylcholine and 7-ketocholesterol in mouse endothelial cells, Endothelium 13(3) (2006) 213-26.
[176] L. Trevisi, A. Bertoldo, L. Agnoletto, C. Poggiani, F. Cusinato, S. Luciani, Antiapoptotic and proliferative effects of low concentrations of 7beta-hydroxycholesterol in human endothelial cells via ERK activation, J Vasc Res 47(3) (2010) 241-51.
[177] M.P. Ares, M.I. Porn-Ares, S. Moses, J. Thyberg, L. Juntti-Berggren, P. Berggren, A. Hultgardh-Nilsson, B. Kallin, J. Nilsson, 7beta-hydroxycholesterol induces Ca(2+) oscillations, MAP kinase activation and apoptosis in human aortic smooth muscle cells, Atherosclerosis 153(1) (2000) 23-35.

[178] H. Sasaki, F. Watanabe, T. Murano, Y. Miyashita, K. Shirai, Vascular smooth muscle cell apoptosis induced by 7-ketocholesterol was mediated via Ca2+ and inhibited by the calcium channel blocker nifedipine, Metabolism 56(3) (2007) 357-62.
[179] M. Xu, X.X. Li, L. Wang, M. Wang, Y. Zhang, P.L. Li, Contribution of Nrf2 to Atherogenic Phenotype Switching of Coronary Arterial Smooth Muscle Cells Lacking CD38 Gene, Cell Physiol Biochem 37(2) (2015) 432-44.
[180] H.C. Lee, Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization, J Biol Chem 287(38) (2012) 31633-40.
[181] Y. Hammoud, T. Rice, J.J. Mackrill, Oxysterols modulate calcium signalling in the A7r5 aortic smooth muscle cell-line, Biochimie 95(3) (2013) 568-77.
[182] A. Berthier, S. Lemaire-Ewing, C. Prunet, T. Montange, A. Vejux, J.P. Pais de Barros, S. Monier, P. Gambert, G. Lizard, D. Neel, 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-apoptotic calcium-dependent transduction pathways, FEBS J 272(12) (2005) 3093-104.
[183] S. Lemaire-Ewing, A. Berthier, M.C. Royer, E. Logette, L. Corcos, A. Bouchot, S. Monier, C. Prunet, M. Raveneau, C. Rebe, C. Desrumaux, G. Lizard, D. Neel, 7beta-Hydroxycholesterol and 25-hydroxycholesterol-induced interleukin-8 secretion involves a calcium-dependent activation of c-fos via the ERK1/2 signaling pathway in THP-1 cells: oxysterols-induced IL-8 secretion is calcium-dependent, Cell Biol Toxicol 25(2) (2009) 127-39.
[184] S. Lordan, N.M. O'Brien, J.J. Mackrill, The role of calcium in apoptosis induced by 7beta-hydroxycholesterol and cholesterol-5beta,6beta-epoxide, J Biochem Mol Toxicol 23(5) (2009) 324-32.
[185] J.J. Mackrill, Oxysterols and calcium signal transduction, Chem Phys Lipids 164(6) (2011) 488-95.
[186] J.W. Li, Y.L. Xiao, C.F. Lai, N. Lou, H.L. Ma, B.Y. Zhu, W.B. Zhong, D.G. Yan, Oxysterol-binding protein-related protein 4L promotes cell proliferation by sustaining intracellular Ca2+ homeostasis in cervical carcinoma cell lines, Oncotarget 7(40) (2016) 65849-65861.
[187] I. Pulli, T. Lassila, G. Pan, D. Yan, V.M. Olkkonen, K. Tornquist, Oxysterol-binding protein related-proteins (ORPs) 5 and 8 regulate calcium signaling at specific cell compartments, Cell Calcium 72 (2018) 62-69.
[188] C. Wang, L. JeBailey, N.D. Ridgway, Oxysterol-binding-protein (OSBP)-related protein 4 binds 25-hydroxycholesterol and interacts with vimentin intermediate filaments, Biochem J 361(Pt 3) (2002) 461-72.
[189] J. Li, X. Zheng, N. Lou, W. Zhong, D. Yan, Oxysterol binding protein-related protein 8 mediates the cytotoxicity of 25-hydroxycholesterol, J Lipid Res 57(10) (2016) 1845-1853.
[190] P. de Medina, M.R. Paillasse, G. Segala, M. Poirot, S. Silvente-Poirot, Identification and pharmacological characterization of cholesterol-5,6-epoxide hydrolase as a target for tamoxifen and AEBS ligands, Proc Natl Acad Sci U S A 107(30) (2010) 13520-5.
[191] M. Hanner, F.F. Moebius, F. Weber, M. Grabner, J. Striessnig, H. Glossmann, Phenylalkylamine Ca2+ antagonist binding protein. Molecular cloning, tissue distribution, and heterologous expression, J Biol Chem 270(13) (1995) 7551-7.
[192] S. Silvente-Poirot, M. Poirot, Cholesterol epoxide hydrolase and cancer, Curr Opin Pharmacol 12(6) (2012) 696-703.
[193] H. Osawa, H. Ohnishi, K. Takano, T. Noguti, H. Mashima, H. Hoshino, H. Kita, K. Sato, H. Matsui, K. Sugano, Sonic hedgehog stimulates the proliferation of rat gastric mucosal cells through ERK activation by elevating intracellular calcium concentration, Biochem Biophys Res Commun 344(2) (2006) 680-7.
[194] S. Nachtergaele, L.K. Mydock, K. Krishnan, J. Rammohan, P.H. Schlesinger, D.F. Covey, R. Rohatgi, Oxysterols are allosteric activators of the oncoprotein Smoothened, Nat Chem Biol 8(2) (2012) 211-20.
[195] S. Hannedouche, J. Zhang, T. Yi, W. Shen, D. Nguyen, J.P. Pereira, D. Guerini, B.U. Baumgarten, S. Roggo, B. Wen, R. Knochenmuss, S. Noel, F. Gessier, L.M. Kelly, M. Vanek, S. Laurent, I. Preuss, C. Miault, I. Christen, R. Karuna, W. Li, D.I. Koo, T. Suply, C. Schmedt, E.C. Peters, R. Falchetto, A. Katopodis, C.

Spanka, M.O. Roy, M. Detheux, Y.A. Chen, P.G. Schultz, C.Y. Cho, K. Seuwen, J.G. Cyster, A.W. Sailer, Oxysterols direct immune cell migration via EBI2, Nature 475(7357) (2011) 524-7.
[196] K.R. Beck, S. Kanagaratnam, D.V. Kratschmar, J. Birk, H. Yamaguchi, A.W. Sailer, K. Seuwen, A. Odermatt, Enzymatic interconversion of the oxysterols 7beta,25-dihydroxycholesterol and 7-keto,25-hydroxycholesterol by 11beta-hydroxysteroid dehydrogenase type 1 and 2, J Steroid Biochem Mol Biol 190 (2019) 19-28.
[197] N. Das, P. Chandran, Microbial degradation of petroleum hydrocarbon contaminants: an overview, Biotechnol Res Int 2011 (2011) 941810.
[198] J.F. Ferguson, J.M. Pietari, Anaerobic transformations and bioremediation of chlorinated solvents, Environ Pollut 107(2) (2000) 209-15.
[199] V. Singh, M. Jain, A. Misra, V. Khanna, M. Rana, P. Prakash, R. Malasoni, A.K. Dwivedi, M. Dikshit, M.K. Barthwal, Curcuma oil ameliorates hyperlipidaemia and associated deleterious effects in golden Syrian hamsters, Br J Nutr 110(3) (2013) 437-46.
[200] A.D. de Grey, P.J. Alvarez, R.O. Brady, A.M. Cuervo, W.G. Jerome, P.L. McCarty, R.A. Nixon, B.E. Rittmann, J.R. Sparrow, Medical bioremediation: prospects for the application of microbial catabolic diversity to aging and several major age-related diseases, Ageing Res Rev 4(3) (2005) 315-38.
[201] B.E. Rittmann, J. Schloendorn, Engineering away lysosomal junk: medical bioremediation, Rejuvenation Res 10(3) (2007) 359-65.
[202] I.V. Kurochkin, Insulin-degrading enzyme: embarking on amyloid destruction, Trends Biochem Sci 26(7) (2001) 421-5.
[203] H. Zhao, U. Dreses-Werringloer, P. Davies, P. Marambaud, Amyloid-beta peptide degradation in cell cultures by mycoplasma contaminants, BMC Res Notes 1 (2008) 38.
[204] Y. Wu, J. Zhou, N. Fishkin, B.E. Rittmann, J.R. Sparrow, Enzymatic degradation of A2E, a retinal pigment epithelial lipofuscin bisretinoid, J Am Chem Soc 133(4) (2011) 849-57.
[205] S.G. Ramani K, Production of lipase from Pseudomonas gessardii using blood tissue lipid and thereof for the hydrolysis of blood cholesterol and triglycerides and lysis of red blood cells, Bioprocess Biosyst Eng 35(6) (2012) 885-896.
[206] J. Mathieu, J. Schloendorn, B.E. Rittmann, P.J. Alvarez, Microbial degradation of 7-ketocholesterol, Biodegradation 19(6) (2008) 807-13.
[207] J.M. Mathieu, W.W. Mohn, L.D. Eltis, J.C. LeBlanc, G.R. Stewart, C. Dresen, K. Okamoto, P.J. Alvarez, 7-ketocholesterol catabolism by Rhodococcus jostii RHA1, Appl Environ Microbiol 76(1) (2010) 352-5.
[208] S. Ghosh, S.K. Khare, Biodegradation of cytotoxic 7-Ketocholesterol by Pseudomonas aeruginosa PseA, Bioresour Technol 213 (2016) 44-49.
[209] S. Ghosh, S.K. Khare, Biodegradation of 7-Ketocholesterol by Rhodococcus erythropolis MTCC 3951: Process optimization and enzymatic insights, Chem Phys Lipids 207(Pt B) (2017) 253-259.
[210] R.M. Perveen I, Sehar S, Naz I, Memon MI, Ahmed S., Studies on Degradation of 7-ketocholesterol by Environmental Bacterial Isolates, Appl Biochem Microbiol 54(3) (2018) 262-268.
[211] S.S. Perveen I, Naz I, Raza MA, Jahangir A, Biodegradation of 7-ketocholestrol (7-KC) by Thermobifidafusca IP1, Int J Biosci 8(4) (2016) 83-93.
[212] W. Jessup, A.J. Brown, Novel routes for metabolism of 7-ketocholesterol, Rejuvenation Res 8(1) (2005) 9-12.
[213] J.M. Mathieu, F. Wang, L. Segatori, P.J. Alvarez, Increased resistance to oxysterol cytotoxicity in fibroblasts transfected with a lysosomally targeted Chromobacterium oxidase, Biotechnol Bioeng 109(9) (2012) 2409-15.
[214] S. Ghosh, R. Ahmad, V.K. Gautam, S.K. Khare, Cholesterol-oxidase-magnetic nanobioconjugates for the production of 4-cholesten-3-one and 4-cholesten-3, 7-dione, Bioresour Technol 254 (2018) 91-96.

[215] I.A. Machorro-Mendez, A. Hernandez-Mendoza, V. Cardenia, M.T. Rodriguez-Estrada, G. Lercker, F. Spinelli, A. Cellini, H.S. Garcia, Assessment of in vitro removal of cholesterol oxidation products by Lactobacillus casei ATCC334, Lett Appl Microbiol 57(5) (2013) 443-50.
[216] D.J. Peet, B.A. Janowski, D.J. Mangelsdorf, The LXRs: a new class of oxysterol receptors, Curr Opin Genet Dev 8(5) (1998) 571-5.
[217] M. Cariello, S. Ducheix, S. Maqdasy, S. Baron, A. Moschetta, J.A. Lobaccaro, LXRs, SHP, and FXR in Prostate Cancer: Enemies or Menage a Quatre With AR?, Nucl Recept Signal 15 (2018) 1550762918801070.
[218] D.H. Volle, K. Mouzat, R. Duggavathi, B. Siddeek, P. Dechelotte, B. Sion, G. Veyssiere, M. Benahmed, J.M. Lobaccaro, Multiple roles of the nuclear receptors for oxysterols liver X receptor to maintain male fertility, Mol Endocrinol 21(5) (2007) 1014-27.
[219] A. Ouvrier, G. Alves, C. Damon-Soubeyrand, G. Marceau, R. Cadet, L. Janny, F. Brugnon, A. Kocer, A. Pommier, J.M. Lobaccaro, J.R. Drevet, F. Saez, Dietary cholesterol-induced post-testicular infertility, PLoS One 6(11) (2011) e26966.
[220] Q. Wang, S. Wang, Y. Shi, M. Yao, L. Hou, L. Jiang, Reduction of Liver X Receptor beta expression in primary rat neurons by antisense oligodeoxynucleotides decreases secreted amyloid beta levels, Neurosci Lett 561 (2014) 146-50.
[221] D.W. Russell, R.W. Halford, D.M. Ramirez, R. Shah, T. Kotti, Cholesterol 24-hydroxylase: an enzyme of cholesterol turnover in the brain, Annu Rev Biochem 78 (2009) 1017-40.
[222] M.A. Burlot, J. Braudeau, K. Michaelsen-Preusse, B. Potier, S. Ayciriex, J. Varin, B. Gautier, F. Djelti, M. Audrain, L. Dauphinot, F.J. Fernandez-Gomez, R. Caillierez, O. Laprevote, I. Bieche, N. Auzeil, M.C. Potier, P. Dutar, M. Korte, L. Buee, D. Blum, N. Cartier, Cholesterol 24-hydroxylase defect is implicated in memory impairments associated with Alzheimer-like Tau pathology, Hum Mol Genet 24(21) (2015) 5965-76.
[223] F. Djelti, J. Braudeau, E. Hudry, M. Dhenain, J. Varin, I. Bieche, C. Marquer, F. Chali, S. Ayciriex, N. Auzeil, S. Alves, D. Langui, M.C. Potier, O. Laprevote, M. Vidaud, C. Duyckaerts, R. Miles, P. Aubourg, N. Cartier, CYP46A1 inhibition, brain cholesterol accumulation and neurodegeneration pave the way for Alzheimer's disease, Brain 138(Pt 8) (2015) 2383-98.
[224] L. Boussicault, S. Alves, A. Lamaziere, A. Planques, N. Heck, L. Moumne, G. Despres, S. Bolte, A. Hu, C. Pages, L. Galvan, F. Piguet, P. Aubourg, N. Cartier, J. Caboche, S. Betuing, CYP46A1, the rate-limiting enzyme for cholesterol degradation, is neuroprotective in Huntington's disease, Brain 139(Pt 3) (2016) 953-70.
[225] S. Theofilopoulos, W.A. Abreu de Oliveira, S. Yang, E. Yutuc, A. Saeed, J. Abdel-Khalik, A. Ullgren, A. Cedazo-Minguez, I. Bjorkhem, Y. Wang, W.J. Griffiths, E. Arenas, 24(S),25-Epoxycholesterol and cholesterol 24S-hydroxylase (CYP46A1) overexpression promote midbrain dopaminergic neurogenesis in vivo, J Biol Chem 294(11) (2019) 4169-4176.
[226] A.E. Baek, Y.A. Yu, S. He, S.E. Wardell, C.Y. Chang, S. Kwon, R.V. Pillai, H.B. McDowell, J.W. Thompson, L.G. Dubois, P.M. Sullivan, J.K. Kemper, M.D. Gunn, D.P. McDonnell, E.R. Nelson, The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells, Nat Commun 8(1) (2017) 864.
[227] W. Tian, W. Pang, Y. Ge, X. He, D. Wang, X. Li, H. Hou, D. Zhou, S. Feng, Z. Chen, Y. Yang, Hepatocyte-generated 27-hydroxycholesterol promotes the growth of melanoma by activation of estrogen receptor alpha, J Cell Biochem 119(3) (2018) 2929-2938.
[228] O. Weingartner, C. Husche, H.F. Schott, T. Speer, M. Bohm, C.M. Miller, F. McCarthy, J. Plat, D. Lutjohann, U. Laufs, Vascular effects of oxysterols and oxyphytosterols in apoE -/- mice, Atherosclerosis 240(1) (2015) 73-9.

[229] M. Nixon, D.J. Wake, D.E. Livingstone, R.H. Stimson, C.L. Esteves, J.R. Seckl, K.E. Chapman, R. Andrew, B.R. Walker, Salicylate downregulates 11beta-HSD1 expression in adipose tissue in obese mice and in humans, mediating insulin sensitization, Diabetes 61(4) (2012) 790-6.
[230] T. Kipari, P.W. Hadoke, J. Iqbal, T.Y. Man, E. Miller, A.E. Coutinho, Z. Zhang, K.M. Sullivan, T. Mitic, D.E. Livingstone, C. Schrecker, K. Samuel, C.I. White, M.A. Bouhlel, G. Chinetti-Gbaguidi, B. Staels, R. Andrew, B.R. Walker, J.S. Savill, K.E. Chapman, J.R. Seckl, 11beta-hydroxysteroid dehydrogenase type 1 deficiency in bone marrow-derived cells reduces atherosclerosis, FASEB J 27(4) (2013) 1519-31.
[231] R.A. Garcia, D.J. Search, J.A. Lupisella, J. Ostrowski, B. Guan, J. Chen, W.P. Yang, A. Truong, A. He, R. Zhang, M. Yan, S.E. Hellings, P.S. Gargalovic, C.S. Ryan, L.M. Watson, R.A. Langish, P.A. Shipkova, N.L. Carson, J.R. Taylor, R. Yang, G.C. Psaltis, T.W. Harrity, J.A. Robl, D.A. Gordon, 11beta-hydroxysteroid dehydrogenase type 1 gene knockout attenuates atherosclerosis and in vivo foam cell formation in hyperlipidemic apoE(-)/(-) mice, PLoS One 8(2) (2013) e53192.
[232] C.D. Pereira, I. Azevedo, R. Monteiro, M.J. Martins, 11beta-Hydroxysteroid dehydrogenase type 1: relevance of its modulation in the pathophysiology of obesity, the metabolic syndrome and type 2 diabetes mellitus, Diabetes Obes Metab 14(10) (2012) 869-81.
[233] S.P. Hong, D. Han, K.H. Chang, S.K. Ahn, A novel highly potent and selective 11beta-hydroxysteroid dehydrogenase type 1 inhibitor, INU-101, Eur J Pharmacol 835 (2018) 169-178.
[234] L. Xu, K. Mirnics, A.B. Bowman, W. Liu, J. Da, N.A. Porter, Z. Korade, DHCEO accumulation is a critical mediator of pathophysiology in a Smith-Lemli-Opitz syndrome model, Neurobiol Dis 45(3) (2012) 923-9.
[235] Y. Zong, J. Gao, H. Feng, B. Cheng, X. Zhang, Toxicity of 7-ketocholesterol on lethality, growth, reproduction, and germline apoptosis in the nematode Caenorhabditis elegans, J Toxicol Environ Health A 77(12) (2014) 716-23.
[236] D.M. Turner, Carbon monoxide, tobacco smoking, and the pathogenesis of atherosclerosis, Prev Med 8(3) (1979) 303-9.
[237] D.M. Turner, P.N. Lee, F.J. Roe, K.J. Gough, Atherogenesis in the White Carneau pigeon. Further studies of the role of carbon monoxide and dietary cholesterol, Atherosclerosis 34(4) (1979) 407-17.
[238] M.S.-W. Lee JJ, Simultaneous Analysis of Cholesterol Oxidation Products (COPs) in Powdered Milk Using HPLC/UV-Vis, Bull. Korean Chem. Soc 34(9) (2013) 2787-2794.
[239] M.S. Jacobson, M.G. Price, A.E. Shamoo, F.P. Heald, Atherogenesis in white carneau pigeons. Effects of low-level cholestane-triol feeding, Atherosclerosis 57(2-3) (1985) 209-17.
[240] F. Martinello, S.M. Soares, J.J. Franco, A.C. Santos, A. Sugohara, S.B. Garcia, C. Curti, S.A. Uyemura, Hypolipemic and antioxidant activities from Tamarindus indica L. pulp fruit extract in hypercholesterolemic hamsters, Food Chem Toxicol 44(6) (2006) 810-8.
[241] K. Decorde, E. Ventura, D. Lacan, J. Ramos, J.P. Cristol, J.M. Rouanet, An SOD rich melon extract Extramel prevents aortic lipids and liver steatosis in diet-induced model of atherosclerosis, Nutr Metab Cardiovasc Dis 20(5) (2010) 301-7.
[242] J.H. Suh, C. Romain, R. Gonzalez-Barrio, J.P. Cristol, P.L. Teissedre, A. Crozier, J.M. Rouanet, Raspberry juice consumption, oxidative stress and reduction of atherosclerosis risk factors in hypercholesterolemic golden Syrian hamsters, Food Funct 2(7) (2011) 400-5.
[243] T.Y. Tsai, L.Y. Chen, T.M. Pan, Effect of probiotic-fermented, genetically modified soy milk on hypercholesterolemia in hamsters, J Microbiol Immunol Infect 47(1) (2014) 1-8.
[244] M. Jayachandran, B. Chandrasekaran, N. Namasivayam, Geraniol attenuates oxidative stress by Nrf2 activation in diet-induced experimental atherosclerosis, J Basic Clin Physiol Pharmacol 26(4) (2015) 335-46.
[245] A. Meynier, J. Lherminier, J. Demaison-Meloche, C. Ginies, A. Grandgirard, L. Demaison, Effects of dietary oxysterols on coronary arteries in hyperlipidaemic hamsters, Br J Nutr 87(5) (2002) 447-58.

[246] G.M. Favero, J.L. Paz, A.H. Otake, D.A. Maria, E.G. Caldini, R.S.S. de Medeiros, D.F. Deus, R. Chammas, R.C. Maranhao, S.P. Bydlowski, Cell internalization of 7-ketocholesterol-containing nanoemulsion through LDL receptor reduces melanoma growth in vitro and in vivo: a preliminary report, Oncotarget 9(18) (2018) 14160-14174.
[247] Y.H. Ji, C. Moog, G. Schmitt, P. Bischoff, B. Luu, Monophosphoric acid diesters of 7 beta-hydroxycholesterol and of pyrimidine nucleosides as potential antitumor agents: synthesis and preliminary evaluation of antitumor activity, J Med Chem 33(8) (1990) 2264-70.
[248] M. Christ, Y.H. Ji, C. Moog, X. Pannecoucke, G. Schmitt, P. Bischoff, B. Luu, Antitumor activity of oxysterols. Effect of two water-soluble monophosphoric acid diesters of 7 beta-hydroxycholesterol on mastocytoma P815 in vivo, Anticancer Res 11(1) (1991) 359-64.
[249] P.L. Bischoff, V. Holl, D. Coelho, P. Dufour, D. Weltin, B. Luu, Apoptosis at the interface of immunosuppressive and anticancer activities: the examples of two classes of chemical inducers, oxysterols and alkylating agents, Curr Med Chem 7(7) (2000) 693-713.
[250] P. Holy, A. Kloudova, P. Soucek, Importance of genetic background of oxysterol signaling in cancer, Biochimie 153 (2018) 109-138.
[251] R.A. Schweizer, M. Zurcher, Z. Balazs, B. Dick, A. Odermatt, Rapid hepatic metabolism of 7-ketocholesterol by 11beta-hydroxysteroid dehydrogenase type 1: species-specific differences between the rat, human, and hamster enzyme, J Biol Chem 279(18) (2004) 18415-24.
[252] S. Paradis, V. Leoni, C. Caccia, A. Berdeaux, D. Morin, Cardioprotection by the TSPO ligand 4'-chlorodiazepam is associated with inhibition of mitochondrial accumulation of cholesterol at reperfusion, Cardiovasc Res 98(3) (2013) 420-7.
[253] M. Deiana, A. Rosa, G. Corona, A. Atzeri, A. Incani, F. Visioli, M. Paola Melis, M. Assunta Dessi, Protective effect of olive oil minor polar components against oxidative damage in rats treated with ferric-nitrilotriacetate, Food Chem Toxicol 45(12) (2007) 2434-40.
[254] D.M. Villalpando, M.M. Rojas, H.S. Garcia, M. Ferrer, Dietary docosahexaenoic acid supplementation prevents the formation of cholesterol oxidation products in arteries from orchidectomized rats, PLoS One 12(10) (2017) e0185805.
[255] V. Cardenia, M.T. Rodriguez-Estrada, A. Lorenzini, E. Bandini, C. Angeloni, S. Hrelia, M. Malaguti, Effect of broccoli extract enriched diet on liver cholesterol oxidation in rats subjected to exhaustive exercise, J Steroid Biochem Mol Biol 169 (2017) 137-144.
[256] J. Amaral, J.W. Lee, J. Chou, M.M. Campos, I.R. Rodriguez, 7-Ketocholesterol induces inflammation and angiogenesis in vivo: a novel rat model, PLoS One 8(2) (2013) e56099.
[257] L. Xu, L.G. Sheflin, N.A. Porter, S.J. Fliesler, 7-Dehydrocholesterol-derived oxysterols and retinal degeneration in a rat model of Smith-Lemli-Opitz syndrome, Biochim Biophys Acta 1821(6) (2012) 877-83.
[258] S.Y. Loke, K. Tanaka, W.Y. Ong, Comprehensive gene expression analyses of the rat prefrontal cortex after oxysterol treatment, J Neurochem 124(6) (2013) 770-81.
[259] C. Rakotoarivelo, M. Adamczyk, M. Desgeorges, K. Langley, J.G. Lorentz, A. Mann, D. Ricard, E. Scherrer, A. Privat, M. Mersel, 7beta-hydroxycholesterol blocked at C-3-OH inhibits growth of rat glioblastoma in vivo: comparison between 7beta-hydroxycholesteryl-3beta (ester)-oleate and 7beta-hydroxycholesteryl-3-beta-O (ether)-oleyl, Anticancer Res 26(3A) (2006) 2053-62.
[260] I. Bjorkhem, A. Henriksson-Freyschuss, O. Breuer, U. Diczfalusy, L. Berglund, P. Henriksson, The antioxidant butylated hydroxytoluene protects against atherosclerosis, Arterioscler Thromb 11(1) (1991) 15-22.
[261] K. Honda, T. Matoba, Y. Antoku, J.I. Koga, I. Ichi, K. Nakano, H. Tsutsui, K. Egashira, Lipid-Lowering Therapy With Ezetimibe Decreases Spontaneous Atherothrombotic Occlusions in a Rabbit Model of Plaque Erosion: A Role of Serum Oxysterols, Arterioscler Thromb Vasc Biol 38(4) (2018) 757-771.
[262] R.H. Cox, E.Y. Spencer, The Effect of 7-Ketocholesterol on the Rabbit, Science 110(2844) (1949) 11.

[263] J.S. Sarma, R.J. Bing, The inhibitory effect of 7-ketocholesterol on the cholesterol uptake by the arterial wall, J Mol Cell Cardiol 10(2) (1978) 197-204.
[264] R.J. Bing, J.S. Sarma, S.I. Chan, Inhibition of cholesterol uptake by the arterial wall in the intact animal, Artery 5(1) (1979) 14-28.
[265] G. Santillan, J.S. Sarma, G. Pawlik, A. Rackl, A. Grenier, R.J. Bing, Toxicity, pharmacokinetics, and cholesterol-inhibitory effect of 7-ketocholesterol, Atherosclerosis 35(1) (1980) 1-10.
[266] S.K. Peng, R.J. Morin, P. Tham, C.B. Taylor, Effects of oxygenated derivatives of cholesterol on cholesterol uptake by cultured aortic smooth muscle cells, Artery 13(3) (1985) 144-64.
[267] V. Deckert, L. Persegol, L. Viens, G. Lizard, A. Athias, C. Lallemant, P. Gambert, L. Lagrost, Inhibitors of arterial relaxation among components of human oxidized low-density lipoproteins. Cholesterol derivatives oxidized in position 7 are potent inhibitors of endothelium-dependent relaxation, Circulation 95(3) (1997) 723-31.
[268] M. Shimabukuro, C. Okawa, H. Yamada, S. Yanagi, E. Uematsu, N. Sugasawa, H. Kurobe, Y. Hirata, J.R. Kim-Kaneyama, X.F. Lei, S. Takao, Y. Tanaka, D. Fukuda, S. Yagi, T. Soeki, T. Kitagawa, H. Masuzaki, M. Sato, M. Sata, The pathophysiological role of oxidized cholesterols in epicardial fat accumulation and cardiac dysfunction: a study in swine fed a high caloric diet with an inhibitor of intestinal cholesterol absorption, ezetimibe, J Nutr Biochem 35 (2016) 66-73.
[269] J.R. Guyton, M.L. Lenz, B. Mathews, H. Hughes, D. Karsan, E. Selinger, C.V. Smith, Toxicity of oxidized low density lipoproteins for vascular smooth muscle cells and partial protection by antioxidants, Atherosclerosis 118(2) (1995) 237-49.
[270] O.V. Klimenko, V. Vobruba, P. Martasek, Influence of the lung mechanical ventilation with injurious parameters on 7-ketocholesterol synthesis in Sus Scrofa, BMB Rep 43(4) (2010) 257-62.
[271] A. Stomby, R. Andrew, B.R. Walker, T. Olsson, Tissue-specific dysregulation of cortisol regeneration by 11betaHSD1 in obesity: has it promised too much?, Diabetologia 57(6) (2014) 1100-10.
[272] A. Zarrouk, M. Debbabi, M. Bezine, E.M. Karym, A. Badreddine, O. Rouaud, T. Moreau, M. Cherkaoui-Malki, M. El Ayeb, B. Nasser, M. Hammami, G. Lizard, Lipid Biomarkers in Alzheimer's Disease, Curr Alzheimer Res 15(4) (2018) 303-312.
[273] E. Baum, W. Zhang, S. Li, Z. Cai, D. Holden, Y. Huang, A Novel (18)F-Labeled Radioligand for Positron Emission Tomography Imaging of 11beta-Hydroxysteroid Dehydrogenase (11beta-HSD1): Synthesis and Preliminary Evaluation in Nonhuman Primates, ACS Chem Neurosci 10(5) (2019) 2450-2458.
[274] H. Kimura, Y. Sakai, T. Fujii, Organ/body-on-a-chip based on microfluidic technology for drug discovery, Drug Metab Pharmacokinet 33(1) (2018) 43-48.
[275] T. Sato, R.G. Vries, H.J. Snippert, M. van de Wetering, N. Barker, D.E. Stange, J.H. van Es, A. Abo, P. Kujala, P.J. Peters, H. Clevers, Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, Nature 459(7244) (2009) 262-5.
[276] M.A. Lancaster, M. Renner, C.A. Martin, D. Wenzel, L.S. Bicknell, M.E. Hurles, T. Homfray, J.M. Penninger, A.P. Jackson, J.A. Knoblich, Cerebral organoids model human brain development and microcephaly, Nature 501(7467) (2013) 373-9.
[277] G. Quadrato, T. Nguyen, E.Z. Macosko, J.L. Sherwood, S. Min Yang, D.R. Berger, N. Maria, J. Scholvin, M. Goldman, J.P. Kinney, E.S. Boyden, J.W. Lichtman, Z.M. Williams, S.A. McCarroll, P. Arlotta, Cell diversity and network dynamics in photosensitive human brain organoids, Nature 545(7652) (2017) 48-53.
[278] M. Volkner, M. Zschatzsch, M. Rostovskaya, R.W. Overall, V. Busskamp, K. Anastassiadis, M.O. Karl, Retinal Organoids from Pluripotent Stem Cells Efficiently Recapitulate Retinogenesis, Stem Cell Reports 6(4) (2016) 525-538.
[279] L. Broutier, A. Andersson-Rolf, C.J. Hindley, S.F. Boj, H. Clevers, B.K. Koo, M. Huch, Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation, Nat Protoc 11(9) (2016) 1724-43.

[280] M. Crespo, E. Vilar, S.Y. Tsai, K. Chang, S. Amin, T. Srinivasan, T. Zhang, N.H. Pipalia, H.J. Chen, M. Witherspoon, M. Gordillo, J.Z. Xiang, F.R. Maxfield, S. Lipkin, T. Evans, S. Chen, Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing, Nat Med 23(7) (2017) 878-884.
[281] M. Karimi, S. Bahrami, H. Mirshekari, S.M. Basri, A.B. Nik, A.R. Aref, M. Akbari, M.R. Hamblin, Microfluidic systems for stem cell-based neural tissue engineering, Lab Chip 16(14) (2016) 2551-71.
[282] D. Park, J. Lim, J.Y. Park, S.H. Lee, Concise Review: Stem Cell Microenvironment on a Chip: Current Technologies for Tissue Engineering and Stem Cell Biology, Stem Cells Transl Med 4(11) (2015) 1352-68.
[283] V. van Duinen, S.J. Trietsch, J. Joore, P. Vulto, T. Hankemeier, Microfluidic 3D cell culture: from tools to tissue models, Curr Opin Biotechnol 35 (2015) 118-26.
[284] G. Wang, M.L. McCain, L. Yang, A. He, F.S. Pasqualini, A. Agarwal, H. Yuan, D. Jiang, D. Zhang, L. Zangi, J. Geva, A.E. Roberts, Q. Ma, J. Ding, J. Chen, D.Z. Wang, K. Li, J. Wang, R.J. Wanders, W. Kulik, F.M. Vaz, M.A. Laflamme, C.E. Murry, K.R. Chien, R.I. Kelley, G.M. Church, K.K. Parker, W.T. Pu, Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies, Nat Med 20(6) (2014) 616-23.
[285] J. Ribas, H. Sadeghi, A. Manbachi, J. Leijten, K. Brinegar, Y.S. Zhang, L. Ferreira, A. Khademhosseini, Cardiovascular Organ-on-a-Chip Platforms for Drug Discovery and Development, Appl In Vitro Toxicol 2(2) (2016) 82-96.
[286] B. Miccoli, D. Braeken, Y.E. Li, Brain-on-a-chip Devices for Drug Screening and Disease Modeling Applications, Curr Pharm Des 24(45) (2018) 5419-5436.
[287] J. Park, I. Wetzel, I. Marriott, D. Dreau, C. D'Avanzo, D.Y. Kim, R.E. Tanzi, H. Cho, A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease, Nat Neurosci 21(7) (2018) 941-951.
[288] Y.W. Wang, L.; Guo, Y.; Zhu, Y.; Qin, J., Engineering stem cell-derived 3D brain organoids in a perfusable organ-on-a-chip system, RSC Adv 8 (2018) 1677-1685.
[289] A. Geraili, P. Jafari, M.S. Hassani, B.H. Araghi, M.H. Mohammadi, A.M. Ghafari, S.H. Tamrin, H.P. Modarres, A.R. Kolahchi, S. Ahadian, A. Sanati-Nezhad, Controlling Differentiation of Stem Cells for Developing Personalized Organ-on-Chip Platforms, Adv Healthc Mater 7(2) (2018).
[290] U. Marx, T.B. Andersson, A. Bahinski, M. Beilmann, S. Beken, F.R. Cassee, M. Cirit, M. Daneshian, S. Fitzpatrick, O. Frey, C. Gaertner, C. Giese, L. Griffith, T. Hartung, M.B. Heringa, J. Hoeng, W.H. de Jong, H. Kojima, J. Kuehnl, M. Leist, A. Luch, I. Maschmeyer, D. Sakharov, A.J. Sips, T. Steger-Hartmann, D.A. Tagle, A. Tonevitsky, T. Tralau, S. Tsyb, A. van de Stolpe, R. Vandebriel, P. Vulto, J. Wang, J. Wiest, M. Rodenburg, A. Roth, Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing, ALTEX 33(3) (2016) 272-321.
[291] J. Zhang, X. Wei, R. Zeng, F. Xu, X. Li, Stem cell culture and differentiation in microfluidic devices toward organ-on-a-chip, Future Sci OA 3(2) (2017) FSO187.
[292] W.J. Griffiths, Y. Wang, Oxysterol research: a brief review, Biochem Soc Trans 47(2) (2019) 517-526.

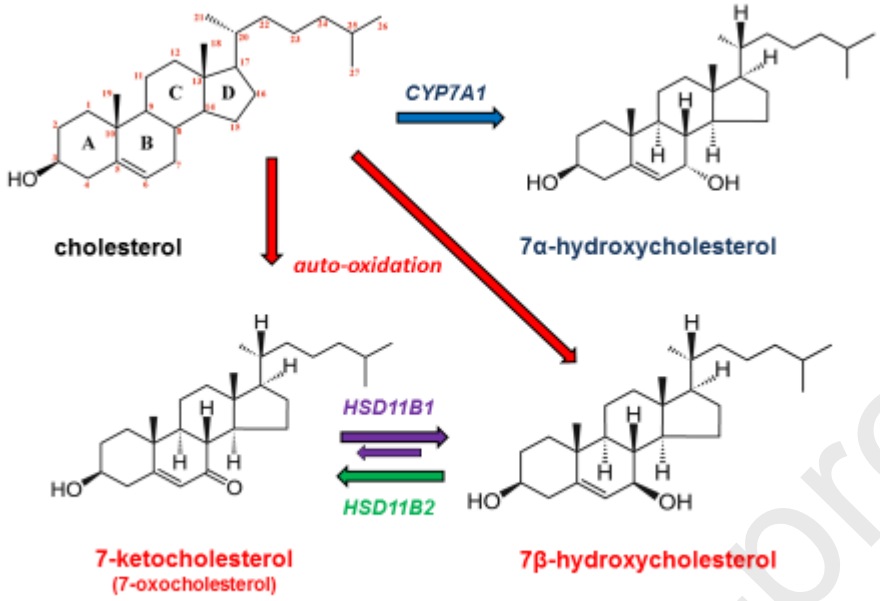


Figure 1 – Vejux et al.

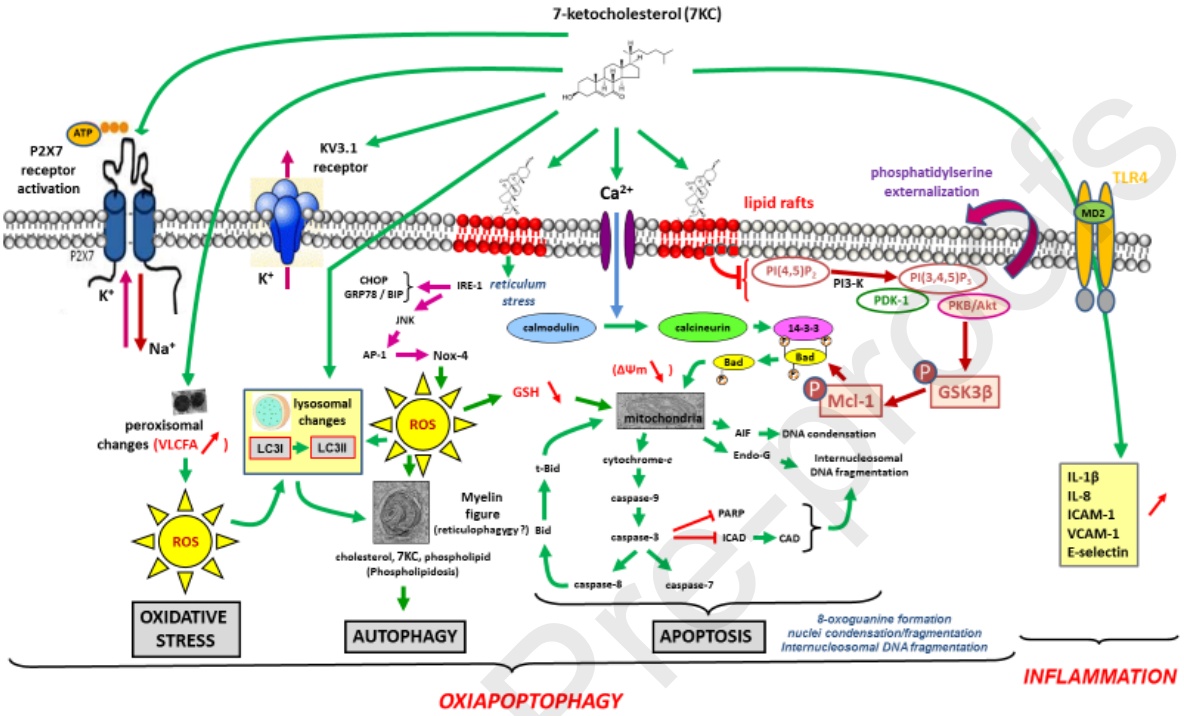


Figure 2 – Vejux et al.

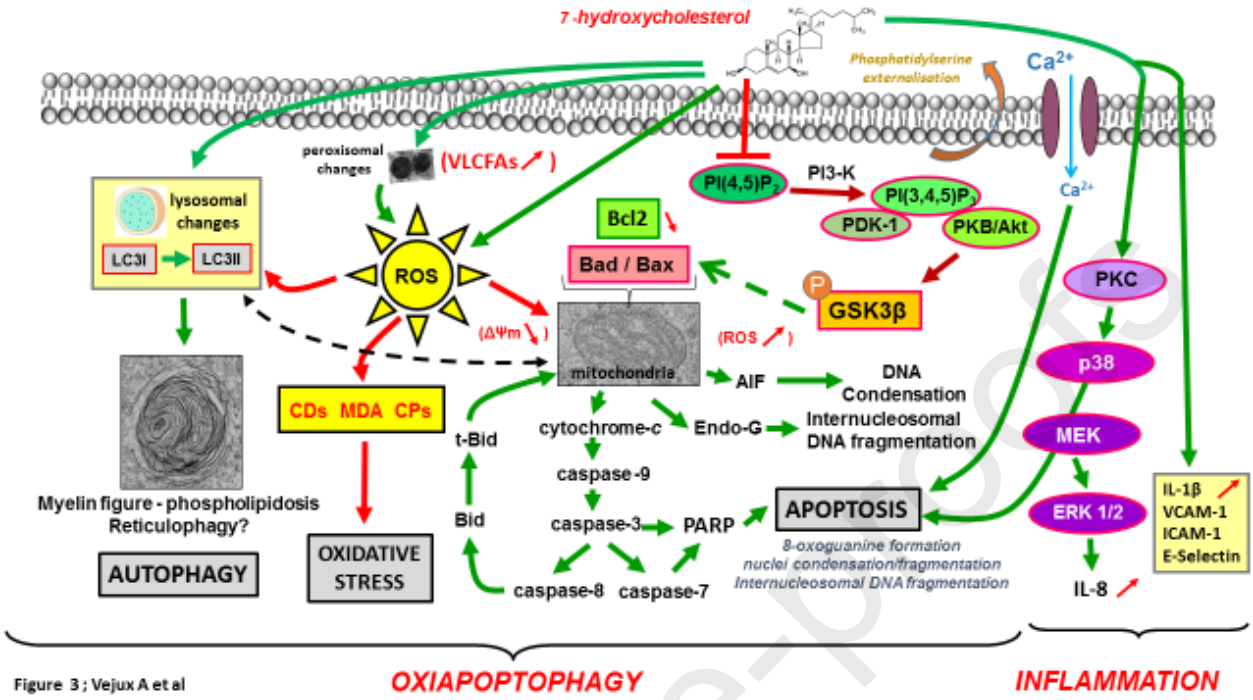
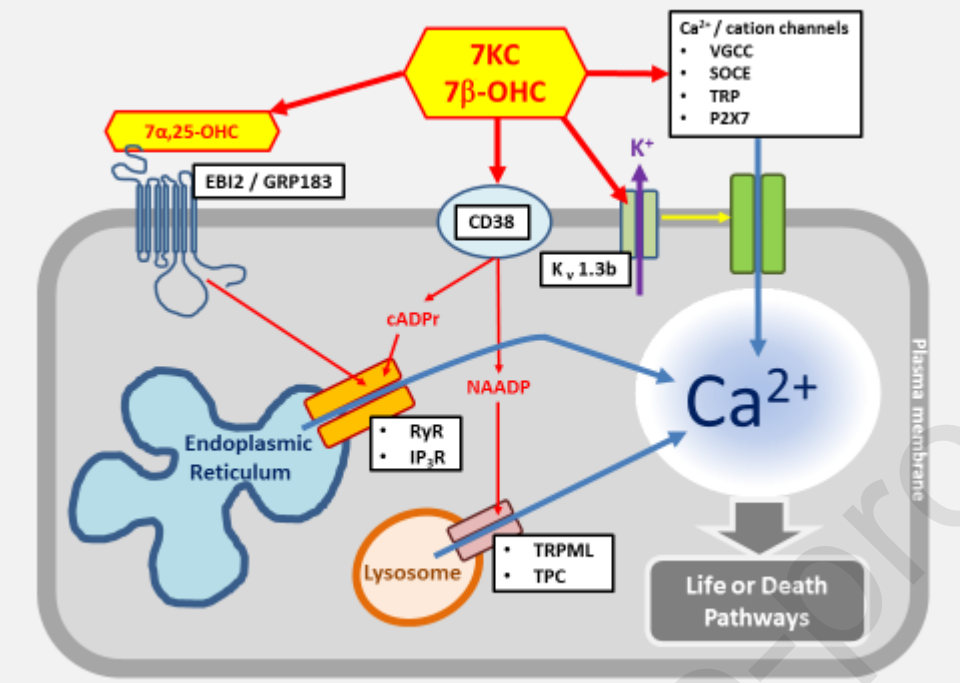


Figure 3 ; Vejux A et al



Vejux et al Figure 4

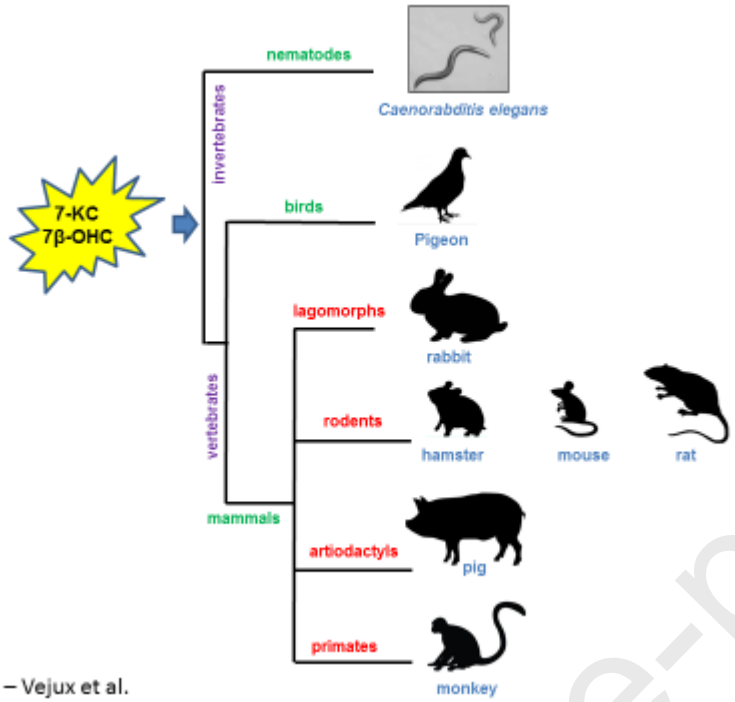


Figure 5 – Vejux et al.