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REVIEW

Sources of 7-ketocholesterol, metabolism and inactivation strategies: food and biomedical applications

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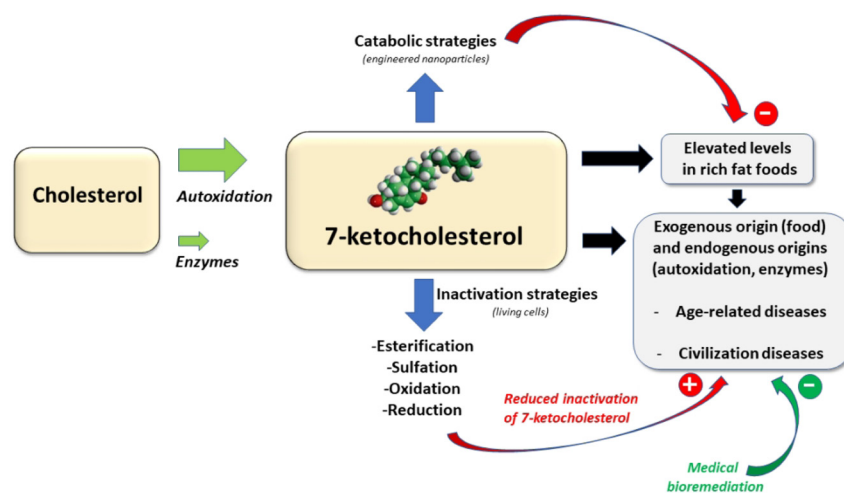
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Graphical abstract



Key Words

- ▶ age-related diseases
- ▶ bioremediation
- ▶ 7-ketocholesterol
- ▶ metabolism
- ▶ nutrition

Abstract

7-Ketocholesterol (or 7-oxocholesterol) is an oxysterol essentially formed by cholesterol autoxidation. It is often found at enhanced levels in the body fluids and/or target tissues of patients with age-related diseases (cardiovascular, neuronal, and ocular diseases) as well as in subjects concerned with civilization diseases (type 2 diabetes, bowel diseases, and metabolic syndrome). The involvement of increased 7-ketocholesterol levels in the pathophysiology of these diseases is widely suspected. Indeed, 7-ketocholesterol at elevated concentrations is a powerful inducer of oxidative stress, inflammation, and cellular degeneration which are common features of all these diseases. It is important to better know the origin of 7-ketocholesterol (diet, incidence of environmental factors, and endogenous formation (autoxidation and enzymatic synthesis)) and its inactivation mechanisms which include esterification, sulfation, oxidation, and reduction. This knowledge will make it possible to act at different levels to regulate 7-ketocholesterol level and counteract its toxicity in order to limit the incidence of diseases associated with this oxysterol. These different points as well as food and biomedical applications are addressed in this review.

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Introduction

7-ketocholesterol (7KC; $C_{27}H_{44}O_2$; PubChem CID 91474; also named 7-oxocholesterol) is a lipid molecule. It is a cholesterol oxide derivative (oxysterol) essentially resulting from the autoxidation of cholesterol which is the most abundant member of a family of polycyclic compounds known as sterols. 7KC was first identified in large quantities in oxidized low-density lipoproteins (LDLox) and in atherosclerotic plaques (Brown & Jessup 1999, Vejux & Lizard 2009). It is also present in high quantities in the retina of patients with age-related macular degeneration (AMD) (Rodríguez & Larrayoz 2010) and in the cortex of Alzheimer's patients (Testa *et al.* 2016). The contribution of 7KC to Alzheimer's disease is well documented (Mahalakshmi *et al.* 2021). 7KC is also increased in the plasma of sarcopenic patients (Ghzaiei *et al.* 2021b). 7KC is also elevated in plasma and tissues of patients with rare diseases such as Smith-Lemli-Opitz syndrome (SLO) (PMID: 26976653), Nieman Pick disease type C (PMID: 29626102), and type B (PMID: 31009661), as well as in patients with severe forms of X-linked adrenoleukodystrophy (X-ALD, PMID: 300100) (Nury *et al.* 2017). *In vitro*, 7KC induces oxidative stress, as well as cytokinetic and non-cytokinetic inflammation, often leading to an apoptotic mode of cell death associated with autophagy criteria. The type of cell death frequently induced by 7KC, as well as by other cytotoxic oxysterols (7 β -hydroxycholesterol, 24S-hydroxycholesterol, 25-hydroxycholesterol, 5,6 epoxycholesterol isomers), is defined as oxiaoptophagy (Nury *et al.* 2021b) and has been observed on different cell types: human monocytic U397

cells (Monier *et al.* 2003), human myeloma cells (Jaouadi *et al.* 2021), nerve cells (158N oligodendrocytes (Nury *et al.* 2014, 2015), murine microglial BV-2 cells (Nury *et al.* 2017), murine neuronal N2a-cells (Yammine *et al.* 2020), human bone marrow mesenchymal stem cells (Paz *et al.* 2019), and L929 mouse fibroblast cells (You *et al.* 2021). Currently, due to the ability of 7KC to trigger cytotoxic activities (oxidative stress, inflammation, cell death induction) characterizing frequent age-related diseases (cardiovascular, ocular, and neurodegenerative diseases), the involvement of this oxysterol in the pathophysiology of these illnesses is well accepted (Zarrouk *et al.* 2014, Samadi *et al.* 2021). Consequently, to efficiently treat these highly disabling diseases which have a high cost for society, a better knowledge of the production and inactivation of 7KC is required. However, if 7KC is mainly studied for its cytotoxic activities, this should not lead to ignore that this molecule could also have beneficial effects on the immune response, control of infectious diseases, and cell proliferation and differentiation (Lembo *et al.* 2016, de Freitas *et al.* 2021, Ghzaiei *et al.* 2021a).

Cholesterol and related sterols autoxidation: formation of 7-ketocholesterol

Cholesterol autoxidation falls within the lipid peroxidation field. Cholesterol oxide derivatives (also named oxysterols) are 27 carbons molecules formed by the addition of oxygen to the cholesterol molecule (an overview of oxysterols

formation is provided on the LipidWeb website: https://lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/chol-der/index.htm; May 2022). This addition of oxygen to cholesterol can be achieved by non-enzymatic and/or enzymatic reactions (Mutemberezi *et al.* 2016a, Brown *et al.* 2021). Electrochemical oxidation of cholesterol-generating numerous oxysterols, including 7KC, in a short time has also been described (Weber *et al.* 2016). The term autoxidation refers to non-enzymatic oxidation which can be considered as a part of chemical reactions which contribute to produce cholesterol derivatives (Morzycki 2014). For cholesterol, a distinction is made between type I autoxidation induced by reactive oxygen species (ROS such as superoxide anion ($O_2^{\cdot-}$) and hydroxyl anion ($HO\cdot$)), reactive nitrogen species (RNS such as nitric oxide (NO) and peroxyxynitrite ONOO-), Fenton reaction ($H_2O_2 + Me^{n+} \rightarrow HO\cdot + OH^- + Me^{(n+1)+}$ where Me is a transition metal such as copper, iron, or aluminium) or Haber-Weiss reaction ($O_2^{\cdot-} + H_2O_2 \rightarrow HO\cdot + HO^- + O_2$), and type II autoxidation induced by ozone (O_3), hypochlorite (HOCl), and singlet oxygen ($^1\Delta gO_2$) (Iuliano 2011). The preferred site for cholesterol autoxidation is at carbon 7 where the carbon-hydrogen bond is weak (Iuliano 2011). The three oxysterols,

7-ketocholesterol (7KC), 7 β -hydroxycholesterol (7 β -OHC), and 7 α -hydroxycholesterol (7 α -OHC), are mainly formed by type I autoxidation and 7KC is the most abundant (Anderson *et al.* 2020, Nury *et al.* 2021a) (Fig. 1A). Hydroperoxycholesterol C4, C5, and C6 are also produced by type I autoxidation and give 4 α -hydroxycholesterol (4 α -OHC), 5 α - or 5 β -hydroxycholesterol (5 α -OHC or 5 β -OHC: 5 α/β -hydroxycholesterol), and 6 α - or 6 β -hydroxycholesterol (6 α -OHC or 6 β -OHC: 6 α/β -hydroxycholesterol), respectively (Zerbinati & Iuliano 2017) (Fig. 1B). 4 β -hydroxycholesterol (4 β -OHC) is enzymatically formed by CYP3A4 and CYP3A5 in humans (Diczfalusy *et al.* 2011, Mutemberezi *et al.* 2016a). As for cholesterol epoxides (5 $\alpha,6\alpha$ -epoxycholesterol and 5 $\beta,6\beta$ -epoxycholesterol), which are stable molecules (Paillasse *et al.* 2012), they can be formed either by type I autoxidation or type II autoxidation (with O_3) and can give cholestane 3 $\beta,5\alpha,6\beta$ -triol (cholestane-triol) (Noguer *et al.* 2017) which can also be enzymatically formed from 5 $\alpha,6\alpha$ -epoxycholesterol by the cholesterol epoxide hydrolase (Ch-EH) (Iuliano 2011, Silvente-Poirot & Poirot 2012, Zerbinati & Iuliano 2017) (Fig. 1B). Noteworthy, whereas the reactions on the cholesterol side chain are mostly enzymatic reactions, 25-hydroxycholesterol

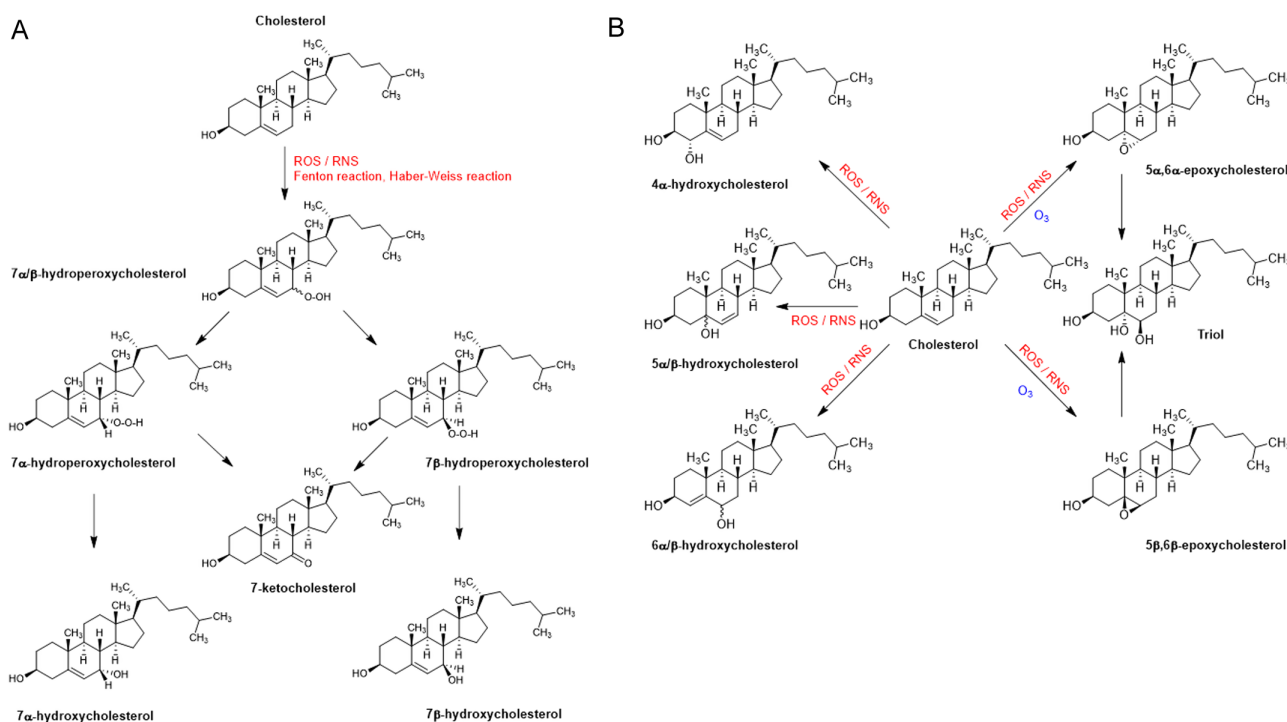


Figure 1

Production of oxysterols, including 7-ketocholesterol, by cholesterol autoxidation. (A) 7-Ketocholesterol can be formed by type I autoxidation as well as Fenton and Haber-Weiss reactions. (B) 4 α -Hydroxycholesterol, 5 α (or 5 β)-hydroxycholesterol (5 α/β -hydroxycholesterol), and 6 α (or 6 β)-hydroxycholesterol (6 α/β -hydroxycholesterol) are formed by cholesterol autoxidation involving type I autoxidation (ROS/RNS), whereas 5 $\alpha,6\alpha$ -epoxycholesterol and 5 $\beta,6\beta$ -epoxycholesterol can be formed by type I autoxidation (ROS/RNS) and type II autoxidation (Ozone: O_3). Triol is formed from 5 $\alpha,6\alpha$ epoxycholesterol or 5 $\beta,6\beta$ epoxycholesterol in an acidic environment.

(25-OHC) can be formed both by autoxidation as well as enzymatically by the enzyme 25-hydroxylase ($\text{cholesterol} + \text{AH}_2 + \text{O}_2 \leftrightarrow \text{25-hydroxycholesterol} + \text{A} + \text{H}_2\text{O}$) (Lund *et al.* 1998).

Exogenous and endogenous sources of 7-ketocholesterol

Dietary origin of 7-ketocholesterol

Oxysterols formed by autoxidation, such as 7KC, are often present in high amounts in manufactured food products (Yan 1999, Rodriguez-Estrada *et al.* 2014, Poli *et al.* 2022). With the following oxysterols (7 α -hydroxycholesterol (7 α -OHC), 7 β -hydroxycholesterol (7 β -OHC), 5 α ,6 α -epoxycholesterol (5 α ,6 α -EPOC), 5 β ,6 β -epoxycholesterol (5 β ,6 β -EPOC), cholestane-3 β ,5 α ,6 β -triol (cholestane-triol), and 25-hydroxycholesterol (25-OHC)), 7KC is one of the oxysterols present in significant quantities in food products of animal origin (Maraschiello *et al.* 1998, Petróñ *et al.* 2003, Canzoneri *et al.* 2022). While 7KC is metabolized by the liver (Lyons *et al.* 1999), the lack of regulation on the content of cytotoxic oxysterols mainly formed by autoxidation (7KC, 7 β -OHC, 5 β ,6 β -EPOC, cholestane-triol) and present in manufactured food products constitutes a real public health problem when they are consumed regularly and in large quantities. Thus, 7KC is abundant in industrial foods associated with a complex manufacturing process involving raw materials where cholesterol is present in large quantities (butter, cream, eggs, meat, milk, milk chocolates) (Clariana & García-Regueiro 2011, Risso *et al.* 2021, 2022). In addition, during the industrial processes, high heating steps and exposure to air favour cholesterol autoxidation (Sabolová *et al.* 2017). Prolonged storage of butter, pastries, shellfish, and meat also promotes the formation of 7KC (Nielsen *et al.* 1996, Lee *et al.* 2001, Mazalli & Bragagnolo 2007, Hernández Becerra *et al.* 2014). In traditional cuisine, the way of cooking can also lead to more or less important 7KC formation (Echarte *et al.* 2005, Lee *et al.* 2006). It is also important to highlight that several oxysterols, including 7KC, have been identified in high quantities in baby's and children's foods (Sander *et al.* 1989, Kilvington *et al.* 2021). Some data support the serious consequences of oxysterols on the intellectual and physical development of children (Kilvington *et al.* 2021) and underline that they could promote metabolic syndrome and obesity (Guillemot-Legrís *et al.* 2016, Mutemberezi *et al.* 2016b), as well as inflammatory bowel disease (Guina *et al.* 2015). Therefore, in the food industry, it seems important to take appropriate

measures to control and limit oxysterol levels in food. In a first step, oxysterols profile (realized by HPLC-mass spectrometry or gas chromatography-mass spectrometry) could be implemented as a guarantee of quality, safety, and nutritional value in the selection of ingredients but also during processing and storage (van de Bovenkamp *et al.* 1988, Razzazi-Fazeli *et al.* 2000, Gorassini *et al.* 2017). In addition, some oxysterols present in the digestive tract, such as 7KC, can also come from cholesterol-rich foods and can be the consequence of digestion in the stomach. Indeed, the acidic pH of the latter, and the presence of free iron and heme proteins, such as myoglobin provided by red meats, make it a very pro-oxidant medium which can favour cholesterol autoxidation (Kanner & Lapidot 2001, Lapidot *et al.* 2005). It is suggested that the presence of 7KC in the stomach could destabilize the gastric epithelial cell barrier (Gajewski *et al.* 2016).

Metabolic origin of 7-ketocholesterol

The production of 7KC by type I autoxidation is the most frequent and it is well established in several age-related diseases (Zarrouk *et al.* 2014, Zerbinati & Iuliano 2017, Nury *et al.* 2021a). However, 7KC can also be obtained enzymatically (Fig. 2). Thus, 7KC can also be formed enzymatically from 7 β -OHC by the hydroxysteroid dehydrogenase type 2 (11 β -HSD2; HSD11B2 gene, OMIM 614232) which is mainly expressed in the kidney, colon, and placenta (Ferrari 2010). In addition, in patients with cerebrotendinous xanthomatosis or with SLO syndrome, 7KC can be formed from 7-dehydrocholesterol (7DHC, a direct precursor in cholesterol biosynthesis belonging to the Kandutsch–Russel pathway (Petrov *et al.* 2016)) by the enzyme cholesterol-7 α -hydroxylase (CYP7A1) (Liu *et al.* 2013, Björkhem *et al.* 2014).

Age-related diseases and civilization diseases associated with 7-ketocholesterol

7KC is greatly increased in the tissues (vascular wall, retina, and certain brain regions) of patients with cardiovascular diseases, AMD, Alzheimer's disease, and Parkinson's disease (Zarrouk *et al.* 2014, Pariente *et al.* 2019), and most likely in X-ALD which is also associated with important oxidative stress (Deon *et al.* 2016, Nury *et al.* 2017). It is now well accepted that 7KC, which triggers oxidative stress and inflammation leading to numerous cell damages, plays key roles in the pathophysiology and in the outcome of

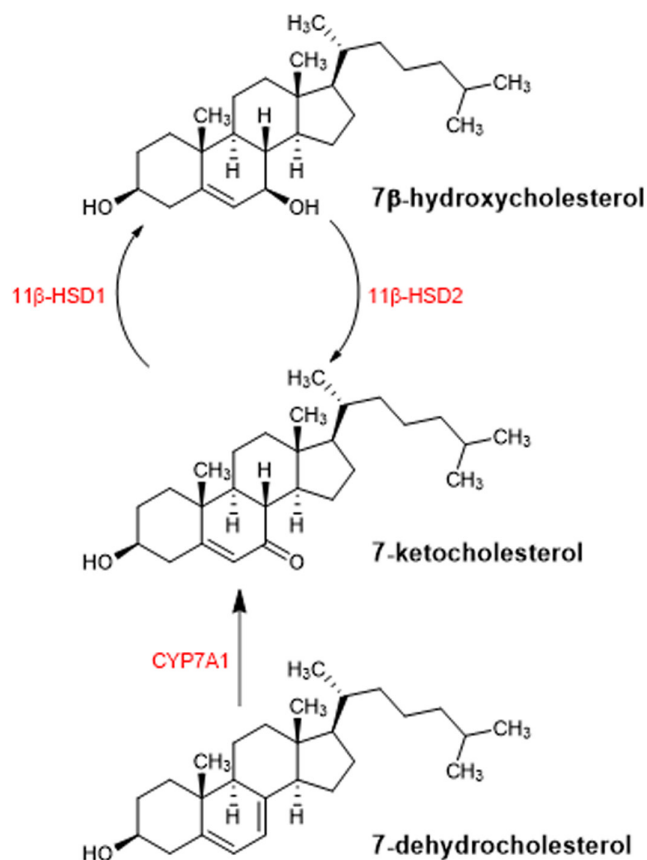


Figure 2

Enzymatic synthesis of 7-ketocholesterol. 7-ketocholesterol can be formed from 7 β -hydroxycholesterol by the 11 β hydroxysteroid dehydrogenase type 2 (11 β -HSD2) and from 7-dehydrocholesterol by cholesterol-7 α -hydroxylase (CYP7A1). 7-Ketocholesterol can also give 7 β -hydroxycholesterol via the 11 β hydroxysteroid dehydrogenase type 1 (11 β -HSD1).

age-related diseases (Samadi *et al.* 2021). Several data also support that 7KC can contribute to ageing (de Medina *et al.* 2022) as well as civilization diseases such as diabetes type 2 (Endo *et al.* 2008, Samadi *et al.* 2019), bowel diseases (Poli *et al.* 2013), metabolic syndrome, and obesity (Murdolo *et al.* 2016) resulting from life habits (diet, physical activity), environmental pollution (air pollution, endocrine disrupters, and obesogens), and neuroemotional pollution (chronic stress). Currently, different types of relationships have been found between environmental pollution that can promote age-related and civilization diseases and 7KC. Thus, it has been reported that 7KC accumulates in vessels as a result of the stress caused by air pollution (Rao *et al.* 2014), is an endogenous modulator for the aryl hydrocarbon receptor which interacts with dioxin (Savouret *et al.* 2001), contributes to cigarette smoke side effects (Steffen *et al.* 2012), could favour asthma (Zanjani *et al.* 2022) as well as silicosis (Aksu *et al.* 2020), and increases the toxicity of nanoparticles (Kahn *et al.* 2010). In civilization diseases, it

is suggested that 7KC, when present at increased levels, may act as an adipokine modulating the adipogenic potential of undifferentiated adipose precursor cells (Murdolo *et al.* 2016). There are also evidences that dietary 7KC accelerates hepatic steatosis and inflammation in obese mice models (Chang *et al.* 2020). Currently, several studies also support that 7KC acts on adipogenic differentiation factor (de Freitas *et al.* 2021). In the context of osteoporosis, it has also been reported that 7KC induces miR107-5p which promotes the differentiation of osteoclasts by downregulating mitogen-activated protein kinase 1 (MKP1) (Li *et al.* 2022).

Characteristics of 7-ketocholesterol-induced cytotoxic effects

7KC has been shown to accumulate in lipid rafts (Royer *et al.* 2009, Ragot *et al.* 2013). 7KC disrupts plasma membrane organization (Kahn *et al.* 2011, Wnętrzak *et al.* 2022) and enhances plasma membrane rigidity (Olkkonen & Hynynen 2009, Vejux *et al.* 2009), as well as permeability (Vejux *et al.* 2020, Nury *et al.* 2021a). 7KC is also a strong inducer of oxidative stress and triggers organelles dysfunctions (mitochondria, peroxisome, lysosome, endoplasmic reticulum), inflammation, and cell death (Nury *et al.* 2021a) (Fig. 3). 7KC activates cell death on different cell types (primary cultures, cell lines) of different species in a concentration range of 25–50 μ M after 24–48 h of culture. 7KC-induced cell death can be either a mode of cell death by apoptosis (caspases-dependent cell death) or a caspases-independent cell death process (Fig. 4). 7KC-induced apoptosis has been described on vascular wall cells, monocytes/macrophages, and nerve cells (neurons, glial cells (oligodendrocytes), and microglial cells). On human monocytic THP-1 cells, 7KC-induced cell death is associated with a sustained increase of Ca²⁺ which elicits the mitochondrial pathway of apoptosis (Berthier *et al.* 2004). A non-apoptotic mode of cell death, without caspases activation, has been described on human fibroblasts, MCF-7 mammary tumor cells, which are deficient in caspase-3, as well as on C6 rat glioblastoma cells (Vejux *et al.* 2020), and in some cases on ARPE-19, a human retinal pigment epithelial cell lines with differentiated properties (Dugas *et al.* 2010). A caspase-independent cell death has also been observed on C2C12 murine myoblasts (Ghzaiei *et al.* 2021b). In all the cases, whether it is an apoptotic mode of cell death or a caspases-independent cell death process, significant mitochondrial and peroxisomal alterations have been found. Thus, topographical and morphological changes

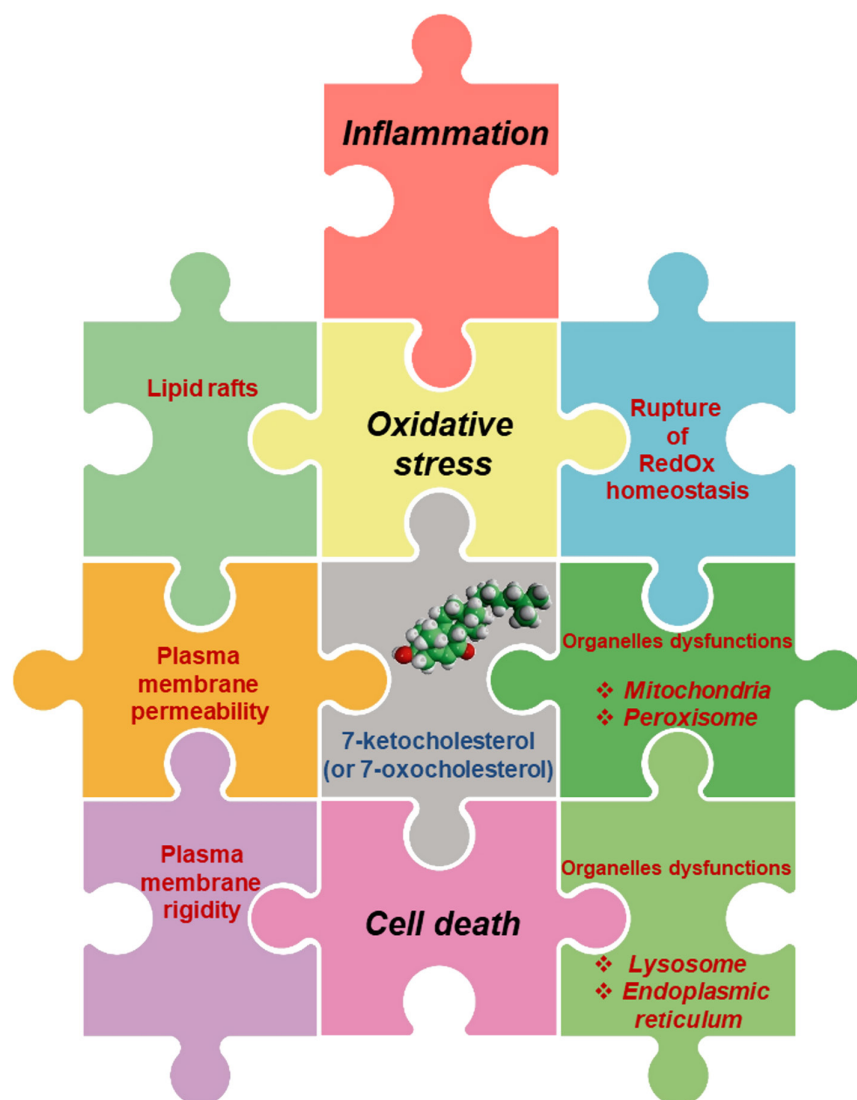


Figure 3

Major characteristics of 7-ketocholesterol-associated cytotoxic effects. The main cytotoxic effects of 7-ketocholesterol, which are also hallmarks of age-related diseases and civilization diseases, are oxidative stress and inflammation which can contribute to trigger cell death.

in mitochondria have been observed; these changes were associated with a drop in transmembrane mitochondrial potential ($\Delta\Psi_m$) (Nury *et al.* 2021a). On A7r5 rat aorta smooth muscle cells, it has been shown that the drop of $\Delta\Psi_m$ induced by 7KC precedes the activation of apoptosis associated with a loss of cell adhesion (Zahm *et al.* 2003). In addition, under treatment with 7KC, the Krebs cycle (also called the citric acid cycle), as well as the oxidative phosphorylation, is also strongly altered leading to lower glycolysis and a decrease in ATP production (Leoni *et al.* 2017). Overproduction of reactive oxygen species (ROS), especially superoxide anions (O_2^-), is also observed at the mitochondrial level (Nury *et al.* 2021a). Like mitochondria, the peroxisome, which is functionally connected to the mitochondria and vice versa (Lismont *et al.* 2015, Franssen *et al.* 2017), is also subject to significant topographical and morphological changes. In addition,

a reduction in peroxisomal mass is always observed, as well as a decrease in peroxisomal β -oxidation which results in a cytoplasmic accumulation of very long-chain fatty acids (VLCFA) known for their cytotoxic and pro-oxidant activities (Zarrouk *et al.* 2012, Nury *et al.* 2018). This alteration of the peroxisome, in particular, the decrease in the levels of peroxisomal proteins, ATP-binding cassette subfamily D member 1 (ABCD1, involved in the transport of VLCFA from the cytoplasm inside the peroxisome) and acyl-CoA oxidase 1 (ACOX1, the first and rate-limiting enzyme in peroxisomal fatty acid β -oxidation of VLCFA), could contribute to 7KC-induced oxidative stress (Trompier *et al.* 2014). Indeed, on 158N rat oligodendrocytes, it has been shown that the decreased expression of ABCD1 and ACOX1 induced by RNA silencing triggered a strong overproduction of ROS and reactive nitrogen species (RNS) (Baarine *et al.* 2012).

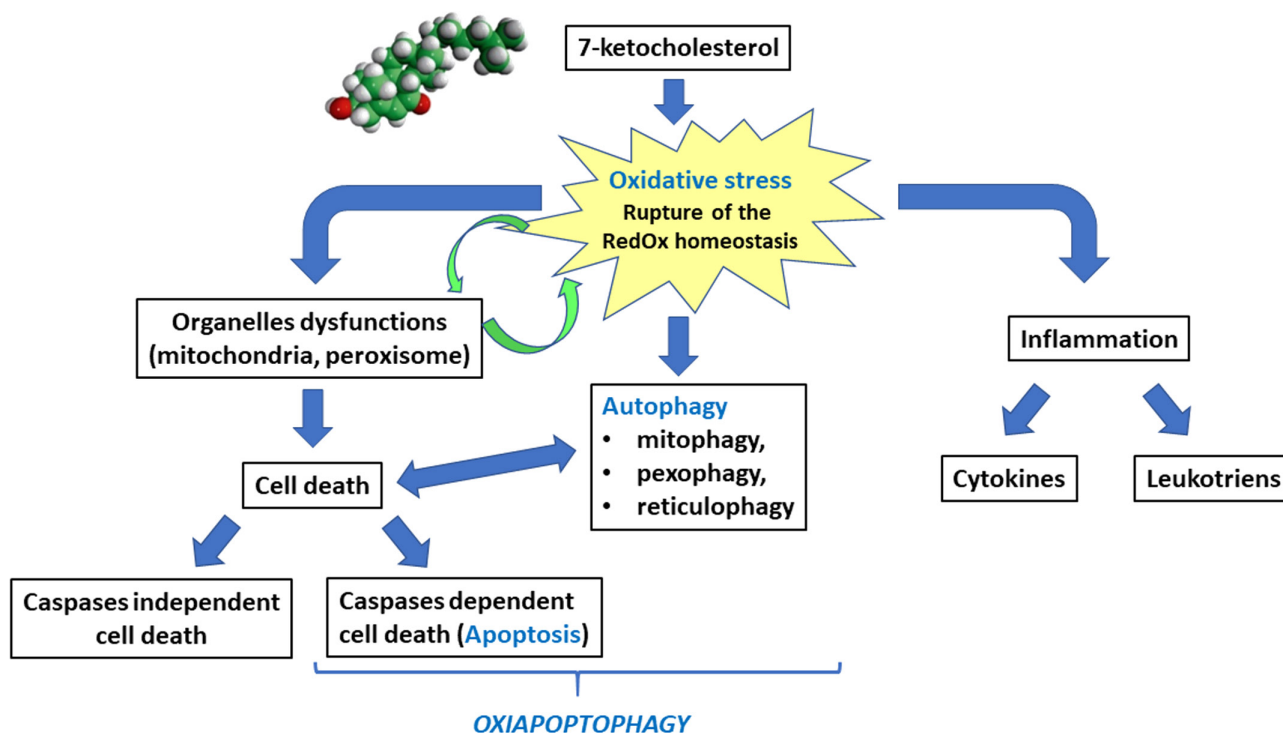


Figure 4

Hypothetical connection between 7-ketocholesterol-induced oxidative stress, inflammation, and cell death. 7-ketocholesterol (also named 7-oxocholesterol) can induce either a caspase-dependent mode of cell death defined as oxiaoptophagy (OXIdative stress + APOPTosis + autoPHAGY) on different cell types from different species (Nury *et al.* 2021b) or a caspase-independent mode of cell death (Dugas *et al.* 2010). These different types of cell death are associated with an important oxidative stress, plasma membrane alterations, and organelles dysfunctions (especially mitochondrial and peroxisomal dyfunctions) leading to cell death. On A7r5 rat smooth muscle cells and 158N oligodendrocytes, an accumulation of 7-ketocholesterol (7KC) in lipid raft has been shown (Royer *et al.* 2009, Ragot *et al.* 2013). Detailed signalling pathways associated with 7KC-induced cytotoxicity are described in the reviews by Vejux *et al.* (2020) and Nury *et al.* (2021a).

Thus, regardless of the type of cell death induced by 7KC (caspases-dependent or independent), mitochondrial and peroxisomal dysfunctions could favour oxidative stress. This latter could not only be due to mitochondrial and peroxisomal dysfunctions but also to the activation of NADPH oxidases. In addition, this oxidative stress is associated with a disruption of the RedOx equilibrium which results (i) in changes in the activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) and (ii) in lipid peroxidation leading to the generation of aldehydes such as malondialdehyde (MDA)-promoting protein carbonylation (Vejux *et al.* 2020, Nury *et al.* 2021a). Therefore, 7KC-induced oxidative stress appears a major element in cellular dysfunctions leading to caspases-dependent and independent cell death. Moreover, among the events associated with cell death, 7KC also activates an autophagic process considered as survival autophagy (Yamine *et al.* 2020, Zhang *et al.* 2020). This latter is observed either during apoptosis or caspases-independent cell death. When autophagy is associated with oxidative stress and apoptosis, the term

‘oxiapoptophagy’ (OXIdation+APOPTosis+autoPHAGY) is used to define 7KC-induced cell death (Fig. 4) (Nury *et al.* 2021b). The signalling pathways associated with this type of cell death have been described in the detail by Vejux *et al.* (2020) and Nury *et al.* (2021a). In the case of oxiapoptophagy, the autophagy observed (mitophagy, pexophagy, and/or reticulophagy) corresponds to survival autophagy (Yuan *et al.* 2016). Indeed, when 7KC is combined with 3-methyl adenine (an autophagy inhibitor), cell death increases, while when combined with rapamycin (an autophagy activator), cell death decreases (Yamine *et al.* 2020). In addition, 7KC also has pro-inflammatory activities. By interacting with the TLR4 receptor (Erridge *et al.* 2007, Huang *et al.* 2014), it promotes the production of inflammatory cytokines (Prunet *et al.* 2006), increases the rate of adhesion molecules (Lemaire *et al.* 1998, Shimozawa *et al.* 2004), and promotes the transition of macrophages from the anti-inflammatory M1 phenotype to the pro-inflammatory M2 phenotype (Buttari *et al.* 2013). On ARPE-19 cells, a link between 7KC-induced autophagy, inflammation, and angiogenesis

has been shown: the inhibition of the mammalian target of rapamycin (mTOR) pathway by rapamycin suppresses 7KC-induced IL-6, IL-8, and vascular endothelial growth factor (VEGF) expression by downregulating the mitogen-activated protein kinase (MAPK) pathway (Yang *et al.* 2022). 7KC also favours non-cytokinic inflammation. Thus, in human mesangial cell, 7KC induces ROS-mediated mRNA expression of 12-lipoxygenase and cyclooxygenase-2 (Watanabe *et al.* 2018). 7KC-induced oxidative stress also suppresses docosahexaenoic acid (DHA)-derived resolvins like RvD1 which prevents atheromatous plaque instability; it is suggested that 7KC could increase the ratio of (nuclear: non-nuclear lipoxygenase) which would decrease RvD1 (Fredman *et al.* 2016). 7KC could also be involved in adipogenesis and vascularization (Chang *et al.* 2020, de Freitas *et al.* 2021).

To reduce the cytotoxic activities of 7KC and to prevent and/or cure the diseases of civilizations and age-related diseases associated with increased levels of 7KC, several strategies are possible. In this context, the identification of natural or synthetic molecules allowing to reduce the toxicity of 7KC takes an important place. Among the natural cytoprotective molecules are many nutrients present in the Mediterranean diet: tocopherols, fatty acids, and polyphenols (Nury *et al.* 2021a). Many Mediterranean oils (argan oil, olive oil, milk thistle seed oil, and *Pistacia lentiscus* L. seed oil) also reduce the toxicity of this oxysterol (Nury *et al.* 2021a). Among the synthetic molecules, dimethyl fumarate used in the treatment of multiple sclerosis under the name Tecfidera as well as its major metabolite, monomethyl fumarate, also has powerful cytoprotective activities (Zarrouk *et al.* 2017). Another strategy, probably more specific, is to promote the catabolism of 7KC to counteract or attenuate its toxicity. This concept was initially validated *in vitro* by Mathieu *et al.* under the name of medical bioremediation, by specifically targeting the degradation of 7KC in the lysosome in which this oxysterol accumulates (Mathieu *et al.* 2008, Schloendorn *et al.* 2009). Better knowledge of the production of 7KC and on its catabolism will contribute to open innovative and promising therapeutic perspectives.

Inactivation of 7-ketocholesterol

Many of the enzymatic pathways which contribute to metabolize cholesterol are also able to act on oxysterols (Gill *et al.* 2008). The major oxysterol metabolite routes for oxysterol inactivation are esterification, sulfation, oxidation, and reduction (Brown & Jessup 2009).

Esterification

Both acyl-CoA cholesterol acyl transferase (ACAT), also named sterol-O-acyltransferase (SOAT), and lecithin-cholesterol acyl transferase (LCAT) can esterify oxysterols in cells and plasma, respectively (Szedlacsek *et al.* 1995, Brown & Jessup 2009, Rogers *et al.* 2015). Excess cholesterol in the cells is esterified under the action of ACAT, which is a normal cellular mechanism for limiting the level of free cholesterol (unesterified cholesterol) in cell membranes to maintain normal membrane structure (Chang *et al.* 2006). The esterified cholesterol is stored in lipid droplets (Luo *et al.* 2020). However, in macrophage foam cells from human atherosclerotic lesions, 7KC levels are the highest in the endosomal and lysosomal compartments (Brown *et al.* 2000), where it inhibits sphingomyelinase and facilitates the intralysosomal accumulation of both sphingomyelin and cholesterol (Maor *et al.* 1995), and in 7KC-treated human monocytic U937 cells, an important 7KC-accumulation has been observed in multilamellar structures named myelin figures (Vejud & Lizard 2009). While ACAT is also able to esterify many oxysterols, cholesterol is superior to 7KC or 7 α -OHC as an allosteric activator of this enzyme (Zhang *et al.* 2003). It has also been reported that the esterification of 7KC to fatty acids involves the combined action of cytosolic phospholipase A2 alpha (cPLA2 α) and sterol O-acyltransferase (SOAT1) (Lee *et al.* 2015). Inhibition of either one of these enzymes ablates 7KC-fatty acid ester (7KFAE) formation. The 7KFAEs are not toxic and do not induce inflammatory responses. An additional function of high-density lipoproteins (HDL) would be to favour the elimination of 7KC by returning 7KFAEs to the liver for bile acid formation. However, 7KC could also inactivate 7 α -hydroxylase (CYP7A1), a major hepatic enzyme involved in bile acid synthesis (Lyons & Brown 1999, Tempel *et al.* 2014).

Sulfation

Cytosolic SULT2 family of cytosolic sulfotransferase family 2 (SULT2B1b) shows a particular affinity for cholesterol and for oxysterols (Javitt *et al.* 2001). SULTs sulfonates at the third position of the ring A of 7KC form 7-ketocholesterol-3- sulphate (7KCS). Sulfation is known to act as a detoxification pathway for the removal of 7KC (Fuda *et al.* 2007, Sanchez *et al.* 2021). Previous work demonstrated that human breast cancer MCF-7 cells expressing high levels of SULT2B1b are significantly more resistant to the cytotoxic effect of 7KC than human embryonic kidney 293T cells that do not express this

isozyme (Fuda *et al.* 2007). Over-expressing SULT2B1b in 293T cells increased sterol and 7KC sulfation and decreased the 7KC-mediated toxicity. Since SULTB1b expression is not universal, this detoxification pathway is not present in some important tissues such as retina where 7KC toxic effects are prominent (Rodríguez & Fliesler 2009, Vejux *et al.* 2011). Expression of SULT2B1b was not observed in neural retinal cells in rats, monkeys, or human (Moreira *et al.* 2009, Rodríguez & Fliesler 2009, Rodríguez & Larrayoz 2010). However, when added to cultured retinal cells *in vitro*, 7KCS attenuates cholesterol transporter, ATP-binding cassette transporter ABCA1 (member 1 of human transporter sub-family ABCA), and vascular endothelial growth factor (VEGF) inductions by 7KC (Moreira *et al.* 2009). Gene transactivation of liver X receptors (LXRs) requires recruitment of co-activators in a ligand-dependent manner and 7KCS inhibited reporter gene activation by LXR α (Song *et al.* 2001). Despite these early investigations, there is more scope to understand the role of 7KCS on cholesterol homeostasis and pathophysiological consequences.

Oxidation

In humans, the enzyme sterol 27-hydroxylase cytochrome P450 27A1 (CYP27A1) eliminates cholesterol and likely 7KC from the retina and many other tissues (Charvet *et al.* 2011, Heo *et al.* 2011). CYP27A1 is a mitochondrial cytochrome P450 enzyme and the first enzyme of the acidic bile acid pathway; CYP27A1 is responsible for the initial metabolism of 7KC by HepG2 cells, a human hepatoblastoma cell line (Lyons & Brown 2001). Thus, via the enzyme CYP27A1, 7KC gives 27-hydroxycholesterol-7-ketocholesterol (27OHC-7KC). The contribution of CYP27A1 in the catabolism of 7KC has also been previously described in the context of the analysis of the cytotoxic effects of 7KC on 158N and BV-2 cells (Bezine *et al.* 2018). In the insect, the enzyme CYP306A1 has 25-hydroxylase activity and allows the formation of 25-hydroxycholesterol-7-ketocholesterol (25OHC-7KC) from 7KC (Pan *et al.* 2021).

Reduction

The enzymatic conversion of 7KC to 7 β -OHC by hydroxysteroid dehydrogenase 11 β -HSD1 (11 β HSD1/EC 1.1.1.146 also known as cortisone reductase; HSD11B1 gene, OMIM 600713) can occur in many tissues (Mitić *et al.* 2013a,b). The conversion of 7KC in 7 β -OHC by 11 β -HSD1 is well established in the arterial wall (Mitić *et al.* 2013a).

Noteworthy, the activity and reaction direction of adipose 11 β -HSD1 are altered in oxysterol excess and could impact the pathophysiology of obesity and its complications (Wamil *et al.* 2008). The contribution of 11 β -HSD1 in the catabolism of 7KC has also been previously described in the context of the analysis of the cytotoxic effects of 7KC on 158N and BV-2 cells (Bezine *et al.* 2018).

Biodegradation with bacterial enzymes: nutritional and biomedical aspects

The catabolic insufficiency of the human body to inactivate and degrade harmful oxysterols, such as 7KC, leads to their progressive accumulation which can have pathophysiological consequences and trigger the development of diseases. An interesting solution termed 'Medical Bioremediation', proposes the use of exogenous enzymes derived from micro-organisms to degrade 7KC either into less toxic metabolites or towards complete mineralization and further explores the delivery of these enzymes in disease conditions. The pioneering works in this field were reported as part of a pilot study funded by the Strategy for Engineered Negligible Senescence (SENS) research foundation (<https://www.sens.org/>; May 2022) where several bacterial strains such as *Pseudomonas aeruginosa*, *Rhodococcus jostii* RHA1, *Sphingomonas* sp. JEM-1, *Nocardia nova*, and *Proteobacterium* Y-134 were explored for their 7KC degradation capability (de Grey *et al.* 2005, Rittmann & Schloendorn 2007, Mathieu *et al.* 2008, 2009, Schloendorn *et al.* 2009). Further, genes responsible for 7KC degradation by *Rhodococcus* RHA1 were studied through transcriptomic studies, and several steroid catabolism gene clusters were found to be expressed, along with enzymes such as dioxygenase (hsaC), 7-keto reductase, and dehydratase (Mathieu *et al.* 2010). Taking a cue, other researchers reported the high 7KC degradation by *Pseudomonas aeruginosa* PseA and *Rhodococcus erythropolis* MTCC 3951, facilitated by the production of the enzymes cholesterol oxidase, lipase, dehydrogenase, and reductase. Some of the identified degradation products were cholesta-3, 5-dien-7-one/cholesta-4, 6-dien-3-one for *P. aeruginosa*, while in case of *R. erythropolis*, chol-5-en-3,7-dione and androsta-4-ene-3,7,17-trione were identified (Ghosh & Khare 2016, 2017, Vejux *et al.* 2020, Ravi *et al.* 2021). Several other 7KC degrading strains were reported including *Thermobifida fusca* IP1, *Alcanivorax jadensis* IP4, *Streptomyces auratus* IP2, and *Serratia marcescens* IP3 (Perveen 2016, Perveen *et al.* 2018). From the above studies, the importance of the first enzyme of the 7KC biodegradation pathway, cholesterol oxidase, cannot be undermined in reducing

7KC cytotoxicity in cells. Thus, a plasmid construct of pEGFP-N3, containing the *Chromobacterium* DS-1 cholesterol oxidase gene fused with the signal sequence and transmembrane domain of the lysosomal membrane protein LAMP1, was found to localize into the lysosome, providing the cytoprotective effect in human fibroblast cells treated with 7KC (Mathieu et al. 2012). Cholesterol oxidase immobilized on magnetic iron (II, III) oxide nanoparticles have also been reported to convert cholesterol and 7KC to 4-cholesten-3-one and 4-cholesten-3, 7-dione, respectively,

in solution, which find applications as pharmaceutically important steroid precursors (Ghosh et al. 2018a,b). An interesting study to mention is the biosorption of 7KC by the probiotic strain *Lactobacillus casei* ATCC334 which could be further explored in inhibiting 7KC absorption via intestine (Machorro-Méndez et al. 2013). Thus, the use of bacteria and their degradative enzyme explore a promising route for remediation of 7KC-mediated cytotoxicity which has important applications in food industry and in pharmacology.

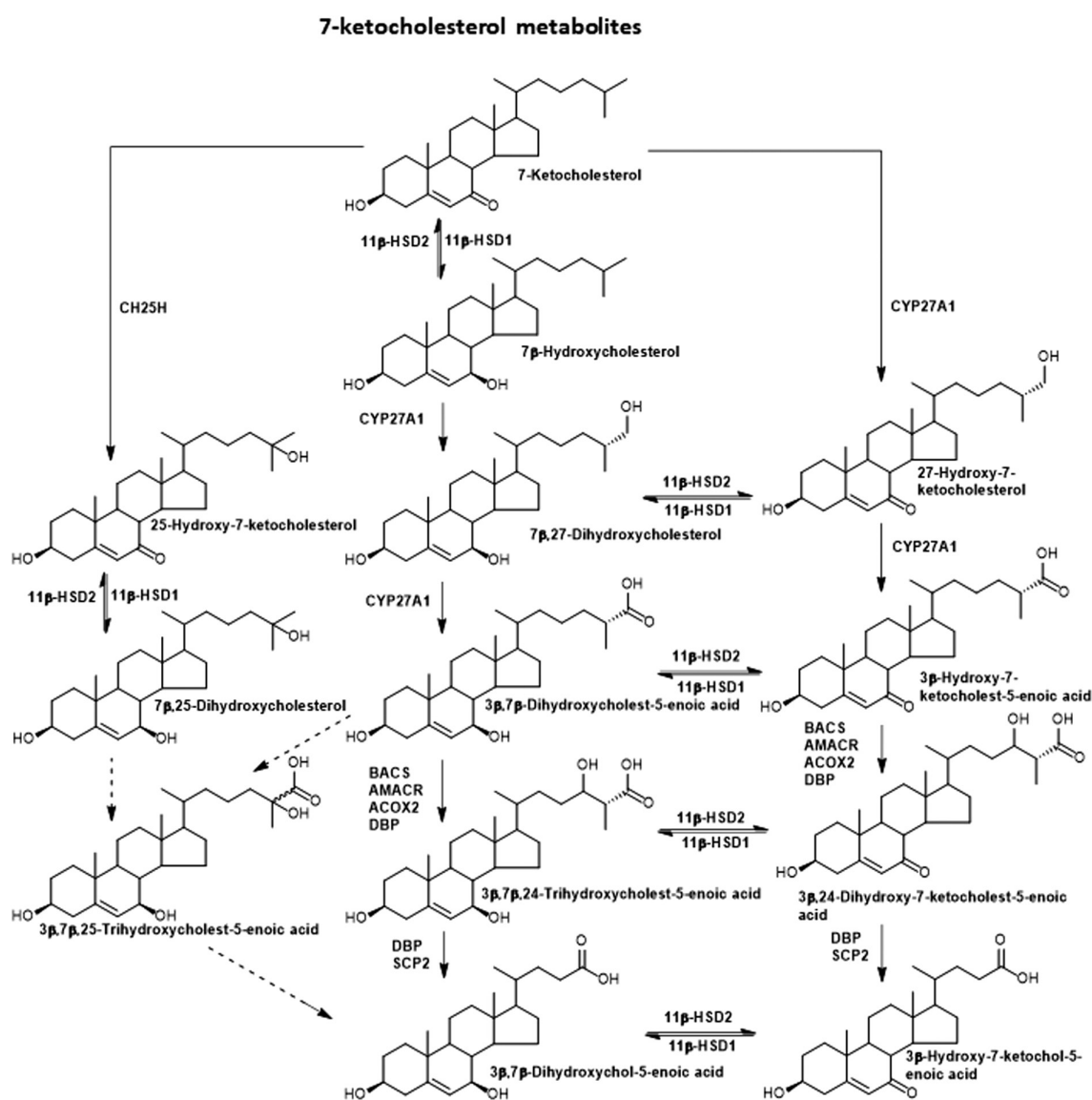


Figure 5

Metabolism of 7-ketocholesterol. The enzymes required for side-chain shortening are presumed to be those utilized in the acidic pathway of bile acid synthesis (Zhou & Hylemon 2014). Reactions catalysed by unknown enzymes are shown by broken arrows. 11β-HSD1, hydroxysteroid dehydrogenase type 1; 11β-HSD2, hydroxysteroid dehydrogenase type 2; CYP27A1, 27-hydroxylase cytochrome P450 27A1. The enzymes involved in bile acid synthesis are peroxisomal enzymes (ACOX2, acyl-coenzyme A oxidase 2; DBP, D bifunctional protein; SCP2, sterol carrier protein 2) and mitochondrial/peroxisomal enzymes (AMACR, alpha-methylacyl-CoA-racemase; BACS, bile acyl CoA-synthase).

Metabolism of 7-ketocholesterol in patients with genetic diseases affecting cholesterol metabolism

The metabolism of 7KC is of interest to limit its accumulation and consequently reduce its side effects. Information on the metabolism of 7KC is often obtained from cell lines (Lyons & Brown 2001, Heo *et al.* 2011) and from samples of patients where levels of 7KC are particularly high as in patients with Niemann–Pick disease type A, B, C1, and C2 (NPA, NPB, and NPC), lysosomal acid lipase deficiency (LALD) (Griffiths *et al.* 2019), and SLO syndrome (Björkhem *et al.* 2014, Griffiths *et al.* 2017). 7KC could also be used as a biomarker of neonatal cholestasis (López de Frutos *et al.* 2021). In the catabolism of 7KC, different pathways have been identified (Fig. 5). Numerous enzymes are involved such as the 11 β hydroxysteroid dehydrogenase type 1 (11 β -HSD1), the 11 β hydroxysteroid dehydrogenase type 2 (11 β -HSD2), the sterol 27-hydroxylase cytochrome P450 27A1 (CYP27A1), and the cholesterol 25 hydroxylase (CH25H). The following enzymes are also involved in bile acid synthesis: several peroxisomal enzymes (acyl-coenzyme A oxidase 2 (ACOX2), D bifunctional protein (DBP), sterol carrier protein 2 (SCP2)), as well as alpha-methylacyl-CoA-racemase (AMACR), localized in the mitochondria and the peroxisome, and also the bile acyl CoA-synthase (BACS) (Griffiths *et al.* 2019).

Overview and conclusion

7KC is the most frequently formed oxysterol by autoxidation (Anderson *et al.* 2020, Nury *et al.* 2021a). This oxysterol, which is a biomarker of oxidative stress (Iuliano *et al.* 2003, Seet *et al.* 2010, Samadi *et al.* 2019), is present in high amounts in several foods (Canzoneri *et al.* 2022) and it is formed in the stomach from cholesterol (Kanner & Lapidot 2001). The increase in oxidative stress during ageing and under the influence of environmental factors (lifestyle habits, stress, and pollution) can also, depending on the individual, contribute to a more or less important accumulation of 7KC in different tissues (Zarrouk *et al.* 2014, de Medina *et al.* 2022). This last aspect can lead to highly disabling diseases (cardiovascular, neurodegenerative, and ocular diseases, as well as metabolic syndrome) with significant societal consequences. It is therefore essential to better know the metabolism of 7KC in humans, and with appropriate models (Vejud *et al.* 2020), to identify molecules counteracting 7KC-induced cytotoxicity (Brahmi *et al.* 2019, Nury *et al.* 2021a), and to develop new strategies to control the level of this oxysterol in food and in

the body in order to avoid its toxic effects: oxidative stress, inflammation, and cell degeneration. Thus, 7KC, which is one of the oldest oxysterols identified, still deserves our full attention.

Declaration of interest

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