Published in final edited form as:
Clin Exp Pharmacol Physiol. 2012 February ; 39(2): . doi:10.1111/j.1440-1681.2011.05641.x.

Mitochondria as a Source and Target of Lipid Peroxidation Products in Healthy and Diseased Heart

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Summary

1. The heart is a highly oxidative organ in which cardiomyocyte turnover is virtually absent, making it particularly vulnerable to accumulation of lipid peroxidation products (LPPs) formed as a result of oxidative damage.
2. Reactive oxygen and nitrogen species are the most common electrophiles formed during lipid peroxidation and lead to the formation of both stable and unstable lipid peroxidation products (LPPs). Of the LPPs formed, highly reactive aldehydes are a well-recognized causative factor in aging and age-associated diseases including cardiovascular disease and diabetes.
3. Recent studies have identified that the mitochondria are both a primary source and target of LPPs, with specific emphasis on aldehydes in cardiomyocytes, and how these affect the electron transport system and Ca^{2+} balance.
4. A number of studies have found that there are functional consequences in the heart as a consequence of exposure to specific aldehydes (acrolein, trans-2-hexanal, 4-hydroxynonenal, and acetaldehyde). Since these LPPs are known to form in heart failure, cardiac ischemia/reperfusion injury, and diabetes, they may have an underappreciated role in the pathophysiology of these disease processes.
5. LPPs are involved in transcriptionally regulating endogenous anti-oxidant systems. Recent evidence has demonstrated that transient increases in LPPs might be beneficial in cardioprotection by contributing to mito-hormesis (i.e. this induction of anti-oxidant systems) in cardiomyocytes. Thus, exploitation of cardioprotective actions of LPPs may represent a novel therapeutic strategy for future treatment of heart disease.

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Disclosures
None of the authors have any conflict of interest to declare.

Keywords

Mitochondria; lipid peroxidation products; aldehydes; heart failure; ischemia; cardiac hypertrophy; diabetes

Introduction

The heart is a post-mitotic, highly oxidative organ in which cell turnover rates are virtually absent, so products formed from oxidative damage of tissue and cellular material accumulate with time. The accumulation of these oxidative products is now a well-recognized causative factor in aging and age-related diseases such as diabetes and cardiovascular disease[1]. In fact, for all diseases where acute and chronic oxidative stress is either a causative factor or deleterious consequence, lipid peroxides are starting to take centre stage as the most potent, persistent and physiologically relevant agents of this stress[2–4].

Oxidation of membrane phospholipids, and other polyunsaturated fatty acids (PUFAs) that are stored or otherwise located in cardiomyocytes, is one of the most prominent manifestations of oxidative stress in the heart. With their long, chain-like structure and large number of unsaturated carbon-carbon bonds, PUFAs are some of the most readily oxidized chemicals in nature, occurring through enzymatic or non-enzymatic pathways. Non-enzymatic peroxidation of PUFAs, particularly those contained within membrane phospholipids, is typically initiated by electrophilic attack on one of the methylene carbons contained within the fatty acid side-chains. Reactive oxygen (ROS) and nitrogen species (RNS) are the electrophiles most commonly involved in lipid peroxidation, and if allowed to proceed unchecked this reaction results in formation of both stable and unstable lipid peroxidation products (LPPs). Of all the LPPs formed in this manner, reactive aldehydes have been shown to have regulatory roles in physiological systems, and have been implicated to play important roles in cardiovascular disease[5].

Because of its insatiable appetite for nutrients and oxygen required to meet its energetic demands, the heart contains a very high mitochondrial content relative to other organs. Mitochondria are the largest source of intracellular ROS in cardiomyocytes[6], as superoxide ($O_2^{\bullet -}$) is continuously formed at sites within the electron transport system during oxidative phosphorylation. Furthermore, mitochondria are double membrane-bound organelles that have an enormous amount of unsaturated phospholipids contained within their inner and outer membranes, notably cardiolipin, a phospholipid exclusive to mitochondria that is highly unsaturated and prone to peroxidation[7, 8]. Thus, due to these characteristics mitochondria are ostensibly the largest endogenous source of LPPs in the heart. To offset the formation of LPPs, mitochondria have a vast network of antioxidant and detoxification systems, ensuring that lipid peroxidation and levels of reactive aldehydes are kept at sub-toxic levels. Over time, however, and under various pathological states, these antioxidant and aldehyde detoxification systems become compromised and/or lost, and the subsequent accumulation of LPPs and aldehydes has profound consequences for the function of mitochondria, the cardiomyocyte and the heart. The focus of this review will be on the role of mitochondria as both a primary *source*, and also a *target*, of LPPs and reactive aldehydes, and on the current state of knowledge regarding the role of LPPs and aldehydes in causing mitochondrial and cardiac dysfunction in pathological conditions. A brief overview of the potential role of LPPs in cardioprotection will also be presented.

I. Mitochondria as a primary source of LPPs in heart

Formation of LPPs and aldehydes in mitochondria

It has been known for decades that mitochondria generate reactive oxygen species (ROS) as a by-product of oxidative phosphorylation[9, 10]. Mitochondrial super oxide ($O_2^{\bullet -}$) is formed when electrons 'leak' from the electron transfer system in the mitochondria and are taken on by molecular oxygen[11]. The exact sites of $O_2^{\bullet -}$ formation within the electron transport system are controversial but there is broad consensus that redox reactions within Complex I and Complex III are both capable of producing $O_2^{\bullet -}$ (shown in detail in Figure 1A). However, it is not clear which of these complexes is the predominant site of superoxide formation *in vivo*[12, 13]. Most superoxide ($O_2^{\bullet -}$) is immediately converted to hydrogen peroxide (H_2O_2) by superoxide dismutase enzymes in either the mitochondrial matrix (by manganese superoxide dismutase (MnSOD)) or in the cytosol (by copper/zinc superoxide dismutase (Cu/ZnSOD)). In addition to the electron transport system, mitochondria also generate H_2O_2 from monoamine oxidase (MAO) bound to the outer membrane[11, 14]. MAO is the enzyme responsible for metabolism of catecholamines, and has recently been shown to be a substantial source of H_2O_2 and oxidative stress in heart failure[15].

Nitric oxide, as it exists with its unpaired electron ($NO^{\bullet}$), is also a free radical and can frequently react with superoxide ($O_2^{\bullet -}$) to form peroxynitrite ($ONOO^{\bullet -}$). Peroxynitrite is considered to be the chief reactive nitrogen species formed in physiological systems[16], being a highly reactive electrophile responsible for nitration and nitrosylation reactions with hydroxyl and thiol groups in proteins during periods of oxidative stress. Though still a matter of some debate, several studies have reported the presence of a nitric oxide synthase (NOS) isoform within mitochondria, distinct from other established isoforms of NOS such as eNOS, nNOS and iNOS[17, 18]. The presence of NOS within mitochondria, coupled to continuous $O_2^{\bullet -}$ formation by the electron transport system would suggest that tight regulation of $ONOO^{\bullet -}$ formation and the presence of $ONOO^{\bullet -}$ scavenging systems are critical to maintaining homeostasis.

Ultimately it is peroxynitrite ($ONOO^{\bullet -}$), in addition to hydroxyl radical $OH^{\bullet}$, formed via Fenton reaction of Fe^{2+} or Cu^{2+} with H_2O_2, that initiates lipid peroxidation by electrophilic attack on mitochondrial phospholipids, particularly the high unsaturated cardiolipin with is prone to peroxidation (see Figure 1 for details). Formation of lipid peroxides from cardiolipin through these reactions has been suggested to be partly responsible for the altered cardiac function seen in the aged heart[19]. These changes occur through disruption in the inner membrane of the mitochondria, where it constitutes about 20% of the total lipid composition, and by altering mitochondrial fission and fusion. This leads to destabilization of cytochrome c and the complexes within the electron transport system, all of which have profound effects on cell energetics and vitality[20–22].

If they are not neutralized by endogenous antioxidants, lipid peroxides will fragment and decompose to form reactive aldehydes such as di-aldehydes (malondialdehyde, MDA) and α,β-unsaturated aldehydes (acrolein; 4-hydroxynonenal (HNE); and 4-hydroxyhexenal (HHE))[23]. The 4-hydroxyalkenals formed from PUFA oxidation (HNE from n-6 PUFAs, HHE from n-3 PUFAs) are highly reactive electrophiles capable of covalently modifying proteins, DNA and other macromolecules, similar to the ROS/RNS that spawned them, though they have unique properties endowing them with distinct roles in biological systems. These 4-hydroxyalkenals are uncharged, lipophilic and chemically stable molecules capable of readily diffusing through membranes. In addition, some of the less hydrophobic aldehydes such as MDA, acrolein and 4-hydroxy-hexanals (HHE) are able to diffuse fairly long distances from their sites of origin, enabling them to act as signalling mediators within cells and tissues under various physiological and pathological contexts[5].

Mechanisms for scavenging LPPs and aldehydes

Mitochondria are endowed with such a highly concentrated and layered antioxidant network that they can be considered to not only be primary 'sources' of ROS/RNS within cells, but also primary 'sinks'[11, 24]. This network includes the glutathione and thioredoxin systems, along with peroxidases, catalase, superoxide dismutase, glutaredoxin, sulfiredoxin, peroxiredoxin, and others (see recent comprehensive review[24–26]).

The major endogenous enzyme responsible for neutralizing lipid peroxides in the heart, as in other cell types, is glutathione peroxidase 4 (GPx4)[27, 28]. Glutathione peroxidase 4 resides in the cytosol, nucleus, and the inner membrane of mitochondria, where it utilizes glutathione (GSH) to reduce lipid peroxides to their corresponding alcohol. Glutathione not only provides the reducing power for a large number of redox enzymes capable of reducing reactive oxygen species[29], but it is also capable of neutralizing electrophilic lipids, such as HNE, after they are formed[30]. Figure 2 illustrates the major enzymatic and non-enzymatic endogenous systems present in mitochondria and the cytosol specific for neutralizing LPPs and aldehydes (i.e. scavenging LPPs and aldehydes once they have already been formed). In the heart, the conjugation of glutathione to HNE is catalysed by the enzyme glutathione-transferase, resulting in export of this conjugate from the cell and into the systemic circulation[31]. The aldehyde dehydrogenase (ALDH) family of enzymes is also capable of neutralizing LPPs. In particular, the cytosolic enzyme ALDH3*a1*, also called fatty aldehyde dehydrogenase (FALDH)[32], and ALDH2[33], a mitochondrial matrix enzyme, are the 2 isoforms of ALDH that are most likely to be the enzymes responsible for detoxification of LPPs *in vivo* (circled in blue in Figure 2), though other isoforms may also play a role. Aldehyde dehydrogenase (ALDH) converts aldehydes to acetate, rendering them far less reactive and virtually benign.

In addition to the endogenous systems in place to neutralize lipid peroxides, chemical agents have been identified that have the capacity to neutralize LPPs and aldehydes (see Figure 2). Lipophilic antioxidants such as vitamin E, α-lipoic acid, and co-enzyme Q10 have the capacity to inhibit lipid peroxidation and prevent adverse downstream effects[34, 35]. Agents that effectively neutralize and/or remove aldehydes in both experimental models of metabolic and cardiovascular diseases as well as clinical studies include taurine[36], histidine analogs[37], and carnosine[38]. These agents provide options for stand-alone or supplemental therapy in treating diseases where suppression or removal of LPPs is desired.

II. Mitochondria as a primary target of LPPs in heart

Studies of the effects of lipid peroxidation in cardiac tissue are sparse, even though this tissue may easily be considered to have the highest capacity for this process. Most research directed at identifying molecular targets of LPPs has been conducted in models of cancer, neuronal disease or in supra-physiological experimental conditions. This complicates extrapolation of these findings to other organs or to systems where lipid peroxidation may be a constitutive process that is fundamental to maintaining homeostasis, such as the heart[39].

Cardiomyocytes possess the highest mitochondrial density of all tissues in the body, and therefore have a higher capacity for reactive oxygen species production. In addition to the high poly-unsaturated fatty acid (PUFA) content distributed across the mitochondrial membrane, there is also an omnipresent pool of free fatty acids, the preferred substrate of cardiac mitochondria. This fatty acid pool closely reflects a person's dietary intake. According to some estimates the n-6:n-3 PUFA ratio approaches 10:1 in the archetypal Western diet[40]. Hence, HNE would be expected to constitute a much higher proportion of LPPs in individuals on such a diet. Approximately 2-8% of HNE produced is involved in largely irreversible cell conjugation reactions with both cellular and mitochondrial

proteins[41]. The following sections discuss the resultant mitochondrial component damage and aberrations in homeostasis.

LPP effects on cellular membranes

The primary source of ROS in cardiomyocytes is the electron transport system located in the mitochondrial inner membrane[42]. Thus, the most immediate target of the 4-hydroxyalkenals are the lipids present in the phospholipid bilayer[43, 44] and its associated proteins[45]. The reaction of 4-hydroxyalkenals with the mitochondrial membrane results in altered lipid-lipid and protein-lipid interactions. This occurs through a variety of mechanisms, including cross-linking of the lipid tails, which limits phospholipid mobility in the bilayer[46]. Of singular importance, the inner mitochondrial membrane mosaic contains cardiolipin, a highly unsaturated phospholipid specifically localized to this compartment, which is particularly prone to peroxidation. Its presence in the inner membrane, constituting ~20% of the lipids here, ensures the efficient function of a battery of mitochondrial components such as the electron transport chain complexes, adenine nucleotide transporter and the acylcarnitine carrier, among others[47, 48]. Cardiolipin levels diminish dramatically in robust lipid peroxidation conditions[49], and diminished levels of cardiolipin have been observed in many pathological conditions including aging, Barth Syndrome, heart failure, ischemia/reperfusion injury, diabetes, and neurodegenerative diseases[47]. If 4-hydroxyalkenals migrate to the cell membrane, the high number of conjugated unsaturated carbon bonds in the cholesterol molecule make it an attractive target for attack[50]. Collectively, studies on the effects on lipid peroxidation on membrane physiology demonstrate a marked reduction in membrane fluidity[46, 51, 52] and an increase in membrane permeability[53, 54]. Moreover, the production of HNE from lipid peroxidation gives rise to a chain of chemical reactions and products. The products formed from electrophilic attack by the 4-hydroxyalkenals degrade to generate more aldehydes, which serve to propagate the cycle[55]. Some of the more stable products include isoprostanes and neuroprostanes following cyclization of the fatty acid tails[45].

LPP affects the Electron Transport Chain and disturbs intracellular Ca2+ in cardiomyocytes

4-hydroxyalkenals target enzymatic, structural, and signalling proteins present in the phospholipid bilayer and mitochondrial matrix. Like all electrophiles, 4-hydroxyalkenals have a characteristic affinity for sulfhydryl groups (e.g. cysteine, methionine), although lysine and histidine are also subject to covalent modification in their amide groups through Michael addition reactions[23, 56–58]. HNE augments the accumulation of damaged proteins by interfering with normal proteolysis pathways[5960]. A recent proteomic study of the relative levels of adducted proteins in rat cardiac mitochondria treated with a spectrum of LPPs including 4-hydroxynonenal (HNE) and 4-hydroxyhexanal (HHE) revealed that proteins of the electron transport chain constituted the greatest percentage of altered proteins. Moreover, this group observed that cysteine constituted an overwhelmingly high percentage of total modified residues (85%), while histidine accounted for 12% and lysine less than 5%[61]. The concentration of 4-hydroxynonenal (HNE) and 4-hydroxyhexanal (HHE) adducts were relatively similar to one another. This study underscores the high propensity that LPPs exhibit towards attacking protein components involved in cellular respiration[41, 62–64], expanding our current knowledge of the negative impact of the LPPS on mitochondrial homeostasis.

Prior to this point there have been multiple studies conducted to determine the effects of reactive aldehydes on mitochondrial components. Most of these studies to date, summarized in Table 1, are from experiments performed in a variety of oxidative tissues like brain, liver, kidney, as well as heart. Questions remain as to whether the observed effects of these LPPs seen in ‘other-than-cardiac’ tissues would also be observed in heart. In the heart, both 4-

hydroxynonenal (HNE) and 4-hydroxyhexanal (HHE) have been shown to affect mitochondrial homeostasis by disturbing the balance of ions[65, 66]. 4-Hydroxynonenal attacks the sulfhydryl groups of the Na+/K+ ATPase[67] resulting in diminished activity and elevated intracellular concentrations of free calcium. Both HNE and HHE have been implicated in inducing Ca^{2+} overload in cardiomyocytes[68]; activation of uncoupling proteins[69] and premature opening of the mitochondrial permeability transition pore (MTP)[70] thus increasing the risk of heart failure[3, 71].

LPP effects on DNA and other cellular targets

The role of the 4-hydroxyalkenals in DNA adduct formation during mutagenesis is well documented with respect to HNE. HNE displays a differential affinity for the respective bases with the highest reactivity toward guanosine[72], cytosine, adenine, and least toward thiamine[73]. Cellular machinery is capable of repairing damage to the DNA inflicted by LPPs by excising the damaged portion, although long term exposure to LPPs leads to an accumulation of damaged DNA. In addition to its well-documented reactivity with DNA, HNE is both directly and indirectly involved in the upregulation of several cell signalling pathways including protein kinase C, mitogen activated protein kinases, IκB Kinase Complex, tyrosine receptor kinases, serine/threonine protein kinases, and C-Jun H-Terminal kinases[57, 71, 74–78].

III. The effects of clinically relevant LPPs on cardiac function

When LPPs are increased in the heart, their effect on cardiac function is uniformly detrimental. However, with respect to the effects of individual LPPs on cardiac function at the molecular level, either as protein adducts or free LPPs, very little is known. The LPPs acrolein, trans-2-hexanal, 4-hydroxynonenal, and acetaldehyde all affect cardiac function and cardiomyocyte viability, each contributing synergistically to adversely affect the heart during times of increased oxidative stress and lipid peroxidation. Since other aldehydes described in previous sections react similar to the LPPs described here, the following findings may act as general examples by which LPPs might affect cardiac function and viability.

Acrolein

Acrolein, the simplest unsaturated aldehyde, is produced widely in different tissues and is found ubiquitously in the environment as a pollutant. It has also recently been identified as a product of lipid peroxidation formed in response to increased oxidative stress. Acrolein binds to cysteine and lysine residues on proteins and has been suggested to be a mediator of oxidative damage in a variety of human diseases[79–81]. Despite the well-known reactivity of unsaturated aldehydes, their effects on cardiac function were only recently described. Mice challenged with intravenous acrolein (0.5 mg/kg) responded with a rapid and reversible left ventricular dilation and dysfunction[82]. In isolated cardiomyocytes, this same study identified that micromolar concentrations of acrolein acutely decreased contractile responses to Ca^{2+}, but did not alter catecholamine sensitivity, paralleling what is found in the stunned myocardium[82]. Attenuation of acrolein's effects on cardiac function and protein adduct formation could be achieved by pre-treatment with N-acetylcysteine, indicating that ROS mediates some of the effects of acrolein on the heart[82]. Mass spec analysis identified that acrolein formed protein adducts on both sarcomeric proteins (cardiac α-actin, desmin) and proteins involved in energy metabolism, including creatine kinase-2 and ATP synthase[82]. Experimental acrolein therefore induces cardiac dysfunction, selective myofilament impairment, and modifies proteins involved in energy metabolism, which possibly affects their function. Cardiac pathologies that produce acrolein in response to oxidative stress may therefore have some or all of these mechanisms at work to inhibit cardiac function.

Trans-2-hexanal

Like acrolein, trans-2-hexanal is a ubiquitous pollutant as well as an endogenous product of lipid peroxidation[83], which similarly has potent effects on cardiac function. When 8 week old ICR mice were fed trans-2-hexanal for 4 weeks, significant impairment in cardiac function ensued[84]. Echocardiographic studies identified impaired contractile function within 4 weeks of trans-2-hexanal feeding and histological analysis of the heart identified myofibril disarray. Trans-2-hexanal feeding also resulted in increased cardiomyocyte apoptosis and aldehyde-protein adduct formation[84]. These findings demonstrate that trans-2-hexanal exposure can lead to cardiac dysfunction by its effects on sarcomere stability and apoptosis, resulting in significant functional deficits[84]. The specific mechanisms by which these effects are mediated, either through trans-2-hexanal adducts or through direct aldehyde effects on signalling, have not yet been determined.

4-hydroxynonenal

In cardiac ischemia reperfusion injury, the production of excess reactive oxygen species trigger increases in lipid peroxidation, resulting in the generation of α,β-unsaturated aldehydes, including 4-hydroxynonenal (HNE). Recent studies have started to delineate the role of HNE on cardiac function. While lower concentrations of HNE are not toxic to cultured cardiomyocytes, higher levels of HNE (≥ 20 μM) are[85]. Interestingly, priming cardiomyocytes with sub-lethal concentrations of HNE (5 μM) results in a "pre-conditioning" that protects cardiomyocytes from subsequent cytotoxic concentrations of HNE[85]. This is due, in part, to HNE-induced nuclear translocation of NF-E2 p45 related factor 2 (Nrf2), which increases intracellular glutathione (GSH) levels through its transcriptional upregulation of glutathione-S-transferase (GST)[85]. Nrf2 is a transcription factor which acts as a master regulator of the antioxidant response, transcriptionally inducing antioxidant systems such as glutathione S-transferase (GST)[86]. Therefore, these studies demonstrate that ≥ 20 μM of HNE is cardio-toxic, while levels around 5 μM of HNE induce endogenous anti-oxidant systems through Nrf2 activation. The mechanisms by which HNE induces nuclear translocation of Nrf2, and subsequent cardioprotection, have not yet been completely delineated. The implications of these findings in the context of ischemia-reperfusion injury are discussed in the next section.

Acetaldehyde

Acetaldehyde is formed in cardiac ischemia/reperfusion (I/R) injury and during the metabolism of ethanol in the heart. The direct effects of acute acetaldehyde on the heart after 5-10 minutes of exposure have been reported at least 8 times[87–92]. Acetaldehyde causes vasoconstriction and positive inotropic and chronotropic responses at concentrations ≤ 3 mM. Levels >3 mM induce cardiac dysfunction, vasodilation, and hypotension[93, 94]. The negative inotropy seen with these higher levels of acetaldehyde is associated with decreased release of SR Ca^{2+} [87, 89] and inhibition of voltage-dependent Ca^{2+} channels[95]. Acetaldehyde depresses myocardial contraction, cardiac myocyte shortening and intracellular Ca^{2+} transients by increasing β-adrenergic activity at low doses, and reducing Ca^{2+} entry and/or release at high doses[90]. The decrease in myocardial function by acute acetaldehyde exposure has been reported to be enhanced in the diabetic state[91] and in the presence of nicotine[96]. Acetaldehyde may inhibit cardiac contractility by other mechanisms, including its effects on cytochrome P450 oxidase, xanthine oxidase, and lipid peroxidation[97]. The implications of these findings in the context of ischemia reperfusion injury and ethanol exposure is discussed in the next section.

IV. LPPs in Diseased Heart

Even though the functional consequences of LPPs in the heart have not been studied extensively, their presence in the heart has been widely reported. What has been most extensively studied is how anti-oxidants are cardioprotective in disease[98–100], although the disappointing clinical utility of these therapies illustrates our lack of knowledge of the pathophysiology of oxidative stress, and the limited utility of studies using anti-oxidants[101, 102]. In this section, we focus on LPPs that have been identified in cardiac disease. LPPs have been reported in heart failure and myocardial infarction, in addition to the cardiomyopathies associated with diabetes and ethanol use. While these observations do not constitute direct evidence that LPPs are involved in the pathogenesis of these diseases, they do suggest clinical scenarios by which LPPs should be investigated for evidence that they directly mediate the pathophysiology of disease.

Clinical studies implicating LPPs in cardiovascular disease

Probably the best described role for LPPs in human heart disease is in the etiology of atherosclerosis, where LPPs initiate and sustain inflammatory reactions along the arterial wall[103]. Oxidized phospholipids have a high affinity for lipoproteins (oxLDL), and increased levels of these adducts are seen in serum of patients with cardiovascular disease[104], as were antibodies to them[105]. The correlation between LPPs in serum and cardiovascular diseases is now so strong that they are frequently used as biomarkers of prognosis for patients with acute coronary syndrome[106], or for atherosclerotic development[107].

In addition to the vascular effects of LPPs, a number of studies have shown increased levels of MDA-adducts in myocardial samples of patients with heart failure[108, 109]. Heart failure is associated with numerous metabolic, functional, and structural defects. The role of anti-oxidant stress in heart failure is just beginning to be appreciated. When heart biopsies from dilated cardiomyopathy (DCM) patients were compared to donor controls, increased malondialdehyde (MDA) was found regionally in hearts of DCM patients[110]. MDA concentrations were generally higher in the DCM group compared to controls, but were decreased in the endocardium and aortic samples in the DCM group[110]. These studies indicate not only that in DCM-mediated heart failure ventricular MDA is increased, but also that this increase is not uniform throughout the heart[110]. In a model of chronic heart failure in hamsters (TO-2 strain), recent studies have identified HNE adducts both histologically and by ELISA, paralleling increases in other markers of oxidative stress, such as HSP27, HSP32, and manganese superoxide dismutase[111]. Persistent oxidative stress has been shown to be present in serum of patients with congestive heart failure as well[112], indicating that a more ‘global’ cardiovascular oxidative stress is likely predominant in heart failure. The recent success of the non-selective β-blocker Carvedilol in demonstrating marked improvements in suppressing markers of oxidative stress by reducing the levels of HNE-adducts[113] in hearts of treated patients, highlights the importance of this characteristic in the pathophysiology of heart failure. While the lipid peroxidation products MDA and HNE have both been reported elevated in models of heart failure, their role in the pathophysiology of heart failure has not been elucidated.

Cardiac ischemia reperfusion injury generates HNE, H_2O_2, and MDA

In addition to formation of HNE (described above), cardiac ischemia reperfusion injury also generates hydrogen peroxide (H_2O_2) and MDA. When isolated rat hearts are challenged with I/R injury, they generate elevated concentrations of hydrogen peroxide, MDA, and calcium[114]. The formation of hydrogen peroxide appears to lead to increased lipid peroxidation, evidenced by increased levels of MDA. Pre-treating hearts with scavenger

oxyradicals (superoxide dismutase and catalase) or antioxidants attenuate I/R damage, also implicating ROS in I/R induced damage[115]. The specific mechanisms in cardiac I/R by which LPPs induces membrane defects to affect calcium overload and contractile dysfunction have yet to be elucidated, but may be related to ROS and LPP production.

Diabetes generates glycoaldehyde

While the cardiovascular complications of diabetes account for more than 80% of patient mortality, mechanisms by which glucose intermediates induce diabetic cardiomyopathy have not been fully elucidated. Recent studies suggest that the glycoaldehyde may mediate some of the cardiac defects. Hyperglycemia increases oxidative stress in the diabetic state, which leads to increases in advanced glycation endproducts (AGE) formed from glucose itself. Glucose itself is an aldehyde, which can react non-enzymatically with proteins (via lysine) to make AGE products (adducts) when glucose is elevated chronically. One of the early reactive intermediates formed during the formation of AGE products is glycoaldehyde (GA). Recent studies have identified that glycoaldehyde induces oxidative stress in the heart. When male Wistar rats are challenged with a single injection of GA, decreased catalase and glyoxalase I activities are identified, resulting in increased protein carbonylation and lipid peroxidation[116]. These studies suggest that the short chain glycoaldehyde (GA) produced during the formation of AGE products may play a significant role in diabetic cardiomyopathy. The direct effects of AGE products or the GA intermediate on the heart to mediate diabetic cardiomyopathy has yet to be determined. Hyperglycemia also induces the production of cardiac MDA in vivo. Using streptozotocin (STZ)-induced diabetic male Wistar rats, cardiac tissue was assayed after 4 weeks of hyperglycemia. Hyperglycemia in this model resulted in significant increases in cardiac MDA[117]. However, the role of MDA on cardiac function in this model remains to be determined.

Cardiac Acetaldehyde and Oxysterols are found with chronic ethanol feeding and in ischemia reperfusion injury

The relevance of acetaldehyde in cardiac disease comes from recent studies demonstrating that acetaldehyde is produced in states in which lipid peroxidation is increased, including I/R injury, similar to other reactive aldehydes such as HNE and MDA[80, 118]. Acetaldehyde is also formed in the heart as well as in the liver during ethanol exposure[119]. It is the direct effects of acetaldehyde on the heart, discussed in the previous section, which have been implicated in the pathogenesis of cardiac I/R injury[33, 120–127] and alcoholic cardiomyopathy[90, 91, 96, 97, 128–130].

Acetaldehyde formed during ethanol consumption may also mediate cardiac dysfunction due to its effects on mitochondrial damage and the regulation of the mitochondrial death pathway[129, 130]. This was determined in experiments using mice with cardiac-specific over-expression of alcohol dehydrogenase (ADH), which leads to an increase in the formation of acetaldehyde. When these ADH transgenic mice are challenged with ethanol (3/g/kg/d) for 3 days, myocardial mitochondrial damage and apoptosis occurred, leading to reduced contractility, and enlarged cardiomyocytes[130]. Additional studies using these ADH transgenic mice have also found dysregulation in ER stress and insulin sensitivity. In response to ethanol challenge, ADH transgenic mice develop cardiac hypertrophy and dilated cardiomyopathy, suggesting a role of acetaldehyde in this process[131, 132]. While acetaldehyde is significantly increased in this transgenic mouse model fed ethanol, its direct role in the development of cardiac hypertrophy and dilated cardiomyopathy has yet to be determined.

A role for acetaldehyde in ischemia reperfusion injury has been determined using genetic mouse models[33, 120–127]. Recent studies have found that I/R injury is exacerbated when

aldehyde dehydrogenase 2 (ALDH2) is missing in ALDH2 −/− mice[133]. One of the primary functions of ALDH2 is to metabolize acetaldehyde (among other aldehydes). By decreasing toxic aldehydes, ALDH2 inhibits the production of free radicals, preventing mitochondrial dysfunction[33, 121, 134]. The ALDH2 −/− mice, therefore, have an exaggerated I/R injury due to increased aldehydes and ROS, both of which lead to impairment of the mitochondria. However, ALDH2 has additional beneficial mechanisms in the heart unrelated to its ability to clear aldehydes, including its role in regulating nitro-glycerine bioactivation, mTOR-STAT3-notch signalling, and autophagy[33, 121, 134]. So the cardioprotective effects of ALDH2 in I/R injury may be due to either direct clearance of acetaldehyde or through indirect regulation of other biologies. The exact mechanisms have not yet been delineated.

V. The transcriptional regulation of endogenous anti-oxidant systems by LPPs and their role in Hormesis and Cardioprotection

We previously introduced the endogenous antioxidant systems that are induced in response to LPPs. Here we introduce the underlying mechanisms by which LPPs induce antioxidant system by their induction of the Nrf2 transcription factor activity. One of the prominent sensors of oxidative stress involves the Keap1/Nrf2/ARE signalling network (see recent reviews for details[135–139]). Briefly, under normal conditions, the Nrf2 (NF-E2-related factors 2) transcription factor associates with the suppressor protein Keap1 (Kelchlike ECH-associated protein 1) in the cytosol (34). When Nrf2 is bound to Keap1, it is ubiquitinated by the Cullen3-Rbx1 ubiquitin ligase, which targets this complex for degradation by the 26S proteasome[140]. Keap1 has many cysteines, 27 in all, which can be modified in the presence of oxidative stress, making it an ideal redox sensor. In this way, free aldehydes and other electrophilic LPPs formed in oxidative conditions can attack and form adducts on these cysteines. Chemical modification of Keap1 with free aldehydes leads to its disassociation with Nrf2, allowing Nrf2 to move into the nucleus[139]. Cysteines in the 273, 288, and 151 positions of Keap1 have been reported as particularly sensitive to chemical modification and play a key role in its interaction with Nrf2[141, 142]. Upon entering the nucleus, Nrf2 binds to the antioxidant response element (ARE) found in the promoter regions of key antioxidant enzymes. These include glutathione S-transferase, UDP-glucuronosyl transferase (UGT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and peroxiredoxin[139]. Additionally, Nrf2 activates genes involved in the cellular redox regulation including glutathione synthetase, thioredoxin, thioredoxin reductase, NAD(P)H: quinone oxidoreductase 1 (NQO1), among others[139]. Nrf2 is also regulated by phosphorylation by protein kinases involved in the stress response, such as ERK1/2, PKC, JNK, which enable its disassociation from its repressor Keap1[137].

Understanding how exposure to LPPs initiate the antioxidant response via the Keap1/Nrf2/ARE signalling pathway allows a mechanistic discussion of how exposure to LPPs may initiate a series of protective actions in the heart. This concept has been previously described as Hormesis. Hormesis is the cellular response by a low-dosed sub-lethal stressor, which results in a cellular reaction that over-compensates, allowing the resistance of subsequent lethal doses of the stressor[139, 143]. There are many examples of this concept in biology, including exercise. The “exercise paradox”, which is the observation that exercise increases oxygen consumption and the generation of ROS, also leads to increases in cyto-protective elements, including glutathione as discussed in the previous paragraph[144, 145]. This finding seems to contradict the paradigm that oxidative stress is unconditionally detrimental in physiological systems, and responsible for age-related decline. Perhaps the words of the early Swiss intellectual Paracelsus provide a simple but insightful answer “*Poison is in everything, and nothing is without poison. The dosage makes it either a poison or remedy”*. This concept underscores a growing consensus that the relationship between oxidative stress and aging may not be as clear-cut and unidirectional after all, as highlighted in a recent

review by Ristow and Schmeisser[146]. As our understanding of redox signalling in homeostasis grows, it is becoming increasing evident that the body needs small doses of 'poison' to mount and maintain adequate defences to blunt the effects of a full-on oxidative onslaught. This precarious balance between poison and remedy is a familiar feature of bi-phasic dose-response curves[147] and is called hormesis. The direct relevance of inducing this pre-emptive cytoprotective response specifically in mitochondria has led to the term 'mitohormesis'[148–151].

The concept of mitohormesis causing cardioprotection via Nrf2 (e.g. through induction of anti-oxidant networks in the mitochondria) is new and uncharted ground, yet pioneering studies are very promising. The role of Nrf2 in cardioprotection is, at this point, unequivocal, and has been demonstrated in multiple transgenic and Nrf2−/− mouse models. In an experimental model of pressure overload induced by transverse aortic constriction, overexpression of Nrf2 proved to be protective against cardiac hypertrophy[152]. Furthermore, in the previous section we described how pre-treatment with non-lethal doses of HNE (5 μM) could induce cardioprotection to larger, lethal doses (≥20 μM) of HNE[85]. The role of this pre-conditioning has been investigated in cardiac ischemia *in vivo*. When mice are pre-treated with HNE (4 mg/kg) prior to an I/R challenge, their heart is protected against injury compared to vehicle-treated mice[85]. The mechanism of this protection paralleled *in vitro* findings described in the previous section. HNE activates the transcription factor Nrf2, which in turn up-regulates endogenous anti-oxidant systems, including GST. This in turn appears to increase intramyocardial GSH content and decrease ROS, resulting in a better functional recovery of the LV following I/R injury. The molecular mechanisms by which HNE regulates Nrf2 have yet to be elucidated, but the importance of Nrf2 in cardioprotection has been determined using Nrf2 −/− mice. The HNE induced "pre-conditioning" cardioprotection in I/R injury is absent in Nrf2 −/− mice, implicating Nrf2's role in mediating HNE induced GSH biosynthesis in the heart[85].

VI. Concluding Perspectives

The importance of LPPs in the pathogenesis, and paradoxically, prevention, of cardiac disease remains an area of research that demands renewed attention. Clearly lipid peroxidation and the reactive lipid peroxidation products have context-specific effects on physiology, with differential effects being driven by organ-specific localization, structure and reactivity of LPP in question, dosage and temporal parameters. An increased understanding of these complex questions may lead to better understanding and improved therapies of cardiovascular diseases associated with redox imbalances.

Acknowledgments

The authors' laboratories are supported by the National Institutes of Health R21HL098780 (to E.A.) and R01HL104129 (to M.W.).

Non-standard abbreviations

ADH	alcohol dehydrogenase
AGE	advanced glycation endproducts
ALDH	aldehyde dehydrogenase
ARE	Antioxidant Response Element
Cu/ZnSOD	copper/zinc super oxide dismutase

GSH	glutathione
GST	glutathione-S-transferase
HHE	4-hydroxyhexenal
HNE	4-hydroxyhexenal
H_2O_2	hydrogen peroxide
I/R	ischemia reperfusion
Keap1	Kelchlike ECH-associated protein 1
LPP	lipid peroxidation products
MTP	mitochondria transition pore
MDA	malondialdehyde
MnSOD	Manganese Superoxide Dismutase
NO	nitric oxide
Nrf2	NF-E2 p45 related factor 2
$O_2^{\bullet -}$	superoxide
$ONOO^{\bullet -}$	peroxynitrite
PUFA	poly-unsaturated fatty acids
ROS	reactive oxygen species
RNS	reactive nitrogen species

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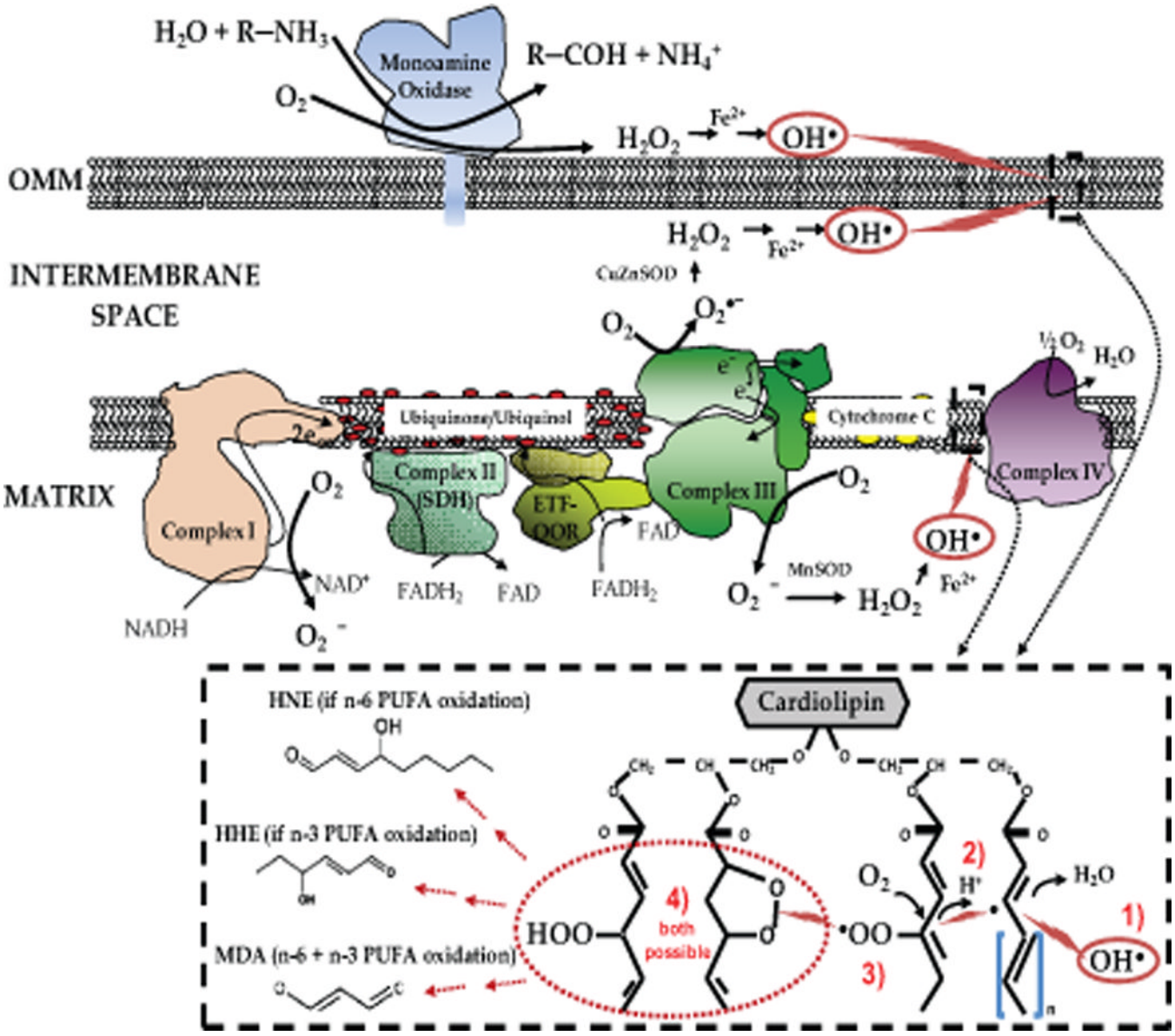

Figure 1.

Top: Primary sites of ROS formation in mitochondria, and reaction scheme for peroxidation of cardiolipin in outer- and inner-mitochondrial membrane. Sites of ROS formation at Complex I and III are depicted, with the formation of hydroxyl radical ($OH^{\bullet -}$) circled in red. Electrophilic attack on cardiolipin from $OH^{\bullet -}$ initiates lipid peroxidation by abstracting hydrogen from a methylene carbon in cardiolipin fatty acyl side-chain. Stepwise reaction scheme and products formed are outlined in dashed box. ***Dashed box:*** The general reaction scheme of non-enzymatic lipid peroxidation in mitochondria with initial step coming from $OH^{\bullet}$ attack of unsaturated fatty acids contained within cardiolipin (step 1). Following initiation by $OH^{\bullet}$, an unstable lipid radical is formed, which can continue to abstract allylic hydrogens from nearby unsaturated fatty acids (step 2), or react with molecular O_2 (step 3) to form a lipo-peroxyl radical that either continues on to react with another fatty acid forming a new radical, or reacts with itself to form a lipid peroxide (step 4).

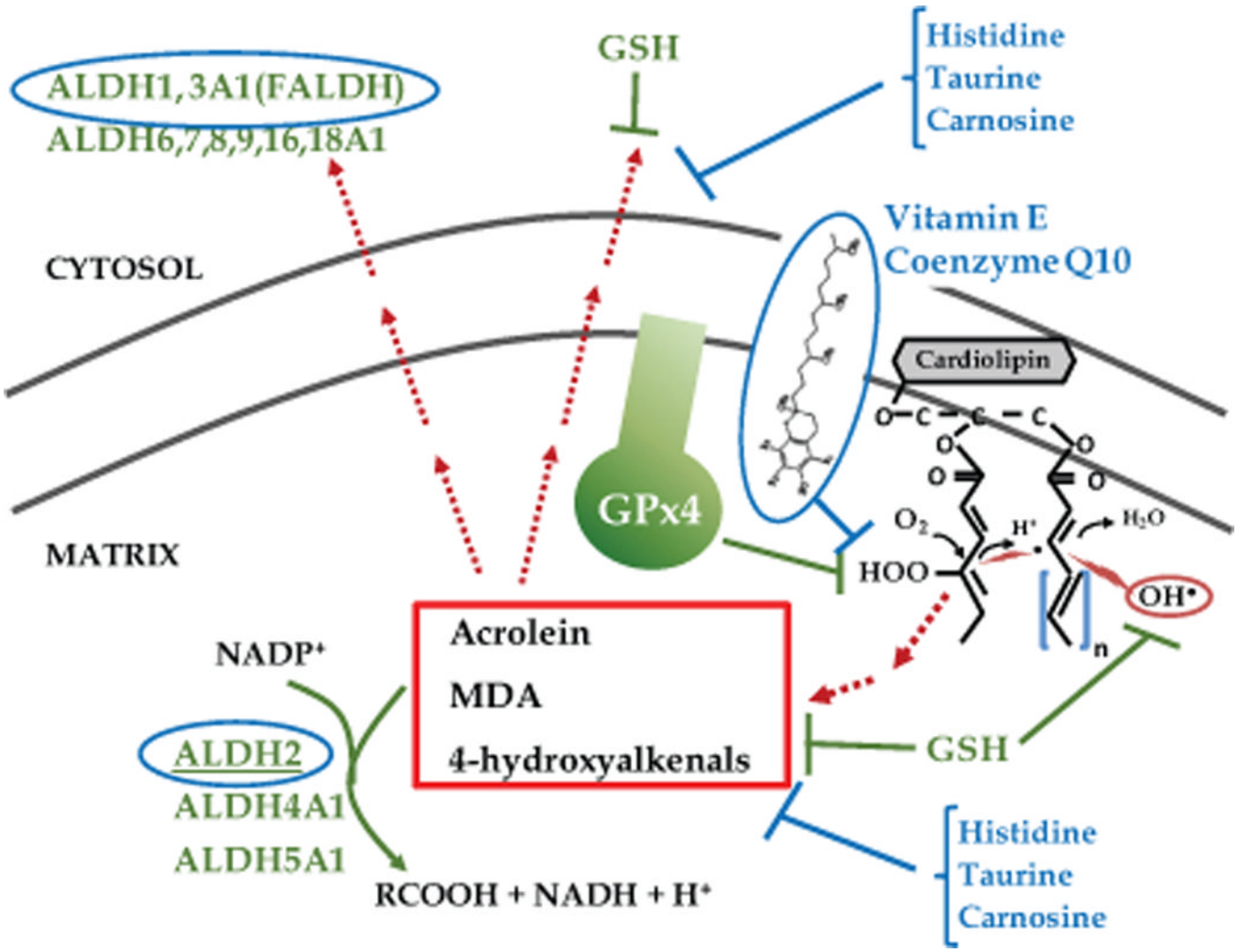

Figure 2.

The major lipid peroxidation product detoxification systems present in the mitochondria and cytosol. Lipid peroxidation can be blunted directly by GSH removal of $OH^{\bullet -}$ prior to imitation of lipid peroxidation. GSH is also used by the enzyme glutathione peroxidase 4 (GPx4), also known as phospholipid hydroperoxide glutathione peroxidase, to neutralize lipid peroxides as they are formed in mitochondrial membrane. Once aldehydes are formed (examples in red box), the most physiologically relevant enzyme in neutralizing these electrophiles is the aldehyde dehydrogenase (ALDH) family, with ALDH2 and ALDH3a1 as most likely candidate enzyme for removal of aldehydes in mitochondrial matrix and cytosol, respectively. Exogenous agents known to suppress lipid peroxidation and/or neutralize aldehydes are listed in blue text.

Table 1

Summary of the of lipid peroxidation products on mitochondria.

Mitochondrial Target	LPP	Model	Effect
UCP	HNE	Heart-mouse Kidney-mouse Skeletal muscle-mouse	↑ uncoupling of respiration at UCP1, UCP2 & UCP3[69, 151]
Complex I	MDA	Liver-rat	↓ activity[153]
	HNE	Heart-rat	↓ activity in model of diabetic cardiomyopathy[154]
	Acrolein	Heart-rat	↓ activity[155]
		Brain-Guinea pig	↓ activity[156]
		Brain & spinal cord-rat	↓ activity[157] ↓ activity[63]
		Liver-rat	80%↓ activity[158]
Complex II	MDA	Liver	↓ activity[153]
	HNE	Heart-rat	↓ activity[154]
	Acrolein	Heart-rat	↓ activity[155]
		Brain-Guinea pig	↓ activity[156]
		Brain-rat	↓ activity[159]
		Liver-rat	80% ↓ activity[158]
Complex III	HNE	Heart-Aging	protein modification, ↓activity especially in interfibrillar mitochondria[160]
Complex IV	HNE	Heart-Aging	protein modification ↓ activity especially in interfibrillar mitochondria[160]
	MDA	Aging/Liver-rat	↓ activity[161]
ATP synthase	MDA	Heart /Skeletal Muscle-Mouse	↓ activity modification targeted at the F1 complex[162]
Cytochrome c oxidase	HNE	Heart - rat	~60%↓activity in rat reperfusion model[163]
	LPPs	Heart-rat	↓activity in rat reperfusion model[49]
Adenine Nucleotide Translocase (ANT)	Acrolein	Brain-Guinea pig	↓activity at concentrations higher than 5uM[156]
	HNE	Flight muscle/Aging-Housefly	↓activity[164]
		Heart/Kidney/Skeletal Muscle	↑uncoupling of respiration at ANT[69, 151]
		Liver - rat	↓ transportation capacity[165]
α-Ketoglutarate dehydrogenase	Acrolein	Liver-rat	↓activity[158]
		Enzyme assay	↓activity[166]
	MDA	Liver-rat	↓ activity[153]
		Heart & Skeletal-Mouse	↓ activity modification at E2 component of a-KD complex[162]
	HNE	Heart-rat	↓activity, modifies E2 component of α-KD[167]

Mitochondrial Target	LPP	Model	Effect
			↓ concentration, potent inhibitor of isolated subsarcolemmal mitochondria[168]
			↓activity in reperfusion model[169]
Succinate dehydrogenase	Acrolein	Liver-rat	↓ activity[170]
		Brain-Guinea pig	↓ activity[156]
	HNE	Heart-rat	30% ↓oxygen consumption, modifies the FAD-containing subunit in diabetic cardiomyopathy[154]
Succinic semialdehyde dehydrogenase	HNE	Brain-rat	↓activity at IC50 110uM[171]
	Acrolein	Brain-rat	↓activity at 15uM respectively[171]
Pyruvate dehydrogenase complex	Acrolein	Liver-rat	↓ activity[158]
		Brain-Guinea pig	↓ activity[166]
		Brain & Spinal Cord-rat	↓ activity[157]
		Enzyme assay	↓ activity[156]
	MDA	Liver-rat	↓ activity[153]
Isocitrate dehydrogenase complex	HNE	Heart-rat	50 uM 85% ↓activity[172] in spontaneously hypertensive rats
Alcohol dehydrogenase	Acrolein	Liver-rat	potent inhibitor of ADH at ↓ concentrations[173]
Aconitase	Acrolein	Brain-Guinea pig	↓activity[156]
	MDA	Heart& Skeletal Muscle-Mouse	↓activity[162]
Heat shock protein 90	HNE	Liver-rat	Inhibits refolding of HSP 90 by modification of cysteine[174]
Mitochondrial permeability transition pore	Acrolein	Liver-rat	↑MTP induction[158]
	HHE	Liver-rat	↑MTP induction[70]
	HNE	Liver-rat	↑MTP induction[70]

Abbreviations: ↓, decreased; ↑, increased; ADH; aldehyde dehydrogenase; HNE, 4-hydroxynonenal; HSP90, heat shock protein 90; LPP, lipid peroxidation products; MDA, malondialdehyde; MTP, mitochondrial transition pore; UCP1-3; uncoupling proteins 1–3