Pathophysiological potential of lipid hydroperoxide intermembrane translocation: Cholesterol hydroperoxide translocation as a special case

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ABSTRACT

Peroxidation of unsaturated phospholipids, glycolipids, and cholesterol in biological membranes under oxidative stress conditions can underlie a variety of pathological conditions, including atherogenesis, neurodegeneration, and carcinogenesis. Lipid hydroperoxides (LOOHs) are key intermediates in the peroxidative process. Nascent LOOHs may either undergo one-electron reduction to exacerbate membrane damage/dysfunction or two-electron reduction to attenuate this. Another possibility is LOOH translocation to an acceptor site, followed by either of these competing reductions. Cholesterol (Ch)-derived hydroperoxides (ChOOHs) have several special features that will be highlighted in this review. In addition to being susceptible to one-electron vs. two-electron reduction, ChOOHs can translocate from a membrane of origin to another membrane, where such turnover may ensue. Intracellular StAR family proteins have been shown to deliver not only Ch to mitochondria, but also ChOOHs. StAR-mediated transfer of free radical-generated 7-hydroperoxycholesterol (7-OOH) results in impairment of (a) Ch utilization in steroidogenic cells, and (b) anti-atherogenic reverse Ch transport in vascular macrophages. This is the first known example of how a peroxide derivative can be recognized by a natural lipid trafficking pathway with deleterious consequences. For each example above, we will discuss the underlying mechanism of oxidative damage/dysfunction, and how this might be mitigated by antioxidant intervention.

1. Introduction

Free cholesterol (Ch) and unsaturated phospholipids in biological membranes and lipoproteins are susceptible to non-enzymatic oxidation (lipid peroxidation) under conditions of oxidative stress [1–4]. Cholesteryl esters bearing unsaturated fatty acyl groups are also susceptible. In mammalian cells, such modifications can occur as negative effects of natural metabolic processes such as electron transport in mitochondrial membranes or NADPH oxidase activation in plasma membranes [5,6]. These reactions may also occur during cell exposure to chemical oxidants such as redox-cycling drugs or physical oxidants such as ultraviolet or ionizing radiation [7,8]. Lipid peroxidation can be triggered by reactive oxygen species (ROS) such as hydroxyl radical (HO$^\bullet$) and singlet oxygen ($^1O_2$) or by reactive nitrogen oxide species such as peroxynitrous acid (ONOOH) and nitrogen dioxide (NO$_2^\bullet$) [9]. All of these except $^1O_2$ initiate lipid peroxidation via abstraction of an allylic hydrogen from an unsaturated lipid, which would be an *sn-2* fatty acyl hydrogen in a phospholipid or a carbon-7 hydrogen in Ch [1,2]. The resulting lipid radical reacts rapidly with O_2 to give a peroxyl radical (e.g. 7-OO$^\bullet$ from Ch), which propagates the reaction by abstracting hydrogen from another lipid, thereby becoming a hydroperoxide species (e.g. 7α/β-OOH from Ch). $^1O_2$, a non-radical, that is typically generated by photodynamic reactions, adds directly to an unsaturated lipid with hydrogen retention and allylic shift in the double bond [2]. $^1O_2$ addition to Ch gives the following positional hydroperoxides: 5α-OOH and 6α/β-OOH, the former one usually in much higher yield than either of the latter ones [10,11]. Structures of free radical- and $^1O_2$-generated cholesterol hydroperoxides (ChOOHs) are shown in Fig. 1. When exposed to free radical initiators or $^1O_2$, unsaturated phospholipids (PLs) are converted to hydroperoxide (PLOOH) species in similar fashion
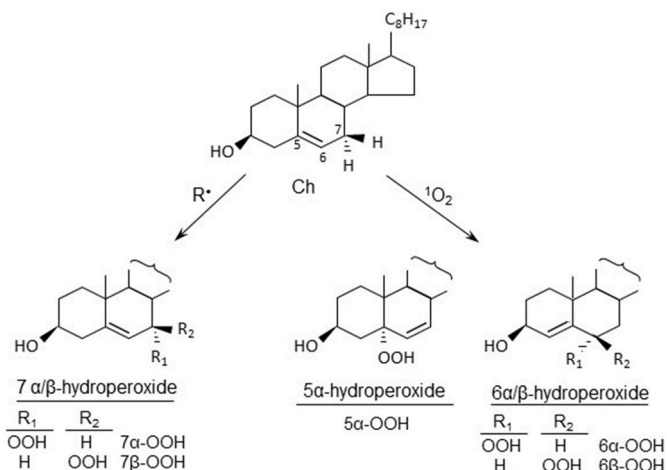
Fig. 1. Structures of cholest-5-en-3β-ol (cholesterol, Ch) and its hydroperoxide species generated by free radical (R•)-mediated reactions: 3β-hydroxycholest-5-ene-7α-hydroperoxide (7α-OOH), 3β-hydroxycholest-5-ene-7β-hydroperoxide (7β-OOH), and by singlet oxygen ($^1O_2$)-mediated reactions: 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH), 3β-hydroxycholest-4-ene-6α-hydroperoxide (6α-OOH)and 3β-hydroxycholest-4-ene-6β-hydroperoxide (6β-OOH).

[1–3]. PLOOHs can also be generated enzymatically, e.g. via 12-lipoxygenase (12-LOX) action on arachidonate-containing PLs, although most LOXs act preferentially on non-esterified unsaturated fatty acids [12, 13]. Free Ch exists naturally as a single molecular species, thus giving rise to far fewer isomeric hydroperoxides than natural phospholipids. Moreover, ChOOHs have been linked to certain pathologic conditions arising from free radical damage/dysfunction at the mitochondrial level. These conditions, the apparent molecular targets involved, and how they might be protected will be discussed in this review.

2. LOOH translocation as a general phenomenon

At relatively low donor membrane levels, non-oxidized PLs and Ch have been shown to translocate to acceptor membranes via an aqueous transit pool rather than via membrane collisions [14,15]. Lipid departure from donor membranes was found to be slow and usually rate-limiting compared with uptake by acceptors. About 20 years ago, Vila et al. [16] predicted that Ch- or PL-derived LOOHs, due to increased hydrophilicity of the peroxide group, should depart a membrane of origin more rapidly than LH precursors. In a cell under mitochondrial oxidative stress, for example, LOOH transfer from mitochondrial membranes to the plasma membrane might be favored by the much larger lipid mass of the latter as an acceptor. In addition to moving from one membrane to another within cells, LOOHs might translocate between lipoproteins in plasma, cells and lipoproteins, or between cells themselves, e.g. via their plasma membranes [16]. Another important prediction is that intracellular LOOH transfer might be accelerated by transfer proteins, which could either promote desorption from donor membranes or act as delivery shuttles to acceptor membranes [14, 17–20]. In the following sections, we will discuss experimental evidence confirming that LOOH transfer between biological membranes does occur, and can be accelerated by intracellular lipid transfer proteins, leading to cellular damage and metabolic dysfunction.

2.1. Spontaneous ChOOH translocation

That spontaneous ChOOH transfer between membranes can occur was first demonstrated using the following model: photodynamically peroxidized red blood cell (RBC) membranes (ghosts) as ChOOH donors and small unilamellar liposomes (SUVs) in 5-10-fold lipid molar excess as acceptors [16]. For tracking ChOOHs, ghost membrane Ch was

[14]C-labeled prior to photoperoxidation, using serum albumin as a labeling vehicle. During transfer incubation, [^{14}C]Ch and [^{14}C]ChOOH acquisition by SUVs was assessed by high-performance thin layer chromatography with phosphorimaging (HPTLC-PI) [21]. Incubations were carried out at 37 °C under redox-inhibited conditions (e.g. desferrioxamine-treated buffers) to prevent LOOH turnover/induction. At various incubation times, ghosts and SUVs were separated, and lipids from the latter were extracted and analyzed by HPTLC-PI. These experiments revealed a striking difference in first-order rate constants for Ch and total ChOOH transfer, the value for the latter being at least 60-times greater than that for Ch [16]. The same difference was observed when photoperoxidized SUVs were used as donors and red cell ghosts as acceptors. In each case, ChOOH departure from donor membranes was found to be rate limiting like that of parent Ch. The ChOOHs monitored in these experiments consisted of a mixture of five different positional isomers (Fig. 1): 5α-OOH with lesser amounts of 6α-OOH and 6β-OOH, plus very small amounts of 7α-OOH and 7β-OOH. The preponderance of 5α-OOH over 7α/β-OOH indicated that the photoperoxidation reaction in donor membranes was mainly $^1O_2$-mediated. In subsequent experiments, ghost-to-SUV transfer kinetics for the different ChOOH positional isomers were compared, using reverse phase high-performance liquid chromatography (Ultrasphere XL-ODS column) with mercury cathode electrochemical detection [HPLC-EC(Hg)] for analysis [22]. In the first reported evidence for this, it was found that the first-order rate constants decreased in the following general fashion: 7α/7β-OOH » 5α-OOH » 6α-OOH > 6β-OOH [23]. Since this progression is the exact opposite of that observed for the reverse-phase HPLC retention times of these species, it was deduced that 7α/7β-OOH are the most hydrophilic and 6β-OOH the least hydrophilic of the ChOOHs examined [23]. Other studies revealed that ChOOHs will not only move from one membrane to another, but also from membranes to lipoproteins and vice-versa. For example, carefully prepared low density lipoprotein (LDL) with little, if any, pre-existing LOOH was found to take up ChOOHs readily from photoperoxidized RBC ghosts, the transfer kinetics exhibiting the same rank order as observed for membrane acceptors (see above) [24]. Circulating RBCs, which are loaded with hemoglobin and possibly some loose iron, are susceptible to oxidative damage, which can be exacerbated by their limited ability to detoxify LOOHs [25]. Thus, it is intriguing to speculate that translocation of pro-oxidant LOOHs such as ChOOHs from RBCs to LDL in the circulation might be one possible means of minimizing such damage. On the other hand, unlimited acquisition of these LOOHs by LDL would promote its atherogenic potential [26].

2.2. Protein-mediated ChOOH translocation

Intracellular and extracellular lipid transfer proteins are known to play important roles in lipid metabolism as well as membrane and lipoprotein biogenesis and homeostasis. Intracellular transfer proteins with differing specificities for fatty acids, PLs, Ch, and Ch esters have been identified and characterized [17–20]. A well-known example is non-specific lipid transfer protein, also known as sterol carrier protein-2 (SCP-2). Mature SCP-2 is a small protein (13.2 kDa) that stimulates inter-membrane translocation of Ch as well as various fatty acids and phospholipids [27–29]. A proposed mechanism involves electrostatic interaction of SCP-2 with the donor membrane, binding of an available lipid, and delivery to an acceptor membrane [30]. There is also evidence that SCP-2 can pick up and transport any desorbed lipids existing in the aqueous compartment [28]. Whether SCP-2 could also recognize and move LOOHs was first investigated by the authors of this review and co-workers, using ChOOHs for initial studies. We showed that ChOOH transfer from photoperoxidized RBC ghosts to SUV acceptors could be substantially accelerated by either natural bovine liver SCP-2 or a recombinant form of human SCP-2 [31]. This was the first reported evidence for stimulation of intermembrane LOOH transport by a lipid trafficking protein. For various ChOOH isomers, the first-order rate

constants for SCP-2-accelerated transfer (spontaneous-corrected) decreased in the same rank order as observed for spontaneous transfer, *viz.* 7α/7β-OOH > 5α-OOH > 6α-OOH > 6β-OOH [31]. Thus, the most hydrophilic peroxides (7α/7β-OOH) were transported the fastest, and the least hydrophilic peroxides (6α/6β-OOH) the slowest. As observed for non-oxidized Ch transfer [28], SCP-2-mediated ChOOH transfer could be enhanced by the following donor membrane properties: (a) increasing lipid unsaturation, and (b) increasing net negative charge, e. g. imposed by phosphatidylserine [31].

It is important to point out that cholesteryl ester trafficking is mediated primarily by cholesteryl ester transfer protein (CETP). Such trafficking has been reported to enhance LDL deposition onto arterial walls, thus promoting atherogenesis [32]. However, whether this might result from CETP's ability to transport peroxidized cholesteryl esters is not yet known.

Proteins in the Steroidogenic Acute Regulatory (StAR) family comprise another important class of intracellular Ch trafficking proteins [33–35]. Each of these contains a unique C-terminal StAR-related lipid binding/transfer (START) domain consisting of ~210 amino acid residues. The structure of this domain has been determined for eight START-containing isoforms, each of which binds a single Ch molecule in highly specific fashion [36]. Thus, in contrast to SCP-2, most StAR proteins are sterol-selective and do not bind and transport PLs or fatty acids. StarD1, the family prototype, localizes in the mitochondrial outer membrane and in conjunction with other proteins [34,35] binds and transfers available free Ch to the inner membrane for metabolic processing. Free Ch could arise via LDL-mediated delivery, hydrolysis of cholesteryl esters in lipid droplets, or *de novo* synthesis in the endoplasmic reticulum [34]. Unlike StarD1 itself, prominent homologues such as StarD4, StarD5, and StarD6, lack organelle-targeting sequences [36]. Therefore, these homologues probably operate in the cytosol, transporting Ch to acceptors such as mitochondria for processing after StarD1-mediated internalization. StAR protein-mediated Ch trafficking is required for various normo-physiologic processes, including (a) sterol hormone synthesis by steroidogenic cells [37,38] and (b) early stage anti-atherogenic reverse Ch transport in vascular macrophages [39–41]. One could predict that under conditions of oxidative stress, StAR proteins (similarly to SCP-2) might transport ChOOHs along with Ch, and that this might have pathophysiologic consequences. Korytowski et al. [42], using [14C]7α-OOH- and [14C]Ch-containing SUVs as donors, isolated mouse liver mitochondria as acceptors, and recombinant human StarD4 provided the first supporting evidence for this. StarD4 was found to markedly increase the first-order rate of 7α-OOH transfer to mitochondria over that for spontaneous transfer, and to a much greater extent than it did for Ch transfer. In contrast, StarD4 had no effect on the translocation rate of non-oxidized or peroxidized phosphatidylcholine, consistent with the protein's binding specificity for sterol-based ligands. The deleterious effects of ChOOH and Ch co-trafficking on steroidogenic cells and on macrophage reverse Ch transport will be discussed subsequently after considering PLOOH translocation and the possible reactions of disseminated LOOHs in general.

2.3. PLOOH translocation: general characteristics

Other model studies have shown that PLOOHs are also capable of spontaneous intermembrane translocation. Like ChOOHs compared with Ch, yet even more so, PLOOH transfer rates far exceed those of parent PLs. In initial experiments, PLOOHs were generated in RBC ghost donors via photosensitized photooxidation, and included species derived from phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM). SUVs in total lipid molar excess over ghosts served as acceptors. SUV acquisition of PCOOH, PEOOH, PSOOH, and SMOOH species was monitored by HPLC-EC(Hg), using an amino (LC-NH₂) column [24]. Each of these peroxides exhibited apparent first-order transfer kinetics, the rate constants for

PCOOH, PEOOH, and PSOOH being approximately the same and about 4-times greater than that for SMOOH [24]. Collectively, PLOOHs transmigrated much more slowly than ChOOHs, consistent with the known slow transfer of PLs compared with Ch [15]. More specifically, the first-order rate constant for PCOOH transfer was found to be ~20% that of 5α-OOH and ~10% that of 7α-OOH [24]. When LDL was used as an acceptor, the rate constant differences were the same. ChOOHs are less susceptible to enzymatic inactivation than PLOOHs (see below), giving the former a longer lifetime for translocation on this basis. Thus, the actual extent of ChOOH *vs.* PLOOH transfer in an oxidatively stressed cells depends on a variety of complex factors, including content of oxidizable PLs *vs.* Ch, relative LOOH hydrophilicity, and relative susceptibility to reductive turnover (see below).

3. Possible fates of translocated LOOHs

Translocated LOOHs have various destinies, the major ones depending on pro-oxidant *vs.* antioxidant conditions in acceptor compartments. In the absence of reducing agents (e.g. NAD(P)H, ascorbate) and redox-active metal ions (e.g. iron, copper), LOOHs will continue to accumulate and in so doing, perturb structure/function of an acceptor membrane or lipoprotein due to increased hydrophilicity. However, if reductants and redox-active metal ions are available, translocated LOOHs may undergo one-electron (Fenton-like) reduction to lipid oxyl radicals (LO•), which can rearrange and rapidly autoxidize to epoxyallylic peroxyl radicals (OLOO•) (Fig. 2). This mechanism has been well-documented for peroxidized fatty acids (FAOOHs) in solution and appears likely in membranes and lipoproteins as well [43,44]. One-electron reduction of primary translocated LOOHs can trigger metal-ion-catalyzed chain lipid peroxidation in acceptor membranes/lipoproteins (Fig. 2). The damaging effects of this chain peroxidation could far exceed those due to primary LOOH uptake alone. Like PLOOHs and other translocated LOOHs, ChOOHs are susceptible to one-electron reduction and in the process are converted to ChO• (or OChOO•) intermediates that can induce chain reactions which damage and impair acceptor functionality [1–3,43,44]. Recent studies have shown that certain ChOOHs (5α-OOH, 6β-OOH) can also undergo acid-catalyzed (Hock) cleavage to give secosterol aldehydes [3,4]. Some of these (Sec A, Sec B) have been detected in atherosclerotic lesions and could serve as highly sensitive reporters of early stage atherogenesis [4, 45].

Another possible fate of translocated PLOOHs and ChOOHs is enzyme-catalyzed two electron reduction (Fig. 2), an antioxidant-type process that competes with pro-oxidant one-electron reduction [2,46].
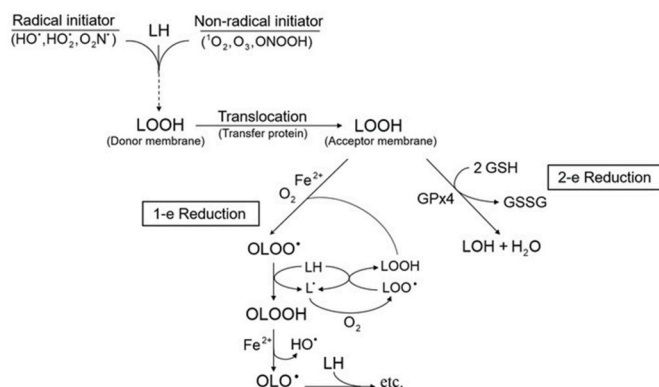
Fig. 2. Two possible mechanisms of translocated LOOH reductive turnover in acceptor membranes. Iron-catalyzed one-electron reduction, followed by O_2 addition, gives an epoxyallylic peroxyl radical which can induce damaging chain peroxidation. Alternatively, two-electron reduction catalyzed by GPx4, or possibly certain peroxiredoxins (e.g. Prx6), converts the LOOH to redox-inert LOH, thereby preventing chain peroxidative damage.

Unlike preventative inactivation of primary ROS (e.g. $O_2^-\bullet$ by superoxide dismutase), two-electron reduction is considered a back-up or reparative detoxification process. Since there are no known specific enzymatic scavengers of $^1O_2$, two-electron reduction would be the only antioxidant option for $^1O_2$-generated LOOHs. The only enzyme with well-established ability to inactivate ChOOHs as well as PLOOHs in membrane and lipoprotein environments is glutathione peroxidase type-4 (GPx4) [25,47,48]. GPx4 is one of approximately four selenium-containing peroxidases that use glutathione (GSH) as a reducing cofactor [49,50]. In mammalian cells, monomeric GPx4 (~20 kDa), which can be found in several compartments, including mitochondria [48], is much less abundant than tetrameric GPx1 (~84 kDa). Unlike GPx4, GPx1 cannot act on membrane-bound ChOOHs or PLOOHs unless the latter are first hydrolyzed to release peroxide-bearing fatty acids [1,2]. Studies by the authors and co-workers [51] revealed a broad range of GPx4 reactivities on various LOOHs, the first-order rate constants decreasing in the following order: PCOOH > 6β-OOH > 7α/β-OOH » 5α-OOH. Importantly, 5α-OOH with the lowest reduction rate was found to be the most cytotoxic of these peroxides, whereas PCOOH with the greatest rate was the least cytotoxic [51]. Free radical-generated 7α/β-OOH is also detoxified relatively slowly by GPx4 (albeit faster than 5α-OOH), and on this basis would be predicted to be more redox-damaging than PLOOHs. In recent years, GPx4 has attracted considerable new attention based on its ability to protect against ferroptosis, a new designation for cell death induced by iron-catalyzed lipid peroxidation [52]. Since GPx4 is found in cytosol and other compartments of eukaryotic cells, one might ask how StarD4-bound 7α/β-OOH, for example, could survive intracellular translocation. One possible explanation is that 7α/β-OOH retains some basic structural characteristics of Ch itself, *viz.* the same hydrophobic "tail" portion and hydrophilic "head" portion with A-ring –OH group and B-ring –OOH group. Since StAR proteins bind and sequester sterols tightly [36], it is unlikely that GPx4 would be able to access StarD4/D1-bound 7α/β-OOH during transit.

Depending on local conditions (e.g. availability of GPx4, catalytic redox metals, and reductants like GSH), one-electron *vs.* two-electron LOOH reduction would also be expected to occur in donor membrane compartments. Not surprisingly, most of the research dealing with lipid peroxidative stress has focused on these originating compartments. One can postulate that LOOH translocation might benefit donor membranes if local metal ion or reductant levels were relatively high. Under these circumstances, translocation could protect donors against redox damage, possibly at the expense of acceptors. Additionally, if GSH/GPx4 levels near acceptors were higher than those near donors, LOOH transfer might serve as an indirect means of LOOH detoxification. Whether such scenarios actually exist at the cellular or tissue level has not yet been investigated in any rigorous way.

LOOH translocation via certain mitochondrial uncoupling proteins (UCPs) has also been reported to be cytoprotective. The mechanism involved is distinct from those discussed thus far because it involves UCP-mediated transfer of PLOOH-derived FAOOHs from the inner to outer mitochondrial membrane. In a study by Jaburek et al. [53], it was found that FAOOH transfer in conjunction with UCP2 uncoupling activity served to relieve proton buildup and oxidative pressure in liposomal membrane models. A later study by Lombardi et al. [54], using isolated muscle mitochondria showed that another uncoupling protein, UCP3, acted in essentially the same manner, i.e. by shuttling arachidonate peroxides and thereby limiting oxidative pressure due to sustained proton motive force. Both studies [53,54] were intriguing, but neither revealed whether parental PLOOHs might also be translocated via UCPs.

4. Negative effects of ChOOH trafficking on steroidogenesis

Non-esterified Ch is an early participant in the synthesis of steroid hormones (progesterone, testosterone, cortisol, etc.) by steroidogenic

cells [37,55]. This Ch may derive from extracellular sources such as LDL via the LDL receptor or high-density lipoprotein (HDL) via scavenger receptors. Upon uptake, cholesteryl esters in LDL/HDL are hydrolyzed by lipases to release free Ch [38]. The latter can also be supplied intracellularly, e.g. from lipid droplets, plasma membrane, or *de novo* synthesis in the endoplasmic reticulum [55]. Free Ch is trafficked to and into mitochondria by StAR family proteins. StarD4 moves Ch through the cytosol to the mitochondrial outer membrane and StarD1 in conjunction with other proteins delivers it to the inner membrane [35–38]. Ch then undergoes hydroxylation and side-chain cleavage by the inner membrane P450$_{scc}$/Cyp11A1 complex to give pregnenolone [35–38]. There is now abundant evidence that pathophysiological conditions associated with oxidative stress, e.g. Type-2 diabetes, ischemia/reperfusion, chronic inflammation, and advanced age can impair steroidogenic function [56,57]. In many cases, this has been associated with diminished and inadequate antioxidant defenses [58]. Model studies have shown that steroidogenic cells *in vitro* become damaged and dysfunctional when exposed to various peroxides other than ChOOHs, e. g. H_2O_2 and fatty acid hydroperoxides [59]. Based largely on these and related studies, Korytowski et al. [60] hypothesized that certain ChOOHs produced by oxidative stress may be trafficked along with Ch to mitochondria of steroidogenic cells and produce damage/dysfunction there due to vigorous one-electron turnover. Strong support for this hypothesis was obtained by exposing mouse testicular MA-10 Leydig cells to [^{14}C]7α-OOH before *vs.* after cell activation with dibutyryl-cyclic AMP (db-cAMP) [60]. The latter stimulated steroidogenesis by strongly upregulating immunodetectable StarD1 as well as StarD4. As shown in Fig. 3A, stimulated cells took up far more [^{14}C]7α-OOH than non-stimulated controls, and in a concentration-dependent fashion. Although not shown here, the [^{14}C]7α-OOH level in mitochondria from stimulated cells exceeded that in non-stimulated counterparts by ~90% [60]. Any mitochondrial damage arising from 7α-OOH uptake would be expected to be reflected in a loss of transmembrane potential. For assessing this, db-cAMP-stimulated MA-10 cells were exposed to 7α-OOH-bearing SUVs for several hours, after which membrane potential ($\triangle\Psi_m$) was evaluated using the fluorophore JC-1 [61]. As shown in Fig. 3B, the fluorescence intensity ratio of stimulated cells was reduced to ~50% that of non-stimulated controls, consistent with 7α-OOH-induced membrane damage [61]. An siRNA-based knockdown of StarD1 prior to MA-10 stimulation resulted in a large decrease in 7α-OOH uptake by mitochondria relative to controls, and $\triangle\Psi_m$ loss was correspondingly reduced [60]. Thus, StarD1 was confirmed to be a key transporter of deleterious 7α-OOH. An Elisa-type assay was used to assess the metabolic ramifications of 7α-OOH import, *viz.* its effects on formation of progesterone, which lies immediately downstream of pregnenolone [55]. With stimulated MA-10 cells under the conditions used, 7α-OOH reduced progesterone output by ~50% relative to Ch-treated controls (Fig. 3C), demonstrating that the peroxide was substantially deleterious to hormone production [60]. After the 3 h of 7α-OOH exposure in this experiment, cells were still attached and showed no obvious signs of cytotoxicity (Fig. 3D). Thus, the indicated drop in progesterone output was attributed to cells that were damaged but still alive during peroxide treatment. However, prolonged 7α-OOH exposure (e.g. 16 h) did result in cell death, and this increased with 7α-OOH concentration [60]. *Tert*-butyl hydroperoxide (t-BuOOH) also killed MA-10 cells in concentration-dependent fashion, but there was no difference between db-cAMP-stimulated and non-stimulated cells [60], consistent with the fact that t-BuOOH is not a StAR protein ligand. Other evidence revealed that SUV-7α-OOH-induced cell death occurred via the intrinsic (mitochondria-initiated) apoptotic pathway. Collectively, these findings provided strong support for the hypothesis that activation of steroidogenic cells under oxidative stress conditions stimulates not only the delivery of unoxidized Ch to mitochondria, but peroxidized Ch as well, and via the same trafficking pathway. Such cells in steroidogenic tissues would be at risk of mitochondrial free radical damage, metabolic dysfunction, and even apoptotic death.
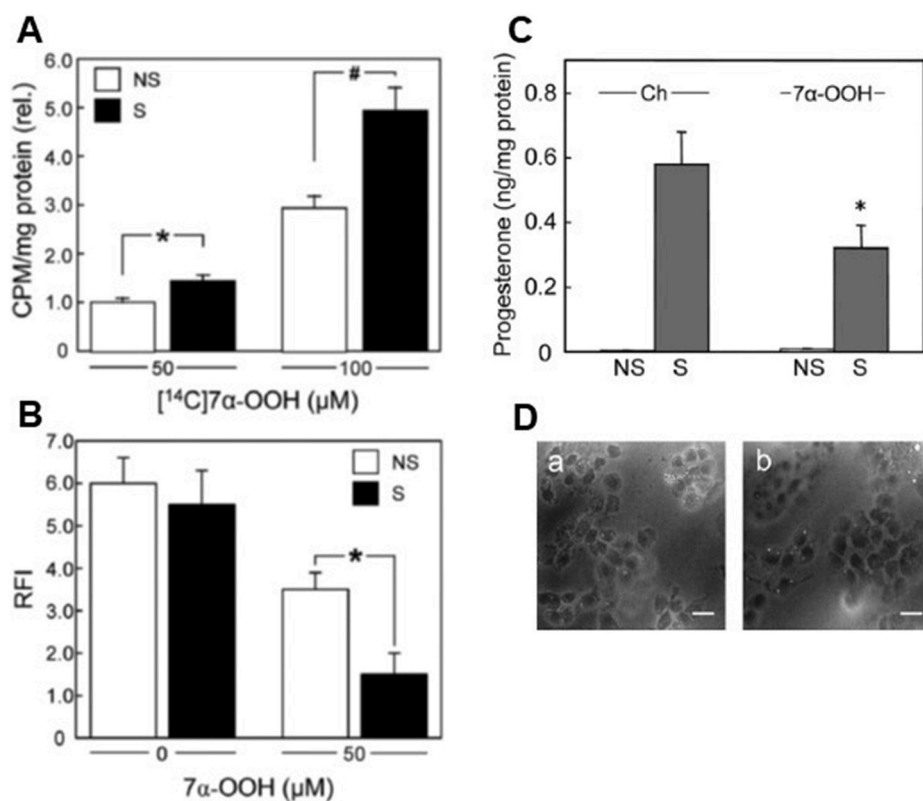
Fig. 3. Deleterious effect of 7α-OOH uptake on mitochondrial integrity and progesterone output of Leydig MA-10 cells. Cyclic AMP-stimulated cells (S) and non-stimulated controls (NS) were incubated for 5 h with SUV-borne [^{14}C]7α-OOH (50 μM or 100 μM), then washed and analyzed for (A) peroxide uptake and (B) mitochondrial membrane potential ($\triangle\Psi_m$), using fluorophore JC-1 (RFI, fluorescence intensity ratio). (C) Progesterone output for Ch- and 7α-OOH-SUV-treated stimulated *vs.* non-stimulated cells, as determined by enzyme immunoassay; n = 3, P < 0.01. (D) Bright field microscopic images of stimulated cells treated with (a) Ch- and (b) 7α-OOH-bearing SUVs; bar: 75 μm. Adapted from different data sets in Ref. 60, with permission.

5. Negative effects of ChOOH trafficking on macrophage reverse cholesterol transport

Elevated oxidative stress lies at the heart of many manifestations of cardiovascular disease, including atherosclerosis [62]. In atherogenesis, unrestricted uptake of oxidized low-density lipoprotein (oxLDL) via scavenger receptors (CD36, SR-BI) in vascular macrophages leads to plaque buildup as a prelude to hypertension and possible infarction [62–64]. Esterified and free Ch comprise a large proportion of the lipids in LDL. In oxLDL, free radical-generated Ch oxides (oxysterols) include the redox-active primary 7-hydroperoxides (7α/β-OOH) and their redox-inactive products, the 7α/β-OH diols and 7-ketone. All of these oxides have been detected in atherosclerotic lesions, the 7α/β-OOH level typically being much lower than that of 7α/β-OH and 7-ketone [65]. However, the relatively small amount of 7α/β-OOH does not signify minor importance, but rather the opposite because it could reflect ongoing one-electron turnover of 7α/β-OOH to 7α/β-OH and 7-ketone, its down-stream products [65,66]. Thus, the levels of these products compared with 7α/β-OOH in atherosclerotic lesions could suggest the extent of damaging chain peroxidation that had occurred. It is important to point out, however, that some small percentage of these species might also arise enzymatically, e.g. CYP7A1-catalyzed oxidation of Ch to 7α-OH. In attempting to limit adverse Ch accumulation, macrophages can export it to acceptors such as HDL or apolipoprotein A-I (apoA-I). This is an early step in overall reverse Ch transport (RCT), which delivers Ch to the liver for disposal [40,41]. In addition to scavenger receptors, vascular macrophages express StAR family proteins, which transport Ch to/into mitochondria for conversion to 27-hydroxycholesterol (27-OH) by 27-hydroxylase (CYP27A1) on the inner membrane [34,67]. 27-OH is a key agonist of the nuclear LXR/RXR transcription factors, which control expression of the ATP binding cassette transporters ABCA1 and ABCG1 on the plasma membrane; ABCA1 mainly exports Ch to apoA-I, while ABCG1 exports it to HDL [68,69]. StAR1 and StAR4 can be induced by loading macrophages with Ch, implicating both in RCT-mediated Ch homeostasis [69]. Based on this background and

findings with steroidogenic cells [60], the authors hypothesized that under oxidative stress conditions, 7α/β-OOH in oxLDL will be caught up in StAR-mediated Ch trafficking to/into mitochondria of vascular macrophages. Upon arrival, these peroxides would induce mitochondrial membrane chain peroxidative damage that disables CYP27A1 and hence early stage RCT. Korytowski et al. [70] provided the first support for the above hypothesis by showing that 7α-OOH uptake by mitochondria of cAMP-activated murine RAW264.7 macrophages was StarD1/D4-dependent. Additionally, this uptake triggered lipid peroxidation, membrane depolarization, and loss of ABCA1 expression [70]. Subsequent studies with activated human monocyte-derived THP-1 macrophages [71] added support to these findings by showing that SUV-7α/β-OOH induced mitochondrial membrane lipid peroxidation, as detected with C11-BODIPY (Fig. 4A) and loss of membrane potential, as detected with JC-1 (Fig. 4B). Of added importance, StarD1 knockdown significantly reduced the extent of lipid peroxidation and loss of membrane potential, consistent with StarD1 trafficking of 7α/β-OOH. Major functional consequences of 7α/β-OOH exposure included substantially reduced CYP27A1 activity, 27-OH output, and ABCA1/G1 expression compared with non-activated controls [71]. In accord with this, activated cells exported significantly less Ch to apoA-I or HDL than non-activated counterparts. When exposed to 7α/β-OOH for sufficiently long periods, THP-1 cells exhibited not only a loss of RCT function, but also increasing death via apoptosis [71]. On the other hand, 7α/β-OH and 7-ketone were completely innocuous, even at relatively high concentrations. These studies were the first to show that a natural Ch trafficking pathway in macrophages can be co-opted by a redox-active Ch oxide (7α/β-OOH), leading to mitochondrial membrane damage and impairment of Ch homeostasis. A scheme illustrating these negative effects of ChOOH trafficking on macrophage RCT is shown in Fig. S1. The inauspicious transport of 7α/β-OOH along with Ch itself can be considered as "stealthy" or "Trojan Horse"-like, since it could be an early event in the development of atherosclerosis and other cardiovascular diseases. It is important to note again that phospholipids can also be shuttled from one membrane to another by PL transfer proteins [17,18].
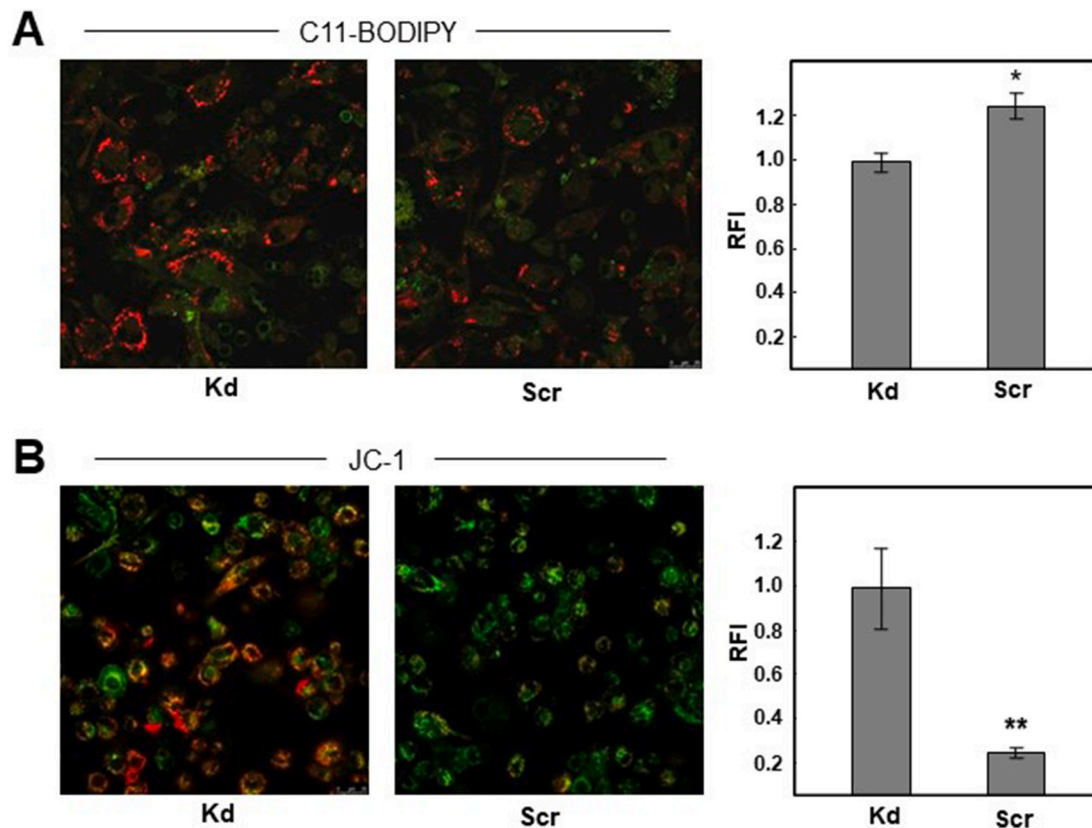
Fig. 4. Mitochondrial lipid peroxidation and loss of membrane potential in 7α-OOH-treated THP-1 macrophages. (A) Lipid peroxidation: StarD1-knockdown cells (Kd) and scrambled controls (Scr) were stimulated with db-cAMP in serum-free medium, then incubated for 4 h with SUVs containing 7α-OOH (50 μM in bulk phase). After washing, the cells were treated with 2 μM C11-BODIPY for 30 min, then examined by confocal fluorescence microscopy; RFI: relative fluorescence (green/red) intensity. Plotted values are means ± SEM, n = 3; *P < 0.05 *vs.* Kd. (B) Membrane potential: StarD1-knockdown cells and scrambled controls were incubated with 7α-OOH-SUVs for 4 h, then washed and treated with JC-1 (5 μg/ml) for 30 min. After a wash, the green/red fluorescence ratio (RFI) was determined. Plotted values are means ± SEM, n = 3; **P < 0.005 *vs.* Kd. Additional details are provided in Ref. 71. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Whether PLOOHs might also be trafficked by these proteins remains to be investigated, along with possible negative consequences.

6. *In vivo* protection against redox damage induced by ChOOH trafficking

As indicated in Sect. 3, no enzyme other than GPx4 is known to be capable of detoxifying ChOOHs in membrane environments [25,47]. Guo et al. [72], found that transgenic apoE(−/−) mice overexpressing active GPx4 in all tissues, including aorta, were more resistant to atherogenesis than wild type controls, and this correlated with reduced lipid peroxidation. Such evidence suggests that GPx4 could play a key role in the ability of vascular cells to cope with trafficked 7-OOH challenges. Similarly, this enzyme might protect steroidogenic tissues against stealthy 7-OOH trafficking. Thus, GPX4's anti-ChOOH activity could be crucial for maintaining mitochondrial integrity and functionality in steroidogenic cells and vascular macrophages. If so, deficiencies in this enzyme due to insufficient dietary selenium or gene mutations would be detrimental to steroid biosynthesis or Ch homeostasis (early stage RCT). Selenium deficiency has been reported to impair steroid synthesis in animal models, and this was attributed to diminished overall activity of glutathione peroxidases; however, GPx4 itself was not examined [73,74]. Prooxidant mitochondrial damage might also be mitigated by pharmacological antioxidants such as mitoquinone (MitoQ), which enters live cells and is driven by electrochemical gradient to accumulate in the mitochondrial inner membrane [75,76]. MitoQ has been reported to protect a variety of cells against mitochondrial oxidative damage by intercepting free radicals [75]. It is known to quench mitochondrial lipid peroxidation [76] and would be expected to do so in connection with deleterious ChOOH/7-OOH trafficking. Although the latter expectation has not been tested yet, several pre-clinical studies have pointed to the cardiovascular benefits of MitoQ as a mitochondria-targeted antioxidant [77,78].

7. Summary and conclusions

We have described how intracellular Ch trafficking pathways can be inappropriately utilized by redox-active hydroperoxide intermediates generated by Ch oxidation, *viz.* 7α- and 7β-OOH. When caught up in StAR protein-mediated Ch trafficking to mitochondria, 7α/β-OOH could induce chain peroxidation of mitochondrial inner membrane lipids, thus disabling at least two physiological functions: steroid hormone synthesis and reverse Ch transport. In steroidogenic cells, early stage metabolism involves Ch processing by inner membrane CYP11A1, whereas in vascular macrophages, Ch homeostasis via reverse transport requires 27-OH formation by CYP27A1. When tightly bound/sequestered by StAR proteins, any ChOOH, including 7α/β-OOH, would be protected against reductive turnover during transit. However, upon arrival at mitochondrial membrane(s), one-electron reduction of 7α/β-OOH could trigger free radical lipid peroxidation with a progressive loss of CYP11A1 or CYP27A1 activity. There is a growing awareness that functionality of steroidogenic cells and Ch-regulating vascular macrophages can be compromised by physiological conditions associated with oxidative stress, e.g. chronic obesity, diabetes, ischemia/reperfusion, or advancing age. Although the mechanism of mitochondrial damage/dysfunction that we describe for *in vitro* systems has yet to be

demonstrated at the *in vivo* level, further investigation would likely reveal its occurrence. Increased recognition of this novel mechanism, which operates via a natural lipid trafficking pathway, is expected to stimulate interest in site-specific protective measures, e.g. use of mitochondria-targeted antioxidants.

Author contributions

The manuscript was drafted by AWG; both authors contributed to revising and finalizing it.

Declaration of competing interest

The authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2021.102096.

References

[1] E. Niki, Lipid peroxidation: physiological levels and dual biological effects, Free Radic. Biol. Med. 47 (2009) 469–484.

[2] H. Yin, L. Xu, N.A. Porter, Free radical lipid peroxidation; mechanisms and analysis, Chem. Rev. 111 (2011) 5944–5972.

[3] C. Zerbinati, L. Iuliano, Cholesterol and related sterol autoxidation, Free Radic. Biol. Med. 111 (2017) 151–155.

[4] E.L. Schaefer, N. Zopyrus, Z.A.M. Zielinski, G.A. Facey, D.A. Pratt, On the products of cholesterol autoxidation in phospholipid bilayers and the formation of secosterols derived therefrom, Angew. Chem. Int. Ed. 59 (2020) 2089–2094.

[5] D. Nolfi-Donegan, A. Braganza, S. Shiva, Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement, Redox Biol.. doi: 10.1016/j.redox.2020.101674.

[6] A.V. Poznyak, A.V. Grechko, V.A. Orekhova, V. Khotina, E.A. Ivanova, A. N. Orekhov, NADPH oxidases and their role in atherosclerosis, Biomedicines 8 (7) (2020) 206, https://doi.org/10.3390/biomedicines8070206.

[7] H. Zhu, S. Sarkar, L. Scott, I. Danelisen, M.A. Trush, Z. Jia, et al., Doxorubicin redox biology: redox cycling, topoisomerase inhibition, and oxidative stress, React. Oxyg. Species (Apex) 1 (2016) 189–198.

[8] R.M. Tyrrell, The molecular and cellular pathology of solar radiation, Mol. Aspect. Med. 15 (1994) 1–77.

[9] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Clarendon Press, Oxford, UK, 1999.

[10] M.J. Kulig, L.L. Smith, Sterol metabolism XXV. Cholesterol oxidation by singlet molecular oxygen, J. Org. Chem. 38 (1973) 3639–3642.

[11] A.W. Girotti, W. Korytowski, Cholesterol peroxidation as a special type of lipid oxidation in photodynamic systems, Photochem. Photobiol. 95 (2019) 73–82.

[12] Y. Takahashi, W.C. Glasgow, H. Susuki, Y. Taketani, S. Yamamoto, M. Anton, et al., Investigation of the oxygenation of phospholipids by porcine leukocyte and human platelet arachidonate 12-lipoxygenases, Eur. J. Biochem. 218 (1993) 165–171.

[13] H. Kuhn, I. Romisch, J. Belkner, The role of lipoxygenases isoforms in atherogenesis, Mol. Nutr. Food Res. 49 (2005) 1014–1029.

[14] M.C. Phillips, W.J. Johnson, G.H. Rothblat, Mechanisms and consequences of cellular cholesterol exchange and transfer, Biochim. Biophys. Acta 906 (1987) 223–276.

[15] R.E. Brown, Spontaneous lipid transfer between organized lipid assemblies, Biochim. Biophys. Acta 1113 (1992) 375–389.

[16] A. Vila, W. Korytowski, A.W. Girotti, Dissemination of peroxidative stress via intermembrane transfer of lipid hydroperoxides: model studies with cholesterol hydroperoxides, Arch. Biochem. Biophys. 380 (2000) 208–218.

[17] D.B. Zilversmit, Lipid transfer proteins, J. Lipid Res. 25 (1984) 1563–1569.

[18] K.W.A. Wirtz, Phospholipid transfer proteins, Annu. Rev. Biochem. J. 60 (1991) 73–99.

[19] T.J. Scallen, A. Patuszyn, B.J. Noland, R. Chanderbhan, A. Kharroubi, G. V. Vanhouny, Sterol carrier and lipid transfer proteins, Chem. Phys. Lipids 38 (1985) 239–261.

[20] P. Cockcroft, Raghu, Phospholipid transport protein function at organelle contact sites, Curr. Opin. Cell Biol. 53 (2018) 52–60.

[21] W. Korytowski, M. Wrona, A.W. Girotti, Radiolabeled cholesterol as a reporter for assessing one-electron turnover of lipid hydroperoxides, Anal. Biochem. 270 (1999) 123–132.

[22] W. Korytowski, P.G. Geiger, A.W. Girotti, High performance liquid chromatography with mercury cathode electrochemical detection: application to lipid hydroperoxide analysis, J. Chromatogr. B Biomed. Sci. Appl. 670 (1995) 189–197.

[23] A. Vila, W. Korytowski, A.W. Girotti, Spontaneous intermembrane transfer of various cholesterol-derived hydroperoxide species: kinetic studies with model membranes and cells, Biochemistry 40 (2001) 14715–14726.

[24] A. Vila, W. Korytowski, A.W. Girotti, Spontaneous transfer of phospholipid and cholesterol hydroperoxides between cell membranes and low-density lipoprotein: assessment of reaction kinetics and prooxidant effects, Biochemistry 41 (2002) 13705–13716.

[25] F. Ursini, M. Maiorino, R. Brigelius-Flohe, K.D. Aumann, B. Roveri, D. Schomberg, et al., Diversity of glutathione peroxidases, Methods Enzymol. 252 (1995) 38–53.

[26] U.P. Steinbrecher, H.F. Zhang, M. Lougheed, Role of oxidatively modified LDL in atherosclerosis, Free Radic. Biol. Med. 9 (1990) 155–168.

[27] B.J. Noland, R.E. Arebalo, E. Hansbury, T.J. Scallen, Purification and properties of sterol carrier protein-2, J. Biol. Chem. 255 (1980) 4282–4289.

[28] A.M. Gallegos, B.P. Atshaves, S.M. Storey, O. Starodub, A.D. Petrescu, H. Huang, et al., Gene structure, intracellular localization, and functional roles of sterol carrier protein-2, Prog. Lipid Res. 40 (2001) 498–563.

[29] N.J. Stolowich, A.D. Petrescu, H. Huang, G.G. Martin, A.L. Scott, F. Schroeder, Sterol carrier protein-2: structure reveals function, Cell. Mol. Life Sci. 59 (2002) 193–212.

[30] J.K. Woodford, S.M. Colles, S.C. Myers-Payne, J.T. Billheimer, F. Schroeder, Sterol carrier protein-2 stimulates intermembrane sterol transfer by direct membrane interaction, Chem. Phys. Lipids 76 (1995) 73–84.

[31] A. Vila, V.V. Levchenko, W. Korytowski, A.W. Girotti, Sterol carrier protein-2-facilitated intermembrane transfer of cholesterol- and phospholipid-derived hydroperoxides, Biochemistry 43 (2004) 12592–12605.

[32] W.A. Targa, H.A. Garda, J.D. Toledo, M.C. Gonzalez, Potential inhibitors of the activity of the cholesteryl ester transfer protein, J. Comput. Biol. 26 (2019) 1458–1469.

[33] B.J. Clark, J. Wells, S.R. King, D.M. Stocco, The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR), J. Biol. Chem. 269 (1994) 28314–28322.

[34] A. Graham, Mitochondrial regulation of macrophage cholesterol homeostasis, Free Radic. Biol. Med. 89 (2015) 982–992.

[35] P. Elustondo, L.A. Martin, B. Karten, Mitochondrial cholesterol import, Biochim. Biophys. Acta 1862 (2017) 90–101.

[36] Y. Tsujishita, J.H. Hurley, Structure and lipid transport mechanism of a StAR-related domain, Nat. Struct. Biol. 7 (2000) 408–414.

[37] M. Calderon-Dominguez, G. Gil, M.A. Medina, W.M. Pandak, D. Rodríguez-Agudo, The StarD4 subfamily of steroidogenic acute regulatory-related lipid transfer (START) domain proteins: new players in cholesterol metabolism, Int. J. Biochem. Cell Biol. 49 (2014) 64–68.

[38] M.B. Rone, J. Fan, V. Papadopoulos, Cholesterol transport in steroid biosynthesis: role of protein-protein interactions and implications in disease states, Biochim. Biophys. Acta 1791 (2009) 646–658.

[39] P.R. Manna, C.L. Stetson, A.T. Slominski, K. Pruitt, Role of the steroidogenic acute regulatory protein in health and disease, Endocrine 51 (2016) 7–21.

[40] M. Cuchel, D.J. Rader, Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? Circulation 113 (2006) 2548–2555.

[41] A.R. Tall, Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high-density lipoproteins, J. Intern. Med. 263 (2008) 256–273.

[42] W. Korytowski, D. Rodriguez-Agudo, A. Pilat, A.W. Girotti, StarD4-mediated translocation of 7-hydroperoxycholesterol to isolated mitochondria: deleterious effects and implications for steroidogenesis under oxidative stress conditions, Biochem. Biophys. Res. Commun. 392 (2010) 58–62.

[43] A.L. Wilcox, L.J. Marnett, Polyunsaturated fatty acid alkoxyl radicals exist as carbon-centered epoxyallylic radicals: a key step in hydroperoxide-amplified lipid peroxidation, Chem. Res. Toxicol. 6 (1993) 413–416.

[44] L.J. Marnett, A.L. Wilcox, The chemistry of lipid alkoxyl radicals and their role in metal-amplified lipid peroxidation, Biochem. Soc. Symp. 61 (1995) 65–72.

[45] J. Brinkhorst, S.J. Nara, D.A. Pratt, Hock cleavage of cholesterol 5α-hydroperoxide: an ozone-free pathway to the cholesterol ozonolysis products identified in arterial plaque and brain tissue, J. Am. Chem. Soc. 130 (2008) 12224–12225.

[46] H. Kühn, A. Borchert, Regulation of enzymatic lipid peroxidation: the interplay of peroxidizing and peroxide reducing enzymes, Free Radic. Biol. Med. 33 (2002) 154–172.

[47] J.P. Thomas, M. Maiorino, F. Ursini, A.W. Girotti, Protective action of phospholipid hydroperoxide glutathione peroxidase against membrane-damaging lipid peroxidation, J. Biol. Chem. 265 (1990) 454–461.

[48] J.P. Thomas, P.G. Geiger, M. Maiorino, F. Ursini, A.W. Girotti, Enzymatic reduction of phospholipid and cholesterol hydroperoxides in artificial bilayers and lipoproteins, Biochim. Biophys. Acta 1045 (1990) 252–260.

[49] H. Imai, Y. Nakagawa, Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells, Free Radic. Biol. Med. 34 (2003) 145–169.

[50] G. Cozza, M. Rossetto, V. Bosello-Travain, M. Maiorino, A. Roveri, S. Toppo, et al., Glutathione peroxidase 4-catalyzed reduction of lipid hydroperoxides in membranes: the polar head of membrane phospholipids binds the enzyme and

addresses the fatty acid hydroperoxide group toward the redox center, Free Radic. Biol. Med. 112 (2017) 1–11.

[51] W. Korytowski, P.G. Geiger, A.W. Girotti, Enzymatic reducibility in relation to cytotoxicity for various cholesterol hydroperoxides, Biochemistry 35 (1996) 8670–8679.

[52] H. Yuan, J. Pratte, C. Giardina, Ferroptosis and its potential as a therapeutic target, Biochem. Pharmacol. 186 (2021) 114486.

[53] M. Jaburek, S. Miyamoto, P. Di Mascio, K.D. Garlid, P. Jazek, Hydroperoxy fatty acid cycling mediated by mitochondrial uncoupling protein UCP2, J. Biol. Chem. 379 (2004) 53097–53102.

[54] A. Lombardi, R.A. Busiello, L. Napolitano, F. Cioffffi, M. Moreno, P. de Lange, et al., UCP3 translocates lipid hydroperoxide and mediates lipid hydroperoxide-dependent mitochondrial uncoupling, J. Biol. Chem. 285 (2010) 16599–16605.

[55] A.H. Payne, D.B. Hales, Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones, Endocr. Rev. 25 (2004) 947–970.

[56] B.R. Zirkin, H. Chen, Regulation of Leydig cell steroidogenic function during aging, Biol. Reprod. 63 (2000) 977–981.

[57] T. Diemer, J.A. Allen, K.H. Hales, D.B. Hales, Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis, Endocrinology 144 (2003) 2882–2891.

[58] S. Azhar, L. Cao, E. Reaven, Alteration of the adrenal cortex antioxidant defense system during aging in rats, J. Clin. Invest. 96 (1995) 1414–1424.

[59] P.H. Kodaman, R.F. Aten, H.R. Behrman, Lipid hydroperoxides evoke antigonadotropic and anti-steroidogenic activity in rat luteal cells, Endocrinology 135 (1994) 2723–2730.

[60] A.W. Korytowski, A. Pilat, J.C. Schmitt, A.W. Girotti, Deleterious cholesterol hydroperoxide trafficking in steroidogenic acute regulatory (StAR) protein-expressing MA-10 Leydig cells, J. Biol. Chem. 288 (2013) 11509–11519.

[61] M. Reers, S.T. Smiley, C. Mottola-Hartshorn, A. Chen, M. Lin, L.B. Chen, Mitochondrial membrane potential monitored by JC-1, Methods Enzymol. 260 (1995) 406–417.

[62] R. Stocker, J.F. Keaney, New insights into oxidative stress in the artery wall, J. Thromb. Haemostasis 3 (2005) 1825–1834.

[63] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, Nat. Rev. Immunol. 13 (2013) 709–721.

[64] S. Vainio, E. Ikonen, Macrophage cholesterol transport: a critical player in foam cell formation, Ann. Med. 35 (2003) 146–155.

[65] A.J. Brown, S.L. Leong, R.T. Dean, W. Jessup, 7-Hydroperoxycholesterol and its products in oxidized low-density lipoprotein and human atherosclerotic plaque, J. Lipid Res. 38 (1997) 1730–1745.

[66] A.J. Brown, W. Jessup, Oxysterols: sources, cellular storage, and metabolism: new insights into their roles in cholesterol homeostasis, Mol. Aspect. Med. 30 (2009) 111–122.

[67] G. Escher, Z. Krozowski, K.D. Croft, D. Sviridov, Expression of sterol 27-hydroxy-lase (CTP27A1) enhances cholesterol efflux, J. Biol. Chem. 278 (2003) 11015–11019.

[68] C. Cavelier, I. Lorenzi, L. Roher, A. von Eckardstein, Lipid efflux by the ATP-binding cassette transporters AVDA1 and ABCG1, Biochim. Biophys. Acta 1761 (2006) 655–666.

[69] F. Borthwick, J.M. Taylor, C. Bartholomew, A. Graham, Differential regulation of the StarD1 subfamily of START lipid trafficking proteins inhuman macrophages, FEBS Lett. 583 (2009) 1147–1153.

[70] W. Korytowski, K. Wawak, P. Pabisz, J.C. Schmitt, A.W. Girotti, Macrophage mitochondrial damage from StAR transport of 7-hydroperoxycholesterol: implications for oxidative stress-impaired reverse cholesterol transport, FEBS Lett. 588 (2014) 65–70.

[71] W. Korytowski, K. Wawak, P. Pabisz, J.C. Schmitt, A. Chadwick, D. Sahoo, et al., Impairment of macrophage cholesterol efflux by cholesterol hydroperoxide trafficking: implications for atherogenesis under oxidative stress, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 2104–2113.

[72] Z. Guo, Q. Ran, L. Jackson Roberts, L. Zhou, A. Richarsson, C. Sharan, et al., Suppression of atherogenesis by overexpression of glutathione peroxidase-4 in apolipoprotein E-deficient mice, Free Radic. Biol. Med. 44 (2008) 343–352.

[73] J.-P. Chanoine, N.A. Compagnone, A.C.K. Wong, S.H. Mellon, Modulation of steroidogenesis by selenium in a novel adrenal cell line developed using targeted tumorigenesis, Biofactors 14 (2001) 229–238.

[74] J.-P. Chanoine, A.C.K. Wong, J.-C. Lavoie, Selenium deficiency impairs corticosterone and leptin responses to adrenocorticotropin in the rat, Biofactors 20 (2004) 109–118.

[75] A.M. James, H.M. Cochemie, R.A.J. Smith, M.P. Murphy, Interactions of mitochondria-targeted and untargeted ubiquinones with the mitochondrial respiratory chain and reactive oxygen species; implications for the use of exogenous ubiquinones as therapies and experimental tools, J. Biol. Chem. 280 (2005) 21295–21312.

[76] A.O. Oyewole, M.A. Birch-Machin, Mitochondria-targeted antioxidants, Faseb. J. 29 (2015) 4766–4771.

[77] M.J. Rossman, J.R. Santos-Parker, C.A.C. Steward, N.Z. Bispham, L.M. Cuevas, H. L. Rosenberg, et al., Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults, Hypertension 71 (2018) 1056–1063.

[78] S.Y. Park, E.J. Pekas, R.J. Headid 3rd, W.M. Son, T.K. Wooden, J. Song J, et al., Acute mitochondrial antioxidant intake improves endothelial function, antioxidant enzyme activity, and exercise tolerance in patients with peripheral artery disease, Am. J. Physiol. Heart Circ. Physiol. 319 (2020) H456–H467.