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Review article

Lipoxidation in cardiovascular diseases

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ABSTRACT

Lipids can go through lipid peroxidation, an endogenous chain reaction that consists in the oxidative degradation of lipids leading to the generation of a wide variety of highly reactive carbonyl species (RCS), such as short-chain carbonyl derivatives and oxidized truncated phospholipids. RCS exert a wide range of biological effects due to their ability to interact and covalently bind to nucleophilic groups on other macromolecules, such as nucleic acids, phospholipids, and proteins, forming reversible and/or irreversible modifications and generating the so-called advanced lipoxidation end-products (ALEs).

Lipoxidation plays a relevant role in the onset of cardiovascular diseases (CVD), mainly in the atherosclerosis-based diseases in which oxidized lipids and their adducts have been extensively characterized and associated with several processes responsible for the onset and development of atherosclerosis, such as endothelial dysfunction and inflammation.

Herein we will review the current knowledge on the sources of lipids that undergo oxidation in the context of cardiovascular diseases, both from the bloodstream and tissues, and the methods for detection, characterization, and quantitation of their oxidative products and protein adducts.

Moreover, lipoxidation and ALEs have been associated with many oxidative-based diseases, including CVD, not only as potential biomarkers but also as therapeutic targets. Indeed, several therapeutic strategies, acting at different levels of the ALEs cascade, have been proposed, essentially blocking ALEs formation, but also their catabolism or the resulting biological responses they induce. However, a deeper understanding of the mechanisms of formation and targets of ALEs could expand the available therapeutic strategies.

1. Lipid peroxidation and formation of advanced lipoxidation end-products (ALEs)

Lipid peroxidation is an endogenous chain reaction that consists in the oxidative degradation of lipids; free radicals or non-radical species attack lipids containing carbon-carbon double bonds, with the hydrogen abstraction from a carbon and oxygen insertion that results in lipid peroxyl radicals and hydroperoxides [1].

Lipid peroxidation generates a wide variety of oxidation products, among which the main primary products are lipid hydroperoxides (LOOH) [2]. Lipid hydroperoxides are non-radical intermediates that originate from phospholipids, unsaturated fatty acids, glycolipids, cholesterol and cholesterol esters.

Both enzymatic and non-enzymatic pathways are involved in lipid hydroperoxides formation [3]. Indeed, lipids can be oxidized by several enzymes like cyclooxygenases, lipoxygenases and cytochrome P450 [4].

On the other hand, non-enzymatic lipid peroxidation involves the generation of free radicals (mainly reactive oxygen species (ROS), such as hydroxyl radical ($HO^{\bullet}$), hydroperoxyl ($HOO^{\bullet}$) and peroxynitrite ($ONOO^-$)) that attack lipids containing carbon-carbon double bonds, like polyunsaturated fatty acids (PUFAs) in cell membranes [2]. PUFAs have multiple double bonds between which there are methylene bridges ($-CH_2-$) that contain reactive hydrogen atoms, and they can be classified in omega-3 (n-3) and omega-6 (n-6) fatty acids. Mitochondria, plasma membrane, endoplasmic reticulum, and peroxisomes are the main sources of ROS; $HO^{\bullet}$ and $HOO^{\bullet}$ are the principal ROS that affect lipids. Lipid peroxidation is propagated in a process that is called "chain reaction mechanism", which usually terminates when an antioxidant molecule reacts with the lipid peroxide radical, as summarized in Fig. 1.

Lipid hydroperoxides are the primary peroxidation products and give rise to the generation of short-chain unesterified aldehydes and a second class of aldehydes still esterified to the parent lipid [1]. Moreover, lipid hydroperoxides can be structurally rearranged and converted into secondary peroxidation products that are highly reactive, known as reactive carbonyl species (RCS). RCS are a heterogeneous group and can be classified into short-chain carbonyl derivatives and oxidized truncated phospholipids [5]. The first group includes α,β-unsaturated aldehydes (e.g. 4-hydroxy-2-nonenal (4-HNE), 4-hydroxy-2-

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Abbreviations

15d-PGJ2	15-deoxy-Δ12,14-prostaglandin J2
15-LOX	15-lipoxygenases
2,5-DBA	2,5-dihydroxy-benzaldehyde
2DE	two-dimensional electrophoresis
4-APC	4-(2-(trimethylammonio)ethoxy)benzenaminium halide
4-HBA	4-hydroxybenzaldehyde
4-HNE	4-hydroxy-2-nonenal
8-epi-PGF2a	15(S)-8-iso-prostaglandin F2alpha
AA	arachidonic acid
ACE	angiotensin-converting enzyme
ACR	acrolein
AGE	advanced glycation end-products
AIDA	alternate isotope-coded derivatization assay
ALEs	advanced lipoxidation end-products
ApoB	apolipoprotein B
ApoE	apolipoprotein E
ATP	adenosine triphosphate
BHT	butylated hydroxytoluene
CE	capillary electrophoresis
CHD	1,3-cyclohexandione
CoA	coenzyme A
COX	cyclooxygenase
CPT1	carnitine palmitoyl transferase 1
CVD	cardiovascular diseases
CyPG	cyclopentenone prostaglandin
DAG	diacylglycerol
DDB	1,2-diamino-4,5-dimethoxybenzen
DHA	docosahexaenoic acid
DNPH	2,4-dinitrophenylhydrazine
DPPP	diphenyl-1-pyrenylphosphine
EAT	epicardial adipose tissue
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid
ER	endoplasmic reticulum
ESR	electron spin resonance
F2-IsoPs	F2-isoprostanes
FABP	fatty acid binding proteins
FACS	fatty acyl-CoA synthetase
$FADH_2$	flavin adenine dinucleotide
FATP	fatty acid transport proteins
FFAs	free fatty acids
FOX	ferrous oxidation-xylenol orange
GC	gas chromatography
G-PC	glutaric acid ester of 1-acyl-*sn*-glycero-3-phosphocholine
GSH	glutathione or γ-L-glutamyl-L-cysteinylglycine
HDL	high-density lipoprotein
HLB	hydrophilic lipophilic balanced
HOCl	hypochlorous acid
HPLC	high performance liquid chromatography
HSP60	heat shock 60 kDa protein
IDL	intermediate-density lipoprotein
IKKβ	IkB kinase β
IR	ischemia/reperfusion
IsoPs	isoprostanes
LC	liquid chromatography
LC-FD	LC-fluorescence detection
LC-UV	LC-ultraviolet
LDLs	low-density lipoproteins
LLE	liquid-liquid extraction
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
LV	left ventricular
MALDI-TOF	matrix assisted laser-desorption ionization-time of flight
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
MRM	multiple reaction monitoring
MS	mass spectrometry
NAC	N-acetyl cysteine
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NO	nitric oxide
OFL	ofloxacin
ONE	4-oxo-nonenal
OV-PC	5-oxovaleric acid ester of 1-acyl-*sn*-glycero-3-phosphocholine
oxLDL	oxidized low-density lipoproteins
oxPL	oxidized phospholipid
PCs	phosphatidylcholines
PDGFR	platelet-derived growth factor receptor
PDGFβ	platelet-derived growth factor receptor-β
PFB-Br	pentafluorobenzyl bromide
PGC-1α	peroxisome proliferator-activated receptor γ coactivator-1α
PM	pyridoxamine
POBN	α-(4-pyridyl-1-oxide)-N-*tert*-butyl nitrone
PPAR	peroxisome proliferator-activated receptor
PTB	N-phenacylthiazolium bromide
PUFAs	polyunsaturated fatty acids
RCS	reactive carbonyl species
RNS	reactive nitrogen species
ROS	reactive oxygen species
SC	subcutaneous
SPE	solid-phase extraction
SRM	selected reaction monitoring
TAG	triacylglycerol
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TLC	thin-layer chromatography
TLR	Toll like receptors
UCPs	uncoupling proteins
VLDL	very low-density lipoprotein

hexenal, nonenal and acrolein (ACR)), di-aldehydes (e.g. malondialdehyde (MDA) and glyoxal) and ketoaldehydes (e.g. methylglyoxal, 4-oxo-nonenal (ONE) and isoketals also called levuglandins). In the second class, the electrophilic moiety remains covalently linked to the phospholipid. Electrophilic prostaglandin metabolites are an additional class of RCS that are characterized by a cyclopentenone. The cyclopentenone prostaglandins are structurally and functionally related to a subset of isoprostanes (IsoPs), a series of prostaglandin-like compounds produced via a non-enzymatic mechanism involving the free radical-mediated peroxidation of PUFAs, mostly arachidonic acid (AA) [6].

RCS exert a wide range of biological effects due to their ability to interact and covalently react with nucleophilic groups on other macromolecules, such as nucleic acids, phospholipids, and proteins, to form irreversible modifications, generating the so-called advanced lipoxidation end-products (Fig. 2) [7].

Covalent adducts of lipid peroxidation products with DNA have been described both at nuclear and mitochondrial DNA level with

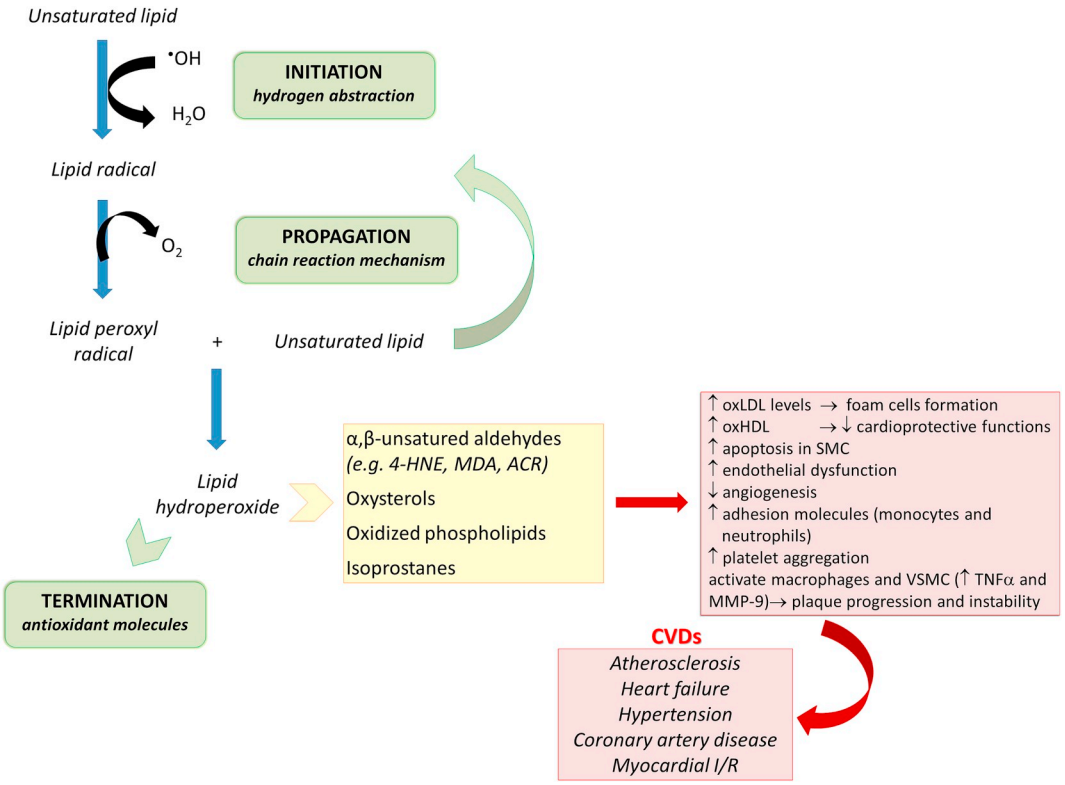

Fig. 1. Scheme of the lipid peroxidation reaction and its effects in the development and progression of CVDs. 4-HNE, 4-hydroxy-2-nonenal; ACR, acrolein; HDL, high-density lipoprotein; I/R, ischemia/reperfusion; LDL, low-density lipoproteins; MDA, malondialdehyde; MMPs, metalloproteinases; TNFα, tumor necrosis factor α; VSMC, vascular smooth muscle cells.

potential mutagenic and carcinogenic effects [2,8]. Additionally, RCS can interact with aminophospholipids, generating covalent adducts such as carboxymethyl-phosphatidylethanolamine or MDA-phosphatidylethanolamine, and starting the reactions occurring in DNA and proteins [7,9].

Finally, both short-chain RCS and oxidized phospholipids can covalently react with proteins generating Michael adducts or Schiff bases, early products that are reversible or can further rearrange forming additional specific reaction compounds [10,11]. However, the protein targets could be different for the two classes, because the short-chain RCS or RCS bound to phospholipids have diverse cellular distribution. The carbonyl group reacts with the nucleophilic side chains on amino acid residues of proteins, such as the imidazole nitrogen of histidine, the thiol group of cysteine, the epsilon amino group of lysine or the guanidine of arginine [5,10]. An example of ALE is represented by carboxymethyl-lysine (CML), a product of the reaction with glyoxal, which derives by both lipid and sugar oxidation pathway and is considered a general marker of oxidative stress and protein damage in cardiovascular diseases and aging [5,12].

Further, bifunctional α,β-unsaturated aldehydes, such as 4-HNE, can act as small crosslinkers between or within proteins leading to the formation of aggregates, besides their effects on protein function [13]. The formation of MDA adducts on proteins generates highly cross-linked undegradable aggregates, such as the fluorescent pigment lipofuscin, which can compromise cell viability [14].

Additionally, prostaglandins present an electrophilic carbon that can interact with the nucleophilic amino acids generating, with a pathway similar to that described for α,β-unsaturated aldehydes, a Michael adduct with effects on cell signaling pathways [10].

1.1. Electrophilic lipid species detectable in cardiovascular diseases

1.1.1. Lipid peroxidation-derived aldehydes

4-hydroxy-2-nonenal (4-HNE) is produced by the oxidation of PUFAs containing n-6 acyl groups, such as AA and linoleic acid, and it is the most abundant lipid-derived RCS. 4-HNE can be produced by the decomposition of lipid peroxidation products that are generated both by enzymatic reactions triggered by 15-lipoxygenases (15-LOX) or from several non-enzymatic oxidative pathways through mechanisms involving the reactions of free radicals and their products [2].

Nowadays 4-HNE is considered one of the major toxic products due to its rapid reaction with thiols and amino groups [15]. 4-HNE usually forms Michael adducts with His, Cys, and Lys residues and the preference for the amino acid modification is Cys ≫ His > Lys.

The degradation of n-6-PUFAs also generates 4-oxo-nonenal (ONE), which derives from the peroxidation of linoleoyl chains of fatty acids. ONE is a more reactive product than 4-HNE and modifies the nucleophilic side chains of Lys, Cys, Arg and His on proteins. ONE can form, at C3 and C2, 4-ketoaldehyde Michael adducts that are relatively unstable and can eventually produce furan derivatives or more stable compounds, such as dihydropyrroles and isomeric 4-ketoamide derivatives [16]. ONE also reacts with Lys forming a Schiff base, which can give the fluorophore pyrrolium Lys-Lys crosslink and Lys-derived pyrrolinone. Unlike 4-HNE, ONE reacts also with Arg residues to yield stable covalent adducts, but this reaction is less favorable than the other amino acid nucleophiles (i.e. preference order is Cys > His > Lys > Arg).

Acrolein (ACR) arises not only by peroxidation of PUFAs but also from the metabolism of amino acids, polyamines, and drugs. Under oxidative stress and inflammation, the endogenous production of acrolein is also controlled by myeloperoxidase-mediated degradation of threonine and the amine oxidase-mediated degradation of spermine and spermidine [5]. Compared to all α,β-unsaturated aldehydes, ACR has the highest reactivity with protein nucleophiles (i.e. the order of

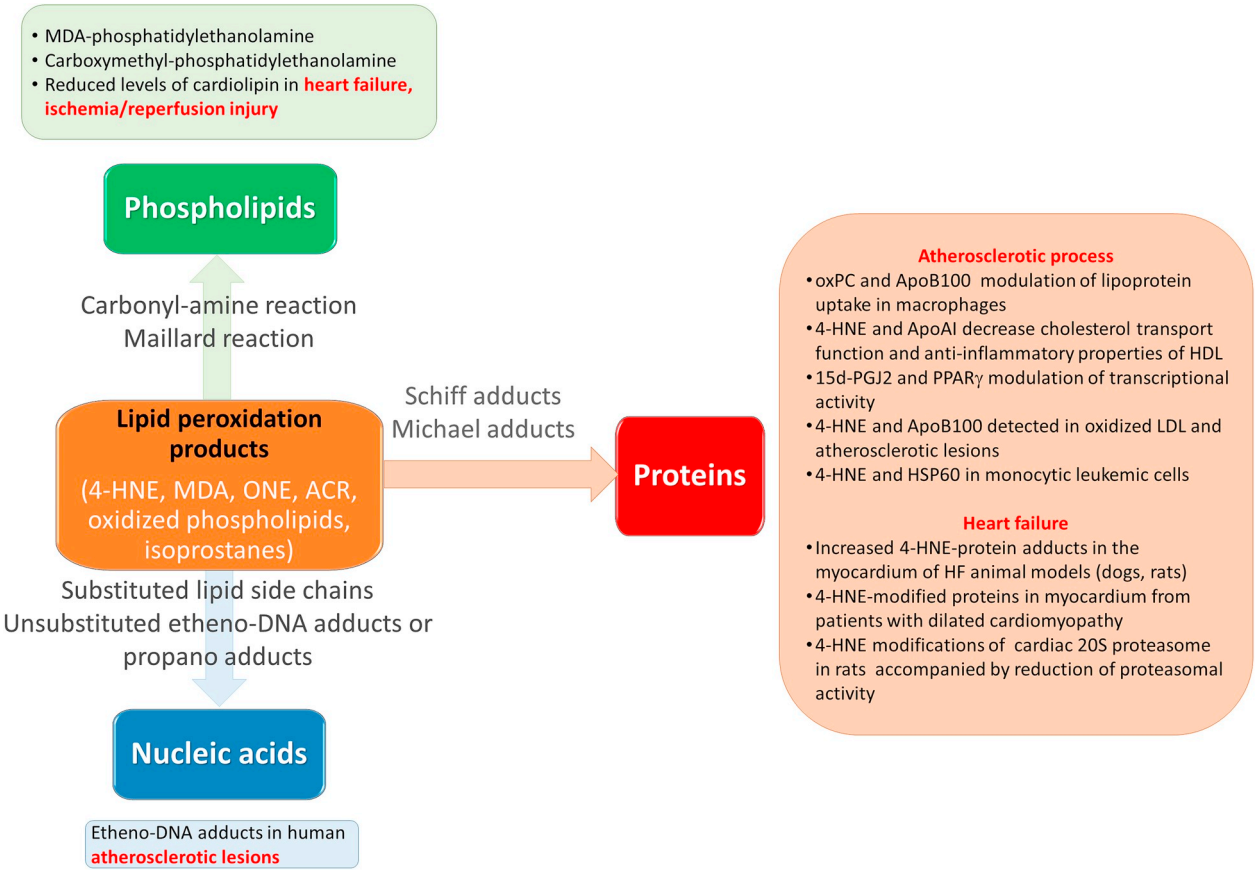

Fig. 2. Mechanisms involved in the formation of advanced lipoxidation end-products. Relevant targets and their involvements in cardiovascular diseases are highlighted. On nucleic acids, the exocyclic amino group of deoxyguanosine, deoxyadenosine, and deoxycytosine can react with α,β-unsaturated aldehyde generating two types of adducts: substituted lipid side chains (for example with MDA, ACR or 4-HNE) and unsubstituted etheno-DNA adducts (for example with 2,3-epoxy-4-hydroxynonanal). On proteins, the reaction of aldehydes with the free amino group of lysine or arginine generates a Schiff base, while the reaction of the electrophilic carbon of α,β-unsaturated aldehydes with the nitrogen lone pair of lysine and histidine or the -SH of cysteine generates a Michael adduct, which is more stable even if both these adducts are reversible.

reactivity is Cys > His > Lys). The formation of ACR adducts on Cys residues is the principal way, but ACR forms additional ALEs with His and Lys, as detected in the oxidized low-density lipoproteins (oxLDL) [17].

Malondialdehyde (MDA) results from lipid peroxidation of PUFAs with two or more methylene-interrupted double bonds. It is a reactive aldehyde that forms interactions with nucleic acids and covalent protein adducts inducing toxic stress in cells [18]. MDA is a highly toxic molecule and it is usually considered a biomarker of oxidative stress in the organism. The most important precursors of MDA are monocyclic peroxides obtained from fatty acids with three or more double bonds. MDA can be also derived by enzymatic processes from prostaglandins during the biosynthesis of thromboxane A2 and 12-l-hydroxy-5,8,10-heptadecatrienoic acid [18]. MDA is able to form adducts with several proteins, introducing cross-links that can alter their biochemical properties. MDA reactivity is pH-dependent: at physiological pH, it can rapidly form the enolate salt, which is of low reactivity; at lower pH, MDA exists as β-hydroxyacrolein, the reactivity increases, and reacts with Lys forming N-propenal-Lys.

1.1.2. Prostaglandin-like compounds: isoprostanes

The IsoPs are a class of prostaglandin-like compounds that are generated *in vivo* from the non-enzymatic free radical-catalyzed peroxidation of essential fatty acids (mainly AA) [19,20]. They are produced in cell membranes at the site of free radical attack, from which they are removed by phospholipases, get into the circulation and then are excreted in urine. They have been found also in other fluids, such as pericardial fluid [21] and cerebrospinal fluid [22,23]. The F2-isoprostanes (F2-IsoPs) are a class of stable molecules that are generated *in vivo* under conditions of oxidative stress, so they can be used as an effective measure of endogenous oxidative stress. Other classes of IsoPs from AA have been described and these classes differ based on the functional groups on the prostane ring. In particular, E2/D2-IsoPs are not terminal products of the IsoPs pathway, but they can rearrange to form A2/J2-IsoPs, also known as cyclopentenone IsoPs, that contain α,β-unsaturated cyclopentenone ring structure [24]. A2/J2-IsoPs react with cellular thiols to form Michael adducts [25], that are metabolized *in vivo* by glutathione transferase enzymes to water-soluble modified glutathione conjugates. Isoprostanes can be also produced from the PUFAs, such as EPA, DHA, adrenic acid, and α-linolenic acid [26,27]. Moreover, the compounds known as isoketals or isolevuglandins are produced *in vivo* by lipid peroxidation and rearrangement of endoperoxide intermediates of the isoprostane pathway [28], and they covalently form adducts with Lys residues on proteins inducing a biological dysfunction. Oxidation of AA can also yield to cyclic peroxides and isofurans, when the intermediate undergoes a 5-exo cyclization reaction with molecular oxygen [29,30].

1.1.3. Oxidized phospholipids (oxPLs)

oxPLs, found in oxidized lipoproteins or in membranes of apoptotic cells, include hundreds of different structures and can be generated both enzymatically, by lipoxygenases, myeloperoxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and non-enzymatically [31]. Oxidized forms of phosphatidylserine and

phosphatidylethanolamine have been described but the most oxidized phospholipid is phosphatidylcholine, which accounts for 40–50% of phospholipids in mammalian cells or lipoproteins.

Alkyl peroxyl radicals or singlet oxygen directly oxidize phospholipid-bound PUFAs to generate fatty acyl derivatives, such as fatty acid hydroperoxides, which are compounds that subsequently and easily undergo decomposition to various reactive products. A common reaction, involving the reduction of fatty acid hydroperoxides, leads also to the generation of truncated phospholipids, such as γ-hydroxyalkenal phosphatidylcholines (PCs). These molecules can undergo another step of fragmentation to the 5-oxovaleric acid ester of 1-acyl-*sn*-glycero-3-phosphocholine (OV-PC) or glutaric acid ester of 1-acyl-*sn*-glycero-3-phosphocholine (G-PC) that have been described in atherosclerotic lesions. Additionally, phospholipid-bound PUFAs are prone to non-enzymatic formation of IsoPs, while they are not a target of cyclooxygenases, and several IsoP-PCs have been described also in atherosclerotic regions [31].

2. Lipid sources in the cardiovascular system

Lipids, with a relevant role in the onset of cardiovascular diseases and susceptible to oxidation, can be originated from many sources, both in the bloodstream as in tissues (Fig. 3).

2.1. Circulating lipoproteins and lipids

Lipoproteins, the major carrier of water insoluble lipids in the blood, are complex particles with a central core of cholesterol esters and triglycerides surrounded by free cholesterol, phospholipids, and apolipoproteins. They are divided into classes according to their size, lipid compositions and apolipoproteins (chylomicrons, chylomicron remnants, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, high-density lipoprotein (HDL), and lipoprotein (a) (Lp(a))). Lipoproteins play a role in the adsorption and transport of dietary lipids from the intestine and endogenous lipids from the liver. HDL are involved in the so-called reverse cholesterol transport from the peripheral tissues to the liver and intestine, thus exerting a protective role. LDL, being the main player in cholesterol transport to the cells, are instead considered a well-established cardiovascular risk factor. This lipoprotein class is responsible for the development of atherosclerosis as a consequence of its modification (oxidation, acetylation, and aggregation) in the sub-endothelial space. Following these modifications, LDL provoke endothelial injury, promote inflammatory cells adhesion and their differentiation to macrophages [32]. Further, a plethora of effects, relevant to the disease progression, is induced, such as endothelial dysfunction, endothelial nitric oxide synthase (eNOS) inhibition, inflammatory cytokines release, and platelet aggregation. Vascular endothelium is a dynamic structure that has many important functions, including regulation of vascular tone, hemostasis, molecular exchange between blood and tissues, and signaling for the immune response and

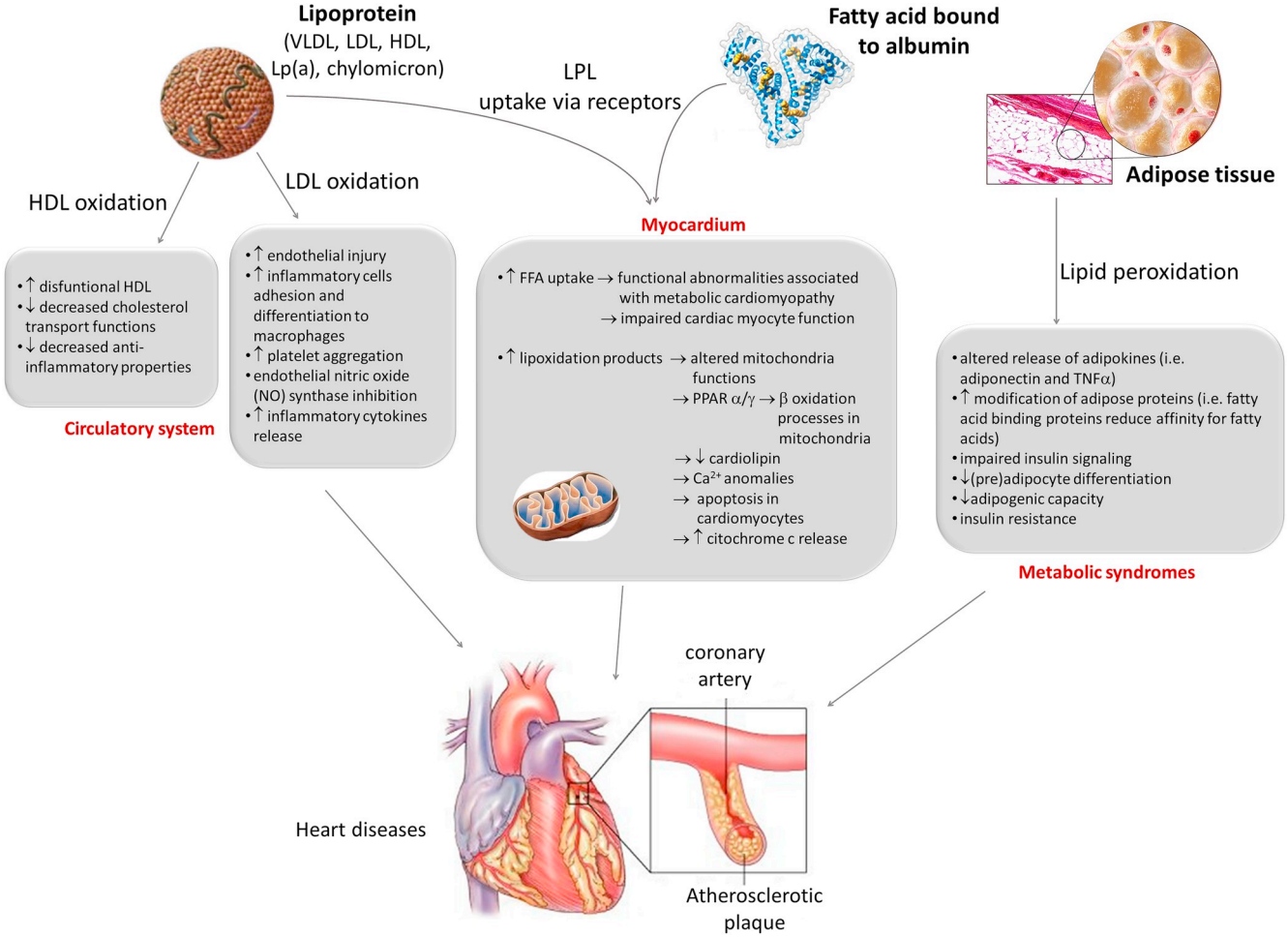

Fig. 3. Relevance of circulating and tissue lipids in the development of cardiovascular diseases. VLDL, very low-density lipoprotein; LDL, low-density lipoproteins; HDL, high-density lipoprotein; Lp(a), lipoprotein(a); LPL, lipoprotein lipase; FFA, free fatty acids; PPAR, peroxisome proliferator-activated receptor; TNFα, tumor necrosis factor α.

inflammation [33,34]. The endothelium maintains vascular tone through the synthesis of vascular dilatory and constricting molecules and controls lipid modification. Alterations of these normal functions lead to atherogenesis. Endothelial dysfunction is clinically characterized by impaired endothelium-dependent vasorelaxation due to a reduced nitric oxide (NO) bioavailability, which is considered an independent factor used for prognosis of patients with cardiovascular disease [35,36]. Endothelial dysfunction has a key role in the development of several cardiovascular diseases and their complications, including atherosclerosis, coronary artery disease, heart failure, as well as diabetes and obesity, which are known risk factors for cardiovascular diseases. The endothelial injury causes the infiltration of LDL-cholesterol into the vessel wall and the oxidation of cholesterol that is a key step in the atherosclerosis development. Cholesterol oxidation products are significantly accumulated in atherosclerotic lesions, such as oxysterols and core-aldehydes. OxLDL uptake and foam cells formation are facilitated by the presence of endothelial scavenger receptors, which have a significant role in promoting atherogenesis. For example, lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) is one of the major endothelial receptor for oxLDL and promotes apoptosis of endothelial cells, increases the expression of monocyte chemotactic protein 1 (MCP-1) and transforming growth factor β1 (TGFβ1), decreases eNOS activity, and induces matrix metalloproteinases production in vascular smooth muscle cells [37,38].

Although the oxidative hypothesis of atherosclerosis was mostly investigated on LDL, there is also evidence that oxidation on other lipoproteins, such as on HDL, can take place in atherogenesis leading to their conversion from atheroprotective, anti-inflammatory, and antioxidant particles to deleterious ones [39]. This HDL remodeling paves the way to the concept that the quality and functionality, instead of the quantity of HDL plasma level, is more relevant, in light of the observations that HDL-raising therapies did not result in cardiovascular risk reduction [40].

Several processes have been involved in the oxidation of lipoproteins, including transition metals, lipoxygenases, hemoglobin, and ROS (reviewed in Ref. [41]). Of note, hypochlorous acid (HOCl) can modify the lipid and protein components of LDL *in vitro* and/or *in vivo* [42] and MDA reacts with the positively charged ε amino group of lysyl residues present in the LDL apolipoprotein ApoB-100, leading to increased uptake by macrophages [43]. ApoAI, the main apolipoprotein of HDL, could be oxidatively modified, leading to dysfunctional HDL and increasing cardiovascular risk [44].

Antibodies against oxidized phospholipids have also been used to detect oxPL-protein adducts in human plasma proteins and LDL. For example, the use of the E0-series of monoclonal auto-antibodies to detect the occurrence of adducts of oxidized phosphatidylcholines, such as 1-palmitoyl2-(5′-oxo)valeroyl-*sn*-glycero-3-phosphocholine, with ApoB-100, showed that the adducts were crucial in inducing uptake of modified lipoproteins by scavenger receptors into foam cells [45].

Very recently, Que et al. suggested that therapies inactivating oxidized phospholipids might be beneficial for reducing a generalized inflammation, including the progression of atherosclerosis, aortic stenosis and hepatic steatosis [46].

Additionally, endothelial cells are highly predisposed to 4-HNE induced damage, which includes increment of proinflammatory factors and failure of the endothelial barrier function. Increased 4-HNE reduces NO bioavailability associated with a decrease in dimethylarginine dimethylamine hydrolase (DDAH) activity [47], which leads to high methylarginine levels, reduction of eNOS-derived NO production, and consequently endothelial dysfunction.

The endothelium expresses also other important targets of lipoxidation, such as soluble epoxide hydrolase (sEH), whose major function is to metabolize the epoxides of AA and linoleic acid. sEH rapidly hydrolyzes epoxyeicosatrienoic acids (EETs), which have been demonstrated to be endothelium-derived hyperpolarizing factors, to dihydroxyeicosatrienoic acids (DHETs) [48]. EETs are vasodilators independent of NO that have protective effects on the vasculature and the heart. Thus, inhibitors of sEH reduce inflammation and prevent the development of atherosclerotic plaques [49].

It has been demonstrated that n-3 PUFAs decrease sEH protein expression in rat aortic strips and endothelial cells [48]. Moreover, 4-hydroxy hexenal (4-HHE), which is a lipid peroxidation product of n-3 PUFAs and a potential activator of the Nrf2 pathway [50], also decreased sEH protein expression increasing the EETs concentration. The antioxidant N-acetyl-L-cysteine (NAC) pretreatment inhibits the reduction in sEH protein induced by n-3 PUFAs or 4-HHE. Oxidative stress induces p38 kinase activation in vascular endothelial cells, thus the inhibition of p38 kinase diminishes the effect of n-3 PUFAs and 4-HHE on sEH protein expression.

Oxysterols, oxidized phospholipids, free and core-aldehydes promote the inflammatory process underlying atherosclerosis, extracellular matrix deposition and arterial wall remodeling [51]. Advanced lipoxidation end-products, such as MDA- and 4-HNE-protein adducts, can promote monocyte activation and vascular complications via induction of inflammatory pathways and networks [52]. In monocytes, ALEs can lead to cellular dysfunction, adhesion to the endothelium, and transmigration into the subendothelial space, through several monocyte-macrophage inflammatory cytokines and chemokines. All these events are important in the pathogenesis of atherosclerosis. Oxidized LDL promotes macrophage proinflammatory gene expression, such as TNFα, IL-1β, and IL-6, through ligand activation of peroxisome proliferator-activated receptor gamma (PPARγ) [53]. Lipid peroxidation products increase the expression of MCP-1, interferon-γ–inducible protein-10, and cyclooxygenase-2 (COX-2), as well as facilitate transcription of nuclear factor (NF)-κB that is a redox-sensitive gene regulatory factor activated by modified LDL internalization.

Leukotrienes, derived from the oxidation of AA, are themselves a family of eicosanoid inflammatory mediators that stimulate the activation of endothelial cells and foam cells.

Acrolein, for example, increased macrophages expression of the atherogenic factors 5-lipoxygenase, leukotriene B4 and matrix metalloproteinase [54,55], and induced mast cell degranulation that can increase inflammatory injury [56].

Additionally, 4-HNE induced the synthesis of metalloproteinases MMP-9 in macrophages [57] and MMP-2 in vascular smooth muscle cells [58], thus contributing to the instability of the atherosclerotic plaque consequently leading to the thrombus formation in atherosclerosis.

Indeed, in smooth muscle cells, 4-HNE stimulates the activity of extracellular matrix-degrading matrix metalloproteinases-1 and -2 [59], protein kinase B (Akt) [58], p38 mitogen-activated protein kinase (MAPK) [60] and induces the phosphorylation of c-jun N-terminal kinase (JNK) [61].

Of note, the balance between ROS and endogenous antioxidants has different effects in different vascular beds, due to the evident differences between arteries and veins in term of cellular composition. ROS regulate multiple cellular functions, among which endothelial and smooth muscle cells growth, proliferation, migration and apoptosis, angiogenesis, vascular tone, and genomic activity through several transcription factors [62].

NAD(P)H oxidase (i.e. Nox enzymes) activity is the major source of ROS in the vasculature, both in human veins and arteries, and it has an important role in the disease development. It has been demonstrated that NAD(P)H oxidase system is the greater source of superoxide in veins, whereas in addition, xanthine oxidase seems to largely contribute to superoxide production in arteries [63]. NAD(P)H oxidase is present both in vascular smooth muscle cells and in endothelial cells, but there are some differences in the component distribution of NAD(P)H oxidases in specific vascular cells, in the extent of superoxide anion radicals production and in the stimuli of NAD(P)H oxidases activation [64]. The molecular composition of vascular NAD(P)H oxidases seems to be different in the vascular cell types and at different phases of

atherosclerotic plaques progression [63]. Moreover, NAD(P)H oxidase is Nox2-based in saphenous veins, while it is proportionally mostly Nox4-based in arteries. The relative contribution of the individual vascular wall segments to the total superoxide production was evaluated, and in human veins, the major contribution comes from the endothelium and adventitia, while in arteries the smooth muscle cells have a key role [63].

Recently, it has been reported that 4-HNE-adducts accumulate mainly in smooth muscle cells in human aorta, and their content increases with the age in the intima and the adventitia, and at a lower amount in the media [65]. The accumulation of 4-HNE-adducts is very high in the intimal aorta and predominantly in patients with high atherosclerosis grade. The 4-HNE expression is also increased in the adventitia, probably associated with the vasa vasorum and microcapillaries in atherosclerotic lesion [66]. Aortic elastin is not modified by 4-HNE, indicating that elastin is not a target of 4-HNE in the vascular wall, but 4-HNE contributes to its degradation and impaired regeneration [67,68].

Myeloperoxidase, lipoxygenases, mitochondria and uncoupled eNOS are other sources of oxidants in the vessel wall that are usually expressed in human atherosclerotic lesions [64].

2.2. Lipids in the myocardium

To sustain its intense metabolism, the myocardium must continually generate, at a high rate, adenosine triphosphate (ATP), which is produced in the mitochondrion utilizing all classes of energy substrates, such as lipids, carbohydrates, amino acids, and ketone bodies (for details see review [69]). In the normal heart, mitochondria are mainly fueled by fatty acyl-coenzyme A (CoA) and pyruvate, which are the primary metabolites of fatty acids and carbohydrates, respectively.

Fatty acids are supplied to the myocardium either attached to the blood albumin or covalently bound to circulating lipoproteins in the form of triacylglycerol. Triglyceride rich lipoproteins (chylomicron and VLDL) are indeed an important source of fatty acids, being chylomicrons the major contributor. The enzyme lipoprotein lipase (LPL) is responsible for the hydrolysis of free fatty acids (FFAs) from chylomicrons, whereas the uptake of VLDL by the VLDL/apolipoprotein E (ApoE) receptors, expressed by the heart, represents an alternative route (reviewed in Ref. [70]). The supply of FFAs to the heart is dependent from alterations in the synthesis, secretion, and transport to the capillary lumen of LPL. Usually, increased activity of LPL, as occurs in fasting conditions, is associated with an increase in fatty acid oxidation.

After dissociation from albumin or lipoproteins, fatty acids are transported to the cardiac muscle through the capillary endothelium, and, after crossing the sarcolemma and cytoplasm, are converted to fatty acyl-CoA at the mitochondrial outer membrane or the sarcoplasmic reticulum. In details, fatty acids enter the cells via transporters (fatty acid transport proteins, FATP) on the cell membrane, such as CD36/FAT and fatty acid binding proteins (FABP) [70]. To allow entry into the mitochondria, a CoA group is added to the fatty acid by fatty acyl-CoA synthetase (FACS) and, subsequently, the conversion to acyl carnitine by carnitine palmitoyl transferase 1 (CPT1) is necessary. Carnitine translocase mediates the entrance into the inner membrane of mitochondria where the long-chain fatty acyl carnitine is then converted back to a fatty acyl-CoA by the CPT2, which finally enters the fatty acid oxidation process [71]. Part of the long-chain acyl CoA can also be used for the synthesis of lipid intermediates such as triacylglycerol (TAG), diacylglycerol (DAG) and ceramides, which have all received considerable interest as implicated in the development of diabetes and cardiovascular diseases. The heart has indeed labile stores of TAG that can undergo dynamic turnover; for example, intramyocardial TAG is accelerated by adrenergic stimulation whilst their synthesis is increased with elevated plasma FFA, as occurs in diabetes or fasting (discussed in Ref. [70]). The production of these intracellular intermediates (TAG, DAG, and ceramides) depends on the FFA supply. In mice over-expressing FATP1, the increased FFA uptake and metabolism resulted in functional abnormalities consistent with metabolic cardiomyopathy, which, rather than inducing cell death, manifests as impaired cardiac myocyte dysfunction [72]. An open question is related to the lipotoxicity induced by a defect of long-chain acyl-CoA removal by the fatty acid oxidation process.

An in-depth description of all steps and factors influencing the fatty acid metabolism in the heart is present in the review by Lopashuk [70]. The metabolism of long-chain acyl-CoA in the mitochondrial matrix occurs via the β-oxidation pathway, which involves sequential metabolism of acyl-CoAs by four enzymes: acyl-CoA dehydrogenase, enoyl-CoA hydratase, L-3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl CoA thiolase. These enzymes are susceptible to feedback inhibition by the same products of the process, that is acetyl CoA, flavin adenine dinucleotide ($FADH_2$), and nicotinamide adenine dinucleotide (NADH). The enzymes involved in the fatty acid β-oxidation are under a tight transcriptional control mainly mediated by the nuclear receptor transcription factors peroxisome proliferator-activated receptor α (PPARα) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). PPARα is abundantly expressed in the heart and regulates genes involved in all aspects of the fatty acid metabolism, from the uptake, binding, and oxidation [73,74]. PPARβ/δ and PPARγ are also expressed in the heart and appear as important regulator of the fatty acid metabolism even if "loss-of-function" and "gain-of-function" studies result in phenotypic differences in comparison with PPARα, likely due to the fact that they do not regulate all the genes involved in fatty acid metabolism as PPARα [75]. Of note, PPARγ is known to be activated by several natural occurring lipid metabolites, including PUFAs, phospholipids, and also oxidized fatty acids [76–78]. 15d-PGJ2 is one of the lipid peroxidation products that is able to covalently bind through a Michael addition to cysteine residues on several proteins (i.e. H-Ras, NF-kB, IkB, AP1) including PPARγ. It has been demonstrated that the formation of an ALE between α,β-unsaturated aldehydes (i.e. 15d-PGJ2, hydroxyeicosatetraenoic acid, hydroxyeicosadecaenoic acid, 4-HNE) and PPARγ can modulate its transcriptional activity and the expression of several genes activated by PPARγ *in vivo* [76,78]. The actions of the nuclear transcription factors are supported by the transcriptional activators PGC-1α and PGC-1β; for example, their upregulation by physiological (i.e. exercise) or pathophysiological (i.e. fasting, diabetes) stimuli results in increased mitochondrial biogenesis, fatty acid β-oxidation, and oxidative phosphorylation. *Vice versa,* in conditions like cardiac hypertrophy, heart failure or in the fetal heart, where the expression of PGC-1 is decreased, also fatty acid β-oxidation and mitochondrial biogenesis are impaired [79].

Because of its immense requirement for nutrients and oxygen, the heart contains a very high content of mitochondria in comparison with other organs. Mitochondria are at the same time the main source of energy and the largest source of intracellular ROS in cardiomyocytes, as superoxide is continuously formed at sites within the electron transport system during oxidative phosphorylation [80]. Thus, due to these characteristics mitochondria are presumably the largest endogenous source of lipid peroxidation in the heart. Although these organelles have robust antioxidant and detoxification systems, ensuring that lipid peroxidation and levels of reactive aldehydes are kept at sub-toxic levels, over time, and under various pathological states, these antioxidant and aldehyde detoxification systems become compromised (reviewed in Ref. [81]). Thus, the resulting accumulation of lipid peroxidation products has profound consequences for the function of mitochondria, the cardiomyocytes, and the heart.

The molecular targets of lipid oxidation are firstly the membrane of mitochondria, which are particularly enriched of cardiolipin, a highly unsaturated phospholipid specifically localized to this compartment, constituting ~20% of the local lipids [82]. Cardiolipin that ensures the efficient function of the electron transport chain complexes, diminishes dramatically in robust lipid peroxidation conditions [82], and decreased levels of cardiolipin have been observed in many pathological

conditions including aging, Barth syndrome, heart failure, ischemia/reperfusion injury, diabetes, and neurodegenerative diseases [82].

Lipid peroxidation products exert many effects on cardiomyocytes, usually detrimental [81]. For example, ACR, the simplest unsaturated aldehyde, that is produced widely in different tissues and is found ubiquitously in the environment as a pollutant, induces contractile dysfunction, Ca^{2+} anomalies, and apoptosis in *in vitro* cultured cardiomyocytes [83,84].

Similarly to ACR, trans-2-hexenal is an endogenous product of lipid peroxidation [85–87], as well as a ubiquitous pollutant, which has potent effects on cardiac function [88]. Trans-2-hexenal exposure results in direct cardiac toxicity through, at least in part, induction of mitochondrial cytochrome c release-mediated apoptosis in murine cardiomyocytes [88].

Interestingly, 4-HNE has been widely accepted as an inducer and mediator of oxidative stress [89], but it is also able, depending on its concentration, to elicit an adaptive response and to protect cells against the oxidative stress through signaling pathways activation and modulation of transcriptional activity (reviewed in Ref. [90]). For example, 4-HNE protects rat neonatal ventricular myocytes against cardiac ischemia/reperfusion (IR) injury via the Nrf2-dependent pathway [91,92].

2.3. The epicardial adipose tissue

Recent studies renewed the importance of ectopic myocardial lipids as the result of the rapid development in the field of non-invasive imaging, which has made possible the quantitation of cardiac fat deposit with high accuracy. The epicardial adipose tissue (EAT), localized between the myocardial surface and the visceral layer of the pericardium, originates from mesodermal cells and is similar to the visceral fat; conversely, the pericardial fat, which has an ectodermal origin, is similar to the subcutaneous fat. This distinction is of great clinical importance [93]. EAT functions span from a mechanical protection role with attenuation of the torsion developed by the myocardium during the contraction, to a role as energy provider [93]. Further, EAT can potentially protect the heart from the cardiotoxic effect of the large amount of FFA due to its capacity to rapidly uptake them; additionally, it represents a paracrine and vasocrine source of inflammatory mediators and adipokines. Adiponectin secreted by EAT improves endothelial function through stimulation of nitrogen monoxide synthase, and reduces oxidative stress and inflammatory cytokines [94]. These properties, and the close proximity to coronary arteries, raised the hypothesis for a direct role of EAT in the development and progression of coronary atherosclerosis [95]. Indeed, a number of cardiovascular diseases, such as coronary artery disease, atrial fibrillation, type 2 diabetes or insulin resistance syndrome, are all associated with an increased amount of epicardial fat (reviewed in Ref. [93]). In this regard, epicardial adipose tissue can contribute increasing oxidative stress. Indeed, it has been shown that the epicardial adipose tissue has more oxidative stress markers than the subcutaneous (SC) adipose tissue in a study conducted with proteomic analysis [96]. Further, EAT may cause an increased risk of cardiovascular diseases by leading to increased oxidative stress in patients with metabolic syndrome [97].

2.4. Adipose tissue

The adipose tissue has recently been reconsidered from a relatively inert organ with a primary role as a storage depot for excess energy, in the form of triglycerides, to a metabolically active organ involved in cross-talk between various systems [98]. Indeed, recently, the term “adiposopathy” has been coined [98]. The central dogma of adiposopathy is based on a shift to visceral adipose tissue distribution (intraperitoneal and retroperitoneal spaces), an onset of ectopic fat deposition (fat store in the liver, pancreas, heart, and skeletal muscle), a dysregulation of the inflammatory and adipokine system, and an insulin resistance.

The adipose tissue, which represents one of the largest body compartments, is classified according to its anatomical location and related functions, and still, an accepted taxonomy is lacking. For example, SC adipose tissue, which mostly contains white adipocytes enriched in triglycerides, stores lipids at a variety of anatomical sites that differ in metabolic and physiological characteristics [99]. On the other hand, visceral adipose tissue is a smaller storage compartment for lipids and it is mechanistically linked to many of the metabolic disturbances and adverse outcomes associated with obesity [100].

The perturbation of the adipose tissue results in a pathological response that directly or indirectly contributes to cardiovascular and metabolic diseases. Cardiovascular disease mortality and morbidity have been shown to be high in individuals who are overweight, mainly with central deposition of adipose tissues [101]. Obesity could be associated with dyslipidemia, hypertension, diabetes, or insulin resistance, and high levels of fibrinogen and C-reactive protein, all of which increase the risk of CVD events [102]. The distribution and the amount of adipose tissue can influence the synthesis and secretion of adipokines and hormones provoking an inflammatory state, and the hydrolysis of triglycerides present in the adipocytes releases free fatty acids which are poured into the plasma. An excessive adiposity causes an increase in the size of liposomes in hepatocytes (steatosis), which leads to a series of pathological states, including nonalcoholic fatty liver disease, steatohepatitis, and cirrhosis, and it can also cause an accumulation of lipid intermediates (e.g., ceramides) in some non-adipose tissues, with cellular dysfunction and apoptosis, due to lipotoxicity (reviewed in Ref. [100]).

Oxidative stress in human and murine adipose tissue, as well as in cultured 3T3-L1 adipocytes, alters the release of adipokines, such as adiponectin and tumor necrosis factor α [103,104]. Further, the modification of adipose proteins by lipid peroxidation products could be one of the contributing factors linking oxidative stress to insulin resistance. Among lipid peroxidation products, oxysterols and 4-HNE received most attention since cross-sectional studies and experimental data indicate their mechanistic implication in the pathophysiology of obesity-linked metabolic diseases (reviewed in Ref. [105]).

In animal models, adipose 4-HNE accumulation *per se* was shown either to promote the obese state or to induce the development of insulin resistance, through carbonylation of key adipocytes proteins involved in lipid metabolism [106]. For example, the adipocyte fatty acid-binding protein, a protein implicated in the regulation of insulin resistance, after modification, reduces its affinity for fatty acids, approximately 10-fold [107]. Furthermore, the exposure of adipose cells to 4-HNE led to a dysfunctional phenotype characterized by an impairment of the insulin signaling, increased lactate and reduced adiponectin production, all features typical of insulin resistance developing [104].

Finally, in differentiating (pre)adipocytes, oxidative stress can induce intracellular 4-HNE production, which, in turn, by activating the MAPK pathway, inhibits adipocyte differentiation, fostering the noxious effect of 4-HNE on adipose homeostasis [108].

Recently, it has been shown that long-term treatment of human primary pre-adipocytes, isolated from SC adipose tissues of obese subjects with physiological concentrations of 4-HNE (up to 10 μM) causes increased oxidative stress, inhibition of cell growth, loss of adipogenic capacity and induction of insulin resistance, thus highlighting the crucial role of 4-HNE in the progression obesity-associated metabolic syndrome [109].

3. Molecular and cellular effects of lipid peroxidation products

Lipid peroxidation products are involved in inflammatory processes, cell proliferation, and viability, and have specific roles in signaling cascades that activate an adaptive response to oxidative damage in order to improve the physiological antioxidant defenses. Shreds of

evidence suggest that 4-HNE is able to activate uncoupling proteins (UCPs) in the mitochondria, inducing negative feedback to lower proton motive forces and to protect the cells from oxidative damage [7,110]. Moreover, lipid peroxidation products can also activate transcription factors, such as Nrf2, through the modification of its inhibitory protein Keap1, inducing genes that modulate the antioxidant response of the cell [7,111].

It has been demonstrated, however, that the effects of lipid peroxidation products are finely tuned and concentration-dependent. Indeed, at a lower concentration, they could have beneficial effects, inducing for example cell growth through epidermal growth factor receptor (EGFR) or platelet-derived growth factor receptor (PDGFR), while, at higher concentration, they can inhibit proliferation and even induce apoptosis [112,113].

Further, oxPLs can exert a variety of biological effects that are different from those of free fatty acids derived from their degradation and are concentration- and cell-type-dependent [114]. They can alter the physical properties of the cell membrane, due to the different chemical-physical characteristics (polarity and shape) in comparison with the corresponding native phospholipids, influencing also the functions of the membrane-bound protein involved, for example, in ion transport [31].

OxPL can be recognized by cell surface receptors, including the scavenger receptor for recognition and uptake of oxLDL, CD36, or toll-like receptors (TLRs), or with circulating LPS binding protein or CD14 [31], activating a cascade of events. These interactions can partially explain their involvement in complex processes ranging from inflammation to apoptosis, affecting endothelial cells, smooth muscle cells and macrophages, as summarized by Fruhwirth et al. [31,115].

However, the ability of lipid peroxidation products to interact with different types of macromolecules, including DNA, phospholipids, and proteins, gives rise to a plethora of biological effects, due to molecular damages.

DNA modification induced by lipid peroxidation products could be repaired by the cells through a nucleotide excision repair pathway, as described for etheno-DNA adducts with 4-HNE [116]. However, these adducts can cause DNA damage associated with carcinogenic effects and profound alterations of the signal transduction pathways involved in particular, in the control of the cell cycle and gene expression [8]. In the context of cardiovascular diseases, oxidative DNA damage could induce apoptosis and regulate angiogenesis [117] and it has been demonstrated that lipid peroxidation-derived etheno-DNA adducts are present in human atherosclerotic lesions [118].

Phospholipids modifications can result in changes of their physical properties and turnover, leading to an altered distribution of aminophospholipids among the cellular or subcellular membranes with potential effects on the activity of proteins that interact with phospholipids [7]. Further, oxidized phospholipids are also involved in protein modifications, as demonstrated by Horkko et al. who showed that adducts of oxidized phosphatidylcholine with ApoB-100, the major protein of LDL, modulate the lipoprotein uptake by macrophages [45].

Protein lipoxidation can occur on different proteins in relation to the presence of residues susceptible to the formation of adducts with lipid peroxidation products, but also to their molecular conformation and the presence of metal binding sites. These modifications can alter both the structure and the function of the modified proteins influencing their physico-chemical characteristics, their degradation and trafficking introducing crosslinking or even stimulating an autoimmune response [7], as described in details for 4-HNE-modified proteins by Schaur et al. [119].

One of the most studied targets of lipoxidation is represented by LDLs considering the role of oxLDL in several pathological processes including atherosclerosis. Several adducts have been identified with MDA, 4-HNE, glyoxal or acrolein leading to a variety of biological and atherogenic processes [112]. Additionally, protein oxidation by 4-HNE on ApoAI, the principal protein component of HDL, has been associated with a decrease in cholesterol transport function and anti-inflammatory properties of these lipoproteins [120].

Of note, one of the mechanisms of degradation of 4-HNE-modified proteins is mediated by the proteasome, but some protein components of the proteasome have been identified as specific targets of these modifications with a consequent loss of function of the entire proteasome system and accumulation of modified proteins [13].

Regarding the protein modifications due to the formation of adducts with oxidized phospholipids, the literature is sparse, especially regarding the mapping of these adducts *in vivo*, even if the role of phospholipid oxidation products in several diseases, including atherothrombosis, has been extensively documented, mainly using antibodies against oxidized phospholipid adducts [11].

Protein adducts can also be formed with prostaglandins, mainly the cyclopentenone prostaglandin (CyPG), a molecule involved in the response to oxidative stress and in the resolution of inflammatory processes. It covalently reacts with proteins generating irreversible adducts, as demonstrated for various proteins involved in the NF-kB signaling cascade, including IkB kinase β (IKKβ), p65 and p50 subunits of NF-kB [121–123] or PPARγ, whose modification is necessary for the conformational changes of the receptors and the binding with the coactivator [10,124].

4. Involvement of lipid peroxidation in cardiovascular complications of systemic diseases

Obesity, metabolic syndrome, and diabetes are the major cardiovascular disease risk factors, including coronary heart disease, atrial fibrillation, ventricular arrhythmias and heart failure.

The association between obesity and different cardiovascular diseases is complex, but it is well known that obesity can cause coronary atherosclerosis through dyslipidemia, hypertension, and diabetes mellitus 2, as well as other factors like subclinical inflammation, increased free fatty acid turnover, intramyocardial and subepicardial fat deposition, elevated leptin and insulin concentrations [125]. Systemic alterations involved in obesity are increased oxidative damage to proteins and lipids, and severe inflammation as indicated by elevated levels of TNF, interleukin-1β and other pro-inflammatory cytokines [126]. Oxidative stress and inflammation are concurrent processes in many tissues that are affected because oxidative stress induces inflammatory cytokines production that in turn promote ROS generation [127]. Levels of 4-HNE are increased in the blood and muscle tissue of obese subjects in comparison to normal weight subjects [127,128]. Enhanced levels of 4-HNE are also present in adipocytes in obese subjects, where they impair the function of proteins responsible for lipid synthesis and suppression of inflammation [107]. Moreover, it has been demonstrated that high intracellular 4-HNE in adipocyte causes enhanced lipolysis through multifactorial mechanisms, which is the major feature in obesity-related metabolic disorders [129].

Oxidative stress is closely related to glycation: free radical oxidation of glycated residues in proteins generates a permanent and irreversible modification. This process is known as glycoxidation and it is involved in the complications associated with several disorders including diabetes, cardiovascular disease and other several forms of cancer [130].

Both glycoxidation and lipoxidation of vascular wall structural proteins are also implicated in diabetes. Hyperglycemia-induced increase in the production of oxidants and other reactive intermediates is a key mechanism for the development and progression of endothelial dysfunction in diabetic patients [126]. Although oxidative stress has a known important role in diabetic complications and atherosclerosis, it is not clear whether oxidative stress is a primary event that is present at early disease stage or whether it represents a secondary event that is a consequence of the end-stage tissue damage. Glycoxidation products in collagen have been demonstrated to minimize the NO activity, which alters vascular tone and perfusion, and contribute to hypertension. High aortic stiffness has been reported in diabetic patients and it is associated

Table 1
Most common methods for detecting and analyzing the principal lipoperoxidation products and their protein adducts. 4-APC, 4-(2-(trimethylammonio)ethoxy)benzenaminium halide; 4-HNE, 4-hydroxy-2-nonenal; ACR, acrolein; AIDA, alternate isotope-coded derivatization assay; CE, capillary electrophoresis; CVDs, cardiovascular diseases; DNPH, 2,4-dinitrophenylhydrazine; ESR, electron spin resonance; GC, gas chromatography; HLB, hydrophilic-lipophilic balanced; LC, liquid chromatography; LC-FD, LC-fluorescence detection; LC-UV, LC-ultraviolet; LDLs, low-density lipoproteins; LLE, liquid-liquid extraction; MALDI-TOF, matrix assisted laser-desorption ionization-time of flight; MDA, malondialdehyde; MRM, multiple reaction monitoring; MS, mass spectrometry; oxPC, oxidized phosphatidylcholine; SPE, solid-phase extraction; SRM, selected reaction monitoring; TBA, thiobarbituric acid; TLC, thin-layer chromatography.

Electrophilic lipid species	Name	Detection methods	CVDs	Cardiovascular targets
Lipid peroxidation-derived aldehydes	4-HNE	**Free 4-HNE:** – **Spectrophotometric methods** (carbonyl-reactive reagents, e.g. DNPH, biotin-containing probes, fluorescence probes and tritiated sodium borohydride) [137,138] – **LC-MS/MS** [142,143] – **GC-MS/MS** (sample derivatization) [139,140] **4-HNE-protein adducts:** – **Immunochemical methods** (immunocytochemistry and immunohistochemistry, ELISA, Western blotting) [146,147,153,156,304] – **MALDI-TOF MS/MS, LC-MS/MS** and **GC-MS/MS** (enrichment strategies, e.g. biotin hydrazide, immuno-based, click chemistry) [154,158–161,166,305]	– Atherosclerosis [112,151,154,272] – Coronary and peripheral artery disease [196] – Dilated cardiomyopathy [165] – Heart failure [166,295] – Myocardial ischemia and reperfusion injury [306,307]	– Apolipoprotein B-100 (LDLs) [148] – Apolipoprotein AI (HDLs) [120] – Heart fatty acid-binding protein [308] – Mitochondrial proteins (e.g. cytochrome c oxidase subunit I) [161,305] – Platelet-derived – growth factor receptor beta [272] – Carnosine [283,286,288] – Serine/threonine-protein kinase STK11 [309] – Glutathione [260] – Ether-a-go-go-related channels [310]
Lipid peroxidation-derived aldehydes	MDA	**Free MDA:** – **Spectrophotometric methods** (sample derivatization with TBA) [170,172–174] **MDA-protein adducts:** – **Spectrophotometric methods** (sample derivatization with TBA, strong acids, hydrazine-based or non-hydrazine-based reagents) [171,180,183] – **LC-UV** or **LC-FD** [176,177,180] – **LC-MS/MS** (e.g. **SRM/MRM MS** or **label-free**; sample derivatization with AIDA assay, 4-APC) [186–188,194,195] – **GC-MS/MS** (sample derivatization with hydrazine-based or non-hydrazine-based reagents) [178,179,190,191,196] – **CE-UV** [193]	– Atherosclerosis [112] – Coronary and peripheral artery disease [196,311] – Aortic stenosis [265] – Heart failure [312]	– Apolipoprotein B-100 (LDLs) [43] – Apolipoprotein AI (HDLs) [313] – Mitochondrial proteins [314] – Carnosine [282,285]
Lipid peroxidation-derived aldehydes	ACR	**Free ACR:** – Fluorescence methods (e.g. Skraup reaction with m-aminophenol, lanthanide probe) [202,204,205] – LC-FD [206,208] – LC-MS/MS (sample derivatization) [214] **ACR-protein adducts:** – Immunochemical methods [209,211] – GC-MS/MS and LC-MS/MS (e.g. SRM/MRM MS; enrichment strategies, e.g. hydrazide-based reagents, non-hydrazine-based reagents, immuno-based) [212,213,216] – LC with electrochemical detection [215]	– Atherosclerosis [112] – Myocardial dysfunction [83]	– Apolipoprotein B-100 (LDLs) [315] – Mitochondrial proteins [160,216] – Carnosine [284]
Prostaglandin-like compounds	Isoprostanes	– Immunochemical methods (ELISA) [223,225] – GC-MS/MS and LC-MS/MS (e.g. SRM/MRM MS; extraction strategies, e.g. SPE, LLE, affinity column, TLC, silica and HLB cartridges) [219,226–228,230,232–236,239]	– Atherosclerosis [228,316] – Coronary heart disease [317–319] – Acute coronary syndrome [320]	– Thrombomodulin [321] – Thromboxane A2 prostanoid receptor [228,322]
Oxidized phospholipids	oxPC	– Spectroscopy methods (e.g. ESR) [241] – Immunoassays [31,46] – Colorimetric and fluorimetric assays (e.g. iodometric titration, ferrous oxidation-xylenol orange assay, isoluminol-dependent assay, diphenyl-1-pyrenylphosphine and hydrazine-based reagents) [242,243] – MALDI-TOF MS/MS, GC-MS/MS and LC-MS/MS (e.g. SRM/MRM MS; extraction strategies, e.g. SPE and TLC) [114,244–247,251]	– Atherosclerosis and aortic stenosis [31,46] – Atherothrombosis [11] – Familial combined hyperlipidemia and familial hypercholesterolemia [246]	– Apolipoprotein B-100 (LDLs) [45] – Apolipoprotein AI (HDLs) [44]

with increased cross-linking of connective tissue proteins. Glycated collagen specifically binds LDL compared to control collagen, and this increased LDL trapping by AGEs accelerates the atherosclerosis development in patients with diabetes mellitus [131]. Dyslipidemia is a common feature in diabetic patients and elevated levels of plasma lipoproteins might contribute to lipoxidation. AGE lipids have been detected in diabetic plasma lipoproteins and isoprostanes levels are high in diabetic patients [132,133].

5. Methods for identification and quantitation of lipid peroxidation products: strategies and challenges

Lipid peroxidation products can be identified in the free form, but they are more likely to be present in biological samples as covalent adducts with proteins, DNA or aminophospholipids.

Methods for the detection and quantification of free lipid peroxidation products include spectrophotometric methods and gas or liquid chromatography (GC and LC, respectively) coupled to mass spectrometry (MS). However, most attention has been focused in recent years on detecting and quantifying adducts with proteins by employing two main approaches: antibody-dependent techniques and proteomic methods based on MS. Currently, MS is the method of choice for the identification of protein-lipid adducts, because it is possible to detect the mass shift caused by the adducts and to acquire fragmentation spectra that can be used for the identification of specific fragment ions (i.e. reporter ions) for each modified amino acid within the protein sequence [134]. Of course, a method for enriching adducted proteins over non-adducted proteins prior to LC-MS/MS analysis would enhance the overall identification of lower abundance protein compounds.

Many pre-analytical factors can influence the analysis of lipid peroxidation products in plasma, serum, and tissue [135]. Lipid peroxidation product concentrations can be higher than the real physiological amounts, due to their artefactual formation that depends on storage time/conditions. The use of antioxidants, such as butylated hydroxytoluene (BHT), or the use of COX inhibitors to block the enzymatic formation, can be possible strategies to avoid/minimize *ex vivo* formation. Accurate and standardized protocols are essential to control the adverse effects of pre-analytical issues on lipid peroxidation product measurements.

Most common technologies for detecting and quantifying the principal lipid peroxidation products and their protein adducts are summarized in Table 1.

5.1. 4-Hydroxy-2-nonenal analysis

Several methods are available to detect and study both free 4-HNE and 4-HNE-protein adducts (Table 1). Methods for detection and quantitation of free 4-HNE include spectrophotometric methods and mass spectrometry analysis [136]. 4-HNE can be separated from other aldehydes by high performance liquid chromatography (HPCL) and quantified by spectrophotometrically monitoring. Anyway, in complex biological samples, specific carbonyl-reactive probes are commonly used, such as 2,4-dinitrophenylhydrazine (DNPH), biotin-containing probes, fluorescence probes (e.g. 1,3-cyclohexandione (CHD)) [137] and tritiated sodium borohydride [138].

Instead of UV, MS can be applied for the identification of the compound based on its fragmentation pattern. In particular, GC-MS analysis is able to detect and quantify derivatized 4-HNE in negative ion chemical ionization mode using a deuterated 4-HNE as internal standard [139]. In order to avoid losses during sample preparation, many isotopic internal standards have been used till now, such as 2,5-dihydroxy-benzaldehyde (2,5-DBA) [140], 4-hydroxybenzaldehyde (4-HBA) [141] and pentafluorobenzylhydroxylamine-HCl [139]. Instead, LC-MS analysis does not necessarily require derivatization, so the sample preparation is less complex and prone to pre-analytical errors during the manipulation [142,143].

However, the majority of 4-HNE is present as adducts of a variety of biomolecules and many reviews reported the main analytical approaches used to detect them (e.g. Refs. [10,144,145]). The two possible approaches to detect 4-HNE adducts are immunochemical techniques and MS analysis. Sometimes, a reduction step with borohydride is required to stabilize the Schiff bases or Michael adducts before the analysis.

Immunohistochemistry/immunocytochemistry approaches detect 4-HNE bound to histidine and the distribution of the 4-HNE-histidine adducts in cells and tissues using antibodies [146]. The advantage of immunohistochemistry is to evaluate the distribution of 4-HNE-protein conjugates, but their quantitation can be estimated only semi-quantitatively [147].

Several antibodies for the 4-HNE adducts identification have been developed over the years [136,146]. In 1990, Palinski et al. developed polyvalent and monoclonal antibodies against sodium cyanoborohydride-reduced 4-HNE-treated LDL and these antibodies were able to recognize 4-HNE-lysine adducts on apolipoprotein B (ApoB) that were produced during copper-induced oxidation of LDL *in vitro* [148]. A few years later [149], a polyvalent antiserum to human 4-HNE-LDL modified under non-reducing conditions, also showed *in vivo* the recognition of copper-oxidized LDL and cross-reactivity with 4-HNE-albumin and a lower extent 4-HNE-HDL. Uchida et al. produced monoclonal antibodies that reacted strongly with 4-HNE-treated proteins and immunohistochemical analysis of atherosclerotic lesions of human aorta demonstrated their specific reactivity with epitopes present in the foam cells [150]. Later, Itakura's group developed a monoclonal antibody directed to a fluorescent derivative of 4-HNE with Lys residue [151] and reported the presence of the fluorophore in atherosclerotic lesions from the human aorta and in the oxidatively modified LDL. However, another study demonstrated that the majority of the epitopes on the oxidatively modified LDL were 4-HNE-histidine adducts [152]; this is also the case of other 4-HNE adducts on various proteins analyzed, suggesting that 4-HNE-histidine adducts could be the major bioactive form of 4-HNE interactions with proteins and peptides.

Another antibody-based approach to estimate the amounts of 4-HNE-protein conjugates is the Western-blot method, which, being a semi-quantitative immunochemical analysis [153,154], is not a standardized and precise method, and provides only an approximate quantitation [155].

Over the years, the enzyme-linked immunosorbent assay (ELISA) technique has given rise to great interest for the analysis of 4-HNE-protein adducts. An ELISA method was developed to detect 4-HNE-histidine adducts in human lysates of cells subjected to slight or severe oxidative stress [147], and it was also adapted to a commercial antibody for the analysis of human plasma samples [156].

Labelling approaches have been used to determine the 4-HNE-protein conjugates in several studies, combined with MS techniques that were required for the identification of the tagged 4-HNE-protein adducts. While the use of 4-HNE-antibodies in ELISAs and immunostaining allows only the detection and quantitation of adducts, the MS provides also a characterization of 4-HNE adducts. Matrix assisted laser-desorption ionization-time of flight (MALDI-TOF) MS has been used for 4-HNE-adducts detection in biological samples by the increase in mass to charge (m/z) ratio of the peptide compared to the native form (i.e. an increased mass of 138 Da by Schiff's base formation or 156 Da by Michael addition [145,157]). Additionally, MS/MS analysis allows sequencing of proteins and identification of the modification sites [136]. However, the analysis is more complicated when reduced products of the sample, due to the treatment with sodium borohydride or cyanoborohydride, or structural rearrangements occur, and a further increase of the mass should be considered. As an example, Arcaro et al. observed by two-dimensional electrophoresis (2DE) and MALDI-TOF MS that the exposure of human promyelocytic HL-60 cells to non-toxic doses of 4-HNE resulted in a heat shock 60 kDa protein 1 (HSP60) modification by 4-HNE, and suggested a possible involvement of the

HSP60 modification with 4-HNE in the pathogenesis of atherosclerosis [154].

Several proteomic studies on the 4-HNE-protein adducts have been also performed by LC-MS/MS. Methods for enriching 4-HNE-adducted proteins over non-adducted proteins prior to LC-MS/MS analysis often enhance the overall identification of lower abundance protein adducts in biological samples. The use of azido and alkynyl derivatives in combination with click chemistry has been proven to be an efficient approach for the identification of the protein targets of 4-HNE prior to LC-MS/MS analysis [158]. Some years later, a study demonstrated the possibility to use stable-isotope tagging followed by enrichment of 4-HNE-modified peptides by solid-phase hydrazide chemistry and combined with LC-MS/MS analysis for quantitation of 4-HNE-modified proteins in plasma samples [159]. In the same year, Chavez et al. reported the identification of endogenous protein targets of several electrophilic 2-alkenals, among which 4-HNE, in cardiac mitochondria by using a chemical aldehyde/keto specific labeling and affinity strategy in combination with LC-MS/MS [160]. In addition, 2DE followed by LC-ESI-MS/MS has been successfully applied to identify 4-HNE modified [161,162]. Increased levels of 4-HNE-modified proteins have been detected both in animal models and patients with heart failure (HF). For example, 4-HNE-protein adducts were significantly increased in the left ventricular myocardium of HF dogs [163] or in the myocardium from rats with HF [164]. In humans, 4-HNE-modified proteins have been found in the myocardium from patients with dilated cardiomyopathy [165]. Moreover, significantly higher 4-HNE plasma levels have been observed in HF patients with respect to healthy subjects. Increased 4-HNE levels correlated with higher heart rate and impaired left ventricular contractility [166].

5.2. Malondialdehyde analysis

The analytical strategies to analyze MDA, free or bound to proteins, can be divided into derivatization-based and label-free methods (Table 1). In particular, these strategies have been implemented with separation techniques such as LC or GC, in order to facilitate MDA measurement [167]. Methods which require an acidic or basic sample pre-treatment, or the use of strong acids to precipitate the protein fraction, are able to hydrolyze the bound MDA and provide an assessment of the total MDA.

Many derivatization-based assays have been developed for the detection of MDA, among which the most frequently used and simplest is TBARS assay (thiobarbituric acid reactive substances assay) in which thiobarbituric acid (TBA) reacts with MDA to obtain a colored chromogen fluorescent red adduct [18,168]. However, TBARS assay is unspecific which has led to important controversy over its use for quantification of MDA from *in vivo* samples [2]. Indeed, TBA may react with many other compounds in addition to MDA [169] and the condensation process may generate further oxidation of the matrix with consequently an overestimation of the quantitative results. Aimed to reduce the matrix oxidation, the precipitation of protein prior to the TBA reaction is often applied as a pre-treatment of the sample [18].

Over the years, different methods based on TBA derivatization have been proposed, in order to improve specificity, selectivity and to obtain accurate quantification of MDA [170–172]. An interesting on-line analytical system that involves the use of microdialysis perfusion, on-line derivatization, and HPLC analysis was developed for the continuous monitoring of MDA [173], and similarly, an on-line microdialysis sampling system coupled with an HPLC system was also proposed for the simultaneous determination of ofloxacin (OFL) and MDA in whole blood [174]. Cooley et al. described a capillary electrophoresis (CE)-fluorescence method combined with *in vivo* microdialysis sampling [170] to quantify MDA in rat heart, muscle, liver, and brain dialysate.

Since TBARS assay failed to distinguish free and bound MDA and it is intrinsically unspecific, several alternative approaches have been proposed [175]. Hydrazine-based derivatization reagents, such as DNPH [176–179], FMOC-hydrazine [180] or dansylhydrazine [181], have been used in the analysis of MDA by LC- or GC-MS. Non-hydrazine-based reagents have been also employed for the measurement of MDA: 1-methyl-2-phenylindole [182,183], diaminonaphthalene [184] and 2-aminoacridone [185].

Besides the use of LC-ultraviolet (LC-UV) and LC-fluorescence detection (LC-FD) for the MDA derivatives analysis, also LC-MS/MS-based strategies have been reported in several studies. For example, alternate isotope-coded derivatization assay (AIDA) and the reagent 4-(2-(trimethylammonio)ethoxy)benzenaminium halide (4-APC) have been proposed for the quantitative analysis of MDA and other aldehydes using LC-MS/MS [186–188]. Other hydrazine labels, such as 2,4,6-trichlorophenylhydrazine [189] and 2,2,2-trifluoroethylhydrazine [190], have been widely used also for the GC-based methodologies. Similarly, the analysis of MDA by GC-MS and GC-MS/MS has been reported using non-hydrazine-based reagents [175,191].

On the other hand, label-free analysis of free MDA is possible and ranges from UV-based methodologies to LC-MS/MS technique. However, the UV detection of MDA has poor sensitivity and selectivity when the analysis of complex biological matrices is performed and, thus, a separation technique by LC [192] or CE [193] is required in order to simplify the UV-absorbance.

LC-MS/MS technique using selected reaction monitoring/multiple reaction monitoring (SRM/MRM) has been applied in several studies for precise quantitation of MDA in biological fluids, coupled with a SPE for a pre-treatment of the biological fluids before SRM [194] or using 3-nitrophenylhydrazine chemical derivatization and isotope-labeling [195]. Recently, Tsikas et al. applied GC-MS/MS technique to analyze simultaneously MDA and 4-HNE in human serum and plasma, from patients with coronary or peripheral artery disease, after administration of L-arginine [196].

In the cardiac context, *in vitro* studies with isolated adult rat ventricular myocytes demonstrated that MDA impairs cardiac contraction and maximal velocity of cardiac contraction and relaxation [197]. Moreover, plasma MDA levels have been found to be significantly elevated in HF patients [198,199]. HF patients with raised MDA plasma levels presented reduced left ventricular ejection fraction [199] and a higher NYHA functional class [200]. Additionally, high plasma MDA levels were a significant independent predictor of mortality in HF patients [201].

5.3. Acrolein analysis

To date, several methods have been developed for the estimation of ACR levels in biological samples, as reported in Table 1. The conventional method for detecting free ACR in biological samples is the fluorescence analysis based on the Skraup reaction with m-aminophenol [202,203] or using a lanthanide probe [204]. A less expensive and practical fluorescence-based method, using a two-step tethering strategy, for the detection of ACR in human plasma under mild conditions without the use of HPLC, has been also developed [205]. In other studies, HPLC-fluorescence detection in combination with a derivatization reaction was used for the accurate quantification of ACR in human serum and plasma samples [206–208].

ACR-protein adducts or -DNA adducts can be detected using monoclonal antibodies [209–211], which allow the detection of conjugates using gel-based approaches. However, the method is time-consuming and site-specific characterization of oxidative modifications is often not possible with gel-based techniques.

MS-based methods were successfully applied to determine ACR using GC-MS [212] or LC-MS/MS [134,213] and mostly DNPH as a derivatization reagent [214]. However, Imazato et al. developed a method for ACR analysis by HPLC with fluorescence detection after pre-column derivatization using 1,2-diamino-4,5-dimethoxybenzen (DDB) [208], which is not a hydrazine reagent and does not have intrinsic fluorescence itself that can interfere with the detection of ACR

derivative.

Alternatively, HPLC with electrochemical detection method allowed the determination of ACR without any derivatization step [215].

Finally, in 2011, Wu et al. developed an analytical approach that combines the identification and relative quantification of ACR-modified proteins in cardiac mitochondrial proteome samples by nanoLC-MS/MS SRM analysis [216].

5.4. Isoprostanes analysis

The measurement of IsoPs is considered the most reliable index for the assessment of lipid peroxidation in humans [217,218]. In humans, F2-IsoPs are usually measured in plasma and urine, but they can be also measured in other biological fluids (e.g. exhaled breath condensate, amniotic fluid, and saliva) or tissue homogenate [219]. Particular care must be taken during plasma collection and storage for F2-IsoPs analysis because isoprostanes can be also produced in plasma from *ex vivo* oxidation of AA [220]. Indeed, in order to minimize artefactual elevation of plasma F2-IsoPs, Barden et al. recommended blood collection into EDTA tubes containing the antioxidants BHT and reduced glutathione (GSH), in addition to storage at −80 °C [221]. Several other factors are important to consider when measuring F2-IsoPs and include the timing of sample collection, the optimal sample matrix and some key issues regarding their hydrolysis, excretion, and metabolism [222].

Commercially available ELISA kits [223–225] or mass spectrometry combined with gas [226,227] or liquid chromatography [228] are employed for the measurement of F2-IsoPs (Table 1).

Anyway, the comparison between commercially available ELISA kits provided very poor correlation and different results compared to LC/LC-MS/MS approach [218]. GC-MS/MS and LC-MS/MS provide indeed more accurate measurements [229], but often require multiple extraction and purification methods before the analysis. Moreover, the GC-MS approach involves derivatization steps to facilitate the following MS detection.

The extraction from biological samples is very important and several approaches are available including solid phase extraction (SPE) [230], liquid-liquid extraction (LLE) [219,231], affinity column [232], thin-layer chromatography (TLC) [233], silica cartridges [234] and hydrophilic-lipophilic balanced (HLB) cartridges [235]. In 2010, Taylor et al. developed and validated a reproducible and sensitive method for the extraction of 15-series F2-IsoPs from plasma samples, applying a combination of SPE and LLE to obtain a cleaner and more easily concentrated extract and reducing the HPLC separation time, before MRM analysis [236]. Some years later, a LLE method using negative chemical ionization GC-MS/MS have been proposed for the measurement of total isoprostanes in plasma and tissue homogenates, allowing the quantification of both free and esterified isoprostanes in order to have a correct estimation of the oxidative status [219].

In general, a known amount of deuterated internal standards is commonly added in sample preparation, before the purification steps, in order to obtain absolute quantification of the isoprostanes [237], and LC-MS/MS using MRM can yield an accurate measurement monitoring multiple pairs of transition mass ions [238–240].

5.5. oxPLs analysis

As shown in Table 1, analytical approaches to measure oxPLs include spectroscopy methods, immunoassays, colorimetric assays, and mass spectrometry, mainly coupled with different separation techniques [114].

When oxPLs are initially generated by radical attack they can be analyzed by electron spin resonance (ESR) [241], but due to the difficulties of this approach, colorimetric or fluorimetric assays are preferred for complex biological samples. Hydroperoxy groups can be evaluated with iodometric titration, ferrous oxidation-xylenol orange (FOX) assay or isoluminol-dependent assay. Alternatively, DNPH or pentafluorobenzyl hydroxylamine can be employed for the detection of aldehydes or ketones on phospholipids [114]. Of note, these methods can be coupled with tissue imaging as demonstrated for diphenyl-1-pyrenylphosphine (DPPP), a suitable fluorescent probe to monitor lipid peroxidation within cell membranes specifically [242]. In particular, lipid peroxidation was evidenced by DPPP fluorescence with microscopy techniques in isolated ischemic rat lungs perfused with DPPP, evaluating also its co-localization with an endothelial cell marker, DiI-acetylated LDL [243].

In order to improve the specificity of these assays, especially in complex samples, separation steps have been also introduced based on TLC, GC or HPLC interfaced with different detectors [114].

Immunoassays have been extensively applied for the analysis of oxidized phospholipids in biological samples since the introduction of the E0 series of antibodies against oxidized phosphatidylcholine, generated from apoE-deficient mice. In particular, the E06 antibody has been used to detect oxPLs both in their free or protein-bound forms [31] and has been extensively employed in the field of atherosclerosis. Further, it has also been suggested as a potential therapy to counteract the *in vivo* effects of oxPLs, not only in atherosclerosis but also in aortic stenosis or hepatic steatosis [46].

MS-based methods, such as MALDI-TOF or LC-ESI-MS/MS, offer the advantages of less handling artifacts and more structural information. Despite some studies have been performed by MALDI-TOF to identify oxidized phospholipids [244], the lack of the possibility to separate lipids before MS reduces their applicability to complex samples. However, it is worth noting the possibility to couple MALDI with imaging techniques allowing also the precise localization of the specific species [245].

Interestingly, Stubiger et al. used a MALDI-TOF-MS/MS approach to profile atherogenic phospholipids in human plasma and lipoproteins of hyperlipidemic patients and evidenced a positive correlation between oxPCs and markers of CVD, such as the intimal medial thickness that is considered an early marker in this young population [246].

Recently, Gao et al. presented the first systematic investigation of HDL apolipoproteins modifications by exposure to endogenous oxPLs *in vitro* [247]. They made a comparison of oxPLs with short-chain aldehyde 4-HNE using MALDI-TOF MS and demonstrated the high selectivity and efficiency of oxPLs in the modification of HDL apoproteins, providing novel insights into the mechanisms of the loss of HDL atheroprotective capacity.

On the other hand, ESI-MS can be easily coupled to HPLC separation at least to separate different classes of lipids, even if it does not resolve oxidation products from their precursors, and it is still the most convenient method in phospholipidomics [248]. Indeed, oxPLs have been analyzed both with shotgun approaches, to have a global analysis of phospholipids and oxPLs, and targeted MS approaches. These latter are the most preferred approaches to deal with the low concentration of oxPLs *in vivo*, thanks to their selectivity and sensitivity, even if they do not provide a comprehensive analysis of all the species [248]. The main reason for the failure of shotgun analysis has been the lack of bioinformatics tools for the identification of oxPLs. Novel bioinformatics solutions have been recently proposed, for example, with the development of the prediction software, LPPtiger, which can provide a system view of oxidized forms of phospholipids for untargeted analysis [249].

On the other hand, targeted approaches are largely used and are mainly based on a precursor ion acquisition of m/z 184 Da (phosphocholine headgroup) for the detection of PC and sphingomyelins, and the neutral loss of water (−18 Da) for hydroxides and hydrogen peroxide (−34 Da) for hydroperoxides derivatives [114]. Additionally, MRM approaches have been applied to evaluate oxPE induced by lipoxygenases, or the presence of oxPS, through the detection of the typical neutral loss of the phosphoserine headgroup (−87 Da) [114]. Novel strategies have been also developed to enrich oxidized LDL before MS analysis [250] or to introduce labeling of PE to increase the

signal intensity and allow a relative comparison of multiple samples using deuterated forms [251].

6. Molecular strategies to prevent ALEs formation in the cardiovascular system

Lipoxidation and, more specifically, ALEs are now considered not only indexes of oxidative damage, but also potential drug targets, due to their involvement in pathogenic mechanisms of oxidative-based diseases, including diabetes, atherosclerosis, and neurological disorders.

Identification of the products and sources of lipid peroxidation and its enzymatic or non-enzymatic nature have been essential for the design of mechanism-based therapies. Indeed, several therapeutic strategies, acting at different levels of the ALEs cascade, have been proposed essentially blocking ALEs formation, but also their catabolism or the resulting biological responses they induce (Fig. 4) [252].

6.1. Inhibition of ALEs formation

As described above, ALEs are generated through the covalent addition to specific nucleophilic amino acids of RCS, which are generated by lipid peroxidation; thus, several approaches have been proposed to reduce ALEs modulating RCS levels in different manners. Inhibition of ALEs formation can be obtained both with indirect strategies, mainly with antioxidants or metal ion chelators, or directly through RCS quenching.

6.1.1. Antioxidants

Antioxidants are molecules that are able to prevent undesired oxidation through their reaction with oxidants or oxidation intermediates. In this category are included xenobiotics that potentiate the endogenous detoxification machinery, which is composed of different enzymes involved in the inactivation of RCS [252], such as natural flavonoids and antioxidant micronutrients (i.e. β-carotene, retinol, vitamin E, and vitamin C). Vitamin E is, indeed, able to scavenge lipid peroxyl radicals, generating a tocopherol radical recycled to vitamin E by lipoic acid and ascorbic acid, while vitamin C is more effective in trapping oxygen, nitrogen and sulfhydryl radical reducing the generation of lipid hydroperoxide.

Among the antioxidant compounds able to inhibit lipid peroxidation, pyridoindole stobadine was proposed due to its ability to not only scavenge oxygen radicals but also to quench singlet oxygen, to repair oxidized bases and to maintain oxidation of SH groups by one-electron donation [253]. It has been demonstrated that stobadine is able to reduce both lipid peroxidation (i.e. conjugated dyene or MDA) [254] and the consequent protein modifications induced by oxidative stress, and it has been postulated as a novel cardioprotectant [253,255,256]. *In vitro* studies demonstrated that stobadine decreased oxidation of LDL, both at lipid and protein level, suggesting a role in atherosclerosis [255]. Moreover, studies performed *in vitro* or in animal models demonstrated that stobadine could reduce the myocardium impairment attributable to oxidative stress (e.g. myocardial infarction, hypoxia/reoxygenation, catecholamine overexposure) [253] and is able to correct hypertriglyceridemia and hypercholesterolemia in diabetic rats [254]. Of note, it has been demonstrated that the effects of stobadine can be

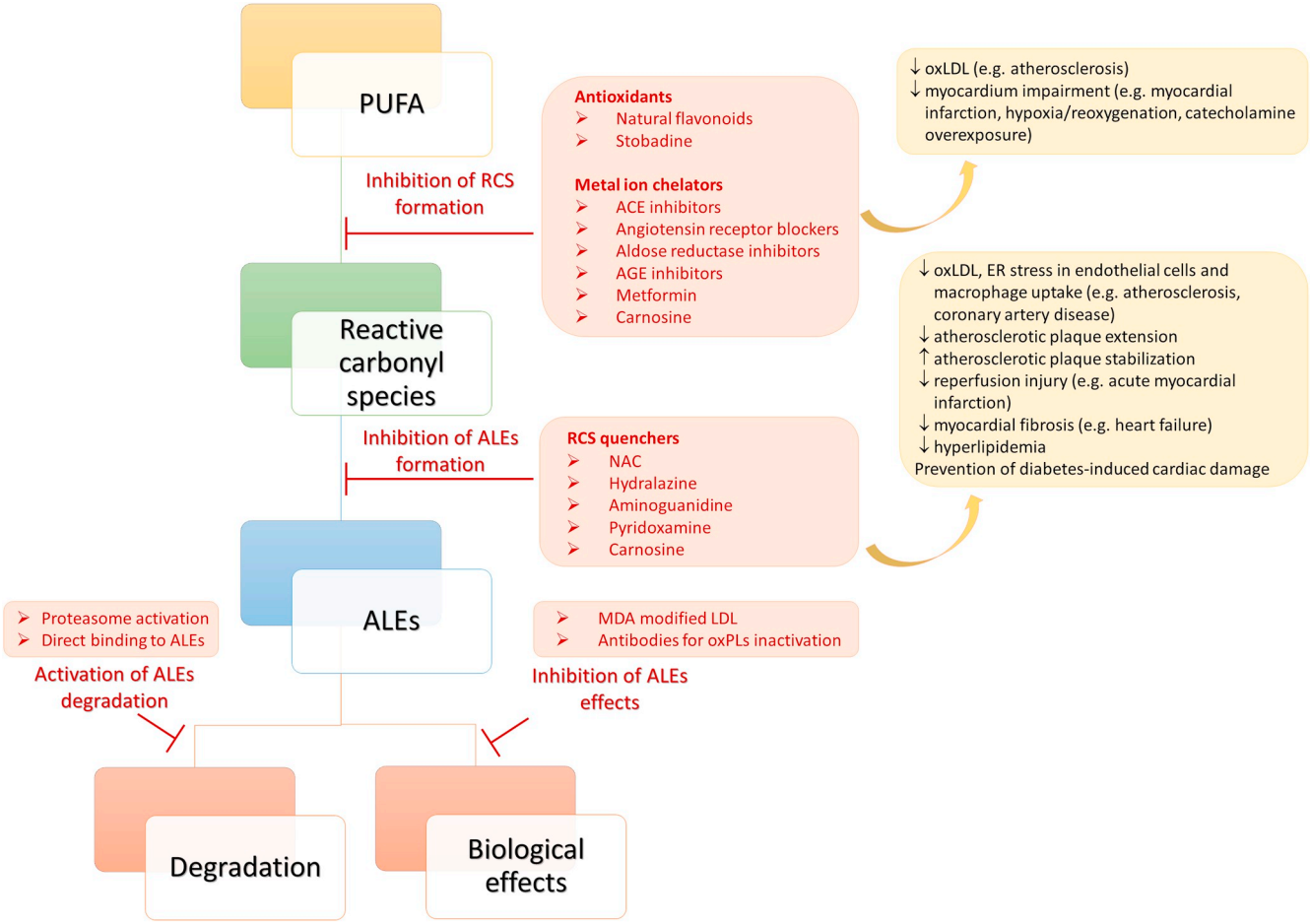

Fig. 4. Strategies to prevent lipoxidation effects and their implication in cardiovascular diseases. ER, endoplasmic reticulum; LDL, low-density lipoproteins; PUFA, polyunsaturated fatty acids; RCS, reactive carbonyl species; ALEs, advanced lipoxidation end-products.

ameliorated by concomitant administration of other antioxidants, such as vitamin E [256], and this is in line with the observation that *in vivo* interactions of antioxidants with endogenous vitamins may contribute to increasing the final antioxidant capacity [252,254]. Other antioxidant compounds include BHT, or Trolox, a soluble analog of alpha-tocopherol that has been shown also to contribute to the recycle of stobadine [257].

Notwithstanding the protective effects of these antioxidant compounds have been *in vitro* and *in vivo* demonstrated, a definitive conclusion regarding their clinical benefits is still lacking.

6.1.2. Metal ion chelators

ALE formation can also be inhibited by metal ion chelators or by multifunctional agents that possess this ability to chelate metal ions, which are the promoter of RCS formation, taking into consideration that complete elimination of all metal ions should not be achieved.

Of note, chelation is one of the most common mechanism to inhibit lipoxidation reactions utilized by many drugs employed to treat cardiovascular diseases, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, aldose reductase inhibitors, hydralazine, and other advanced glycation end-products (AGE) inhibitors, including pyridoxamine, carnosine or metformin [252,258]. However, regarding AGE breakers, N-phenacylthiazolium bromide (PTB) and its analogs, it has been demonstrated that chelating activity could be prevalent at high concentrations, such as those used in *in vitro* studies, but not at therapeutic concentrations in which the carbonyl trapping activity resulted to be more important [259].

6.1.3. RCS quenchers

ALE formation could also be reduced by RCS quenchers, strong reactive nucleophilic molecules that form covalent adducts with electrophilic carbonyl derivatives, including dialdehydes, keto-aldehydes, and α,β-unsaturated carbonyls. Classification of RCS quenchers is essentially based on the number of functional groups and their chemical nature: monoreactive quenchers include thiol-containing compounds, β-dicarbonyl analogs, guanidine and hydrazine derivatives; polyreactive compounds are amino derivatives, heterocycle-based compounds, phenols, and polyphenols. In general, monoreactive compounds are characterized by a lower selectivity than polyreactive molecules, which, on the other hand, display softer reactivity and do not act against physiological carbonyls [252].

The most important example of thiol-containing carbonyl scavenger is represented by the endogenous tripeptide γ-L-glutamyl-L-cysteinylglycine (GSH), a known antioxidant characterized also by a marked sulfur nucleophilicity that allows the formation of covalent adducts with electrophilic compounds [260]. The detoxifying activity is exerted by the mercapturic acid, which is generated after the loss of GSH terminal residues and makes the quenched molecules more hydrophilic, facilitating its secretion. GSH quenching activity could be spontaneous or catalyzed by specific enzymes, glutathione-S-transferases, that are upregulated by oxidative stress to improve detoxification of α,β-unsaturated aldehydes, such as 4-HNE.

A similar quenching activity has also been demonstrated by free cysteine and N-acetyl cysteine (NAC) [137]. NAC is known as a precursor of GSH as well as a direct antioxidant for some oxidant species, such as NO_2 and HOX. It is also able to break disulfides, thus releasing free thiols as well as reduced proteins [261], besides its activity in preventing ALEs accumulation [262].

NAC is employed in several branches of medicine including cardiology, where it has been increasingly used over the past decades, especially to reduce reperfusion injury in the treatment of acute myocardial infarction as demonstrated in several animal models [263]. Indeed, a recent systematic review highlighted the ameliorative properties of NAC in preventing diabetes-induced cardiac damage through inhibition of oxidative stress [264], together with several preclinical studies supporting this effect. Reyes et al. demonstrated in a rat model of long-term ascending aortic stenosis NAC treatment resulted in a reduction of myocardial fibrosis during the transition from compensated left ventricular (LV) hypertrophy to heart failure, paralleled by reduced oxidative stress due to restored glutathione levels and reduced MDA [265]. Giam et al. demonstrated that NAC could blunt cardiac fibrosis and related remodeling in the setting of heart failure, in mice with cardiomyopathy secondary to the cardiac-specific overexpression of mammalian sterile 20-like kinase 1, and this effect was ascribed to reduced oxidative stress [266].

In addition, NAC has also been proposed for the treatment of atherosclerosis. In *in vitro* studies, NAC inhibits lipoxidation- and oxidized LDL-induced endoplasmic reticulum (ER) stress in human endothelial cells implicated in atherogenesis [267]. Moreover, Cui et al. demonstrated that NAC attenuates atherosclerosis and reduces LDL oxidation in a mouse model of atherosclerosis represented by LDL receptor knockout mice. This effect was also evidenced *in vivo* in patients with coronary artery disease in which oxidized LDL, and not native LDL, were reduced by NAC [268]. Moreover, in ApoE deficient mice, NAC was able to induce atherosclerotic plaque stabilization [269].

Among the monoreactive nitrogen-containing compounds, an important role is played by the hydrazine derivatives, which react both with α,β-unsaturated aldehydes and dicarbonyls, even if with low selectivity. This category includes several well-established vasodilators, such as hydralazine, used as antihypertensive drugs, all with an excellent scavenging activity. Thus, several studies have been designed to evaluate the effects of their scavenging activity in oxidative stress-based diseases; for example, many hydrazine derivatives can reduce atherosclerotic lesion development through the carbonyl stress inhibition [270,271]. In particular, Vindis et al. demonstrated that hydralazine traps 4-HNE and reduces the formation of 4-HNE adducts on the platelet-derived growth factor receptor-β (PDGFβ) in smooth muscle cells treated *in vitro* with oxidized LDL. Moreover, this effect was also evidenced *in vivo* in hypercholesterolemic rabbits. Indeed, accumulation of 4-HNE-adducts on PDGFβ was increased in the aorta of hypercholesterolemic rabbits in comparison with normocholesterolemic rabbits, but hydralazine was able to reduce them, mainly in the intima, with a concomitant reduction of the extension of the atherosclerotic plaque [272].

Similarly, aminoguanidine reduces modifications of LDL induced by MDA, thereby preventing macrophage uptake and degradation of oxidized LDL [273], even if this protective effect could be also ascribed to the antioxidant properties of aminoguanidine, not only to its activity as RCS quencher [112].

Pyridoxamine (PM), one of the three natural forms of vitamin B6, is another carbonyl quencher known to prevent the formation of both ALEs and AGEs in various biological systems [112]. Several studies reported that PM can trap lipid peroxidation intermediates, thus reducing the chemical modifications of proteins exerted by peroxidizing agents, *in vitro* but also *in vivo* [274–276]. It has been demonstrated that PM can react with products of lipid peroxidation via its benzoyl amino group, and completely prevented the modifications of lysine residues on the RNase treated with arachidonic acid, and on LDL in a model of copper-catalyzed oxidation [275]. These effects have been ascribed to the ability of PM to intercept early lipid peroxidation products, precursors of reactive carbonyl groups involved in the formation of ALEs [275,276]. In addition, Kang et al. showed that PM interferes with the lipid peroxidation damage directly trapping MDA, under physiological conditions, as demonstrated by its ability to reduce the generation of lipofuscin-like fluorescence resulting from the incubation of MDA with bovine serum albumin [274]. More recently, Lee et al. compared the reaction of PM with ONE and 4-HNE and its effects on lysine modifications on human serum albumin exerted by the two lipid hydroperoxide-derived aldehydes. They demonstrated that the formation of adducts of PM with ONE is preferred, and that albumin modified by ONE, but not by 4-HNE, was decreased by treatment with PM [277]. Protective effects of PM have also been demonstrated in insulin-

secreting cells exposed *in vitro* to NO, where PM was able to inhibit NO-induced apoptosis through the inhibition of ALEs formation and carbonylation of poly (ADP-ribose) polymerase [278]. Finally, PM could also reduce AGE/ALEs formation *in vivo,* in streptozotocin-diabetic rats and in non-diabetic Zucker obese rats, with a consequent reduction of hyperlipidemia and protection against renal and vascular complications [279,280].

Carnosine, a polyreactive quencher, is a dipeptide of β-alanine and His, characterized by a strong antioxidant capacity due to metal ion chelation and RCS scavenging properties. These effects were demonstrated *in vitro* and *in vivo*, both in physiological conditions and in animal models of oxidative stress [281]. Several studies report the ability of carnosine to prevent ALEs formation induced by different precursors, including MDA [282]. It has been demonstrated that carnosine can directly bind to α,β-unsaturated aldehydes, such as 4-HNE [283], acrolein [284] and MDA [285]. Recently, Vistoli et al. evaluated also the quenching activity of carnosine and some of its derivatives towards MDA and methylglyoxal, and demonstrated that derivatives are more reactive with MDA than with methylglyoxal [285]. The quenching activity of carnosine towards 4-HNE have also been demonstrated *in vivo* in Zucker obese rat [286]. Therapeutic use of carnosine and its analogs have been tested in several pathological states, from cancer to Alzheimer's and cardiovascular disease, ischemia or diabetic complications, as previously summarized in the review by Boldyrev et al. [281].

Of note, a recent paper demonstrated the therapeutic value of a carnosinase-resistant analog, carnosinol, which displayed a higher potency and selectivity toward α,β-unsaturated aldehydes (e.g. 4-HNE, ACR) and reduced 4-HNE adduct formation in rodent models of obesity and metabolic syndrome [287]. Of note, Menini et al. showed that D-carnosine was able to protect, *in vitro,* smooth muscle cells from 4-HNE-induced injury and, to reduce, *in vivo*, atherosclerosis, in a mouse model of ApoE null mice fed with a Western diet [288].

6.2. Other therapeutic strategies

Once ALEs are formed, alternative strategies could be employed to reduce their effects, by enhancing, for example, their catabolism or by acting on ALE-induced biological responses, as already widely demonstrated for AGEs [252].

Degradation of ALEs and, in particular, of 4-HNE-modified proteins, has been mainly ascribed to a proteasome-dependent process, even if 4-HNE-protein adducts could form cross-linked aggregates able to inhibit the proteasome system, thus impairing their cellular turnover [289]. Few studies reported alternative ways of degradation based on lysosomal degradation or protease activity [13]. Of note, the above-described RCS quencher carnosine has been shown to react directly with ALEs leading to the formation of carbonyl-carnosine adducts and, thus, reducing crosslinking of oxidized proteins with other unmodified proteins, such as the proteasome components [281,282]. Proteasome activation could also be linked to the ability of carnosine to modulate release, metabolism, and activity of nitric oxide, a known activator of proteasome activity [281].

On the other hand, concerning the possibility of acting on the biological responses raised by ALEs, the extensive knowledge on the role of LDL oxidation in the atherosclerotic process led to the development of MDA-modified homologous LDL (MDA-LDL), able to exert atheroprotective effects in rabbits and mice [148]. More recently, Gonen et al. demonstrated that also small immunogens generated from the condensation of two or more MDA molecules with lysine could lead to atheroprotective responses; therefore, they have been proposed to generate a vaccine to retard or even prevent the development of atherosclerosis [290]. Recent studies suggest also that inactivation of oxPLs with modified forms of E06 antibody could reduce the inflammatory state associated with atherosclerosis [46].

7. Lipid peroxidation in clinical studies

Several oxidative biomarkers are currently measured in research laboratories, but they are not yet used as routine assays in clinical laboratories because of their uncertain diagnostic value [291,292]. The clinical applicability of several oxidative stress markers is influenced by the fact that some protein modifications by excessive ROS have a direct effect on the function of target molecules, while other modifications just reflect the general local degree of oxidative stress [293]. Venous blood and urinary samples are the most commonly used in clinical practice, but sometimes the measurement in specific tissue or cell samples may give more precise information. Measurement of lipid peroxidation products may be a reliable marker, as long as it is characterized by high sensitivity, specificity, and reproducibility.

However, several clinical studies focused on the detection and quantitation of the main lipid peroxidation products are actually reported in the literature. One of the most important clinical studies that evaluated the predictive value of lipid hydroperoxides levels for adverse cardiovascular outcomes in patients with stable CAD was PREVENT (Prospective Randomized Evaluation of the Vascular Effects of Norvasc) Trial [294]. Circulating levels of 4-HNE bound to proteins, for example, were measured by GC-MS in ambulatory symptomatic heart failure patients and control subjects, along with other clinically- and biochemically-relevant parameters, such as other oxidative stress markers, total levels of fatty acids from all classes [295]. Few clinical studies documented also circulating plasma levels of MDA using the highly criticized TBARS method [198,200,296]. Urinary and plasma isoprostanes have been mostly promoted for the *in vivo* estimation of oxidative stress for human studies. Urinary and plasma 8-isoprostane levels were measured in patients scheduled for coronary angiography, in order to investigate their association with the presence and severity of CAD [297,298]. Moreover, a case-control study was performed to measure by LC-MS/MS systemic levels of multiple specific fatty acid oxidation products including F2-isoprostanes in CAD patients and subjects without CAD [299]. A larger case-control study also investigated the association between lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) activity and CAD in relation to oxidative stress markers, in particular urinary 8-iso-PGF2α, in patients with angiographically confirmed CAD versus healthy controls [300]. Indexes of redox status have been also proposed evaluating the oxidative stress by using more than one criterion [301,302]. The OXY-SCORE is a global oxidative stress index, computed by subtracting the protection score (GSH, alpha- and gamma-tocopherol levels, and antioxidant capacity) from the damage score (plasma free and total MDA, GSSG/GSH ratio, and urine F2-IsoPs). Its good performance was demonstrated in CAD patients versus healthy subjects, and the OXY-SCORE was significantly higher in CAD after adjusting for age, gender and smoking [303]. Similarly, Oxidative-INDEX reflects both oxidative and antioxidant counterparts [302].

8. Conclusions

Lipid peroxidation products represent a broad category of highly reactive compounds that exert many biological effects due to direct activation of signaling pathways or modification of other macromolecules, including nucleic acids, phospholipids or proteins.

Their involvement in the context of cardiovascular diseases has been mainly associated with the oxidation of circulating lipoprotein and the role of oxidized LDL in atherosclerosis. However, the heart is a highly oxidative organ in which cardiomyocyte turnover is virtually absent, making it particularly vulnerable to the accumulation of lipid peroxidation products formed as a result of oxidative damage.

Technological advances, mainly in the field of mass spectrometry, allowed overcoming the challenges in the detection, characterization, and quantitation of such complex heterogeneous class of lipid modifications.

Thanks to the expanding knowledge of the products and sources of lipid peroxidation and ALEs formation, several therapeutic strategies have been proposed and successfully applied to different oxidative-based diseases. However, a deeper understanding of the mechanisms of formation and targets of ALEs could expand the available therapeutic strategies. Indeed, it is clear that antioxidants can be used to reduce ALEs formation, but cannot neutralize the effects of ALEs when adducts are already formed, and this aspect could explain why *in vitro* or pre-clinical efficacy of antioxidant treatment is not always confirmed *in vivo,* in human patients.

Declaration of interest

None.

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Appendix A. Supplementary data

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References

[1] T. Shibata, K. Shimizu, K. Hirano, et al., Adductome-based identification of biomarkers for lipid peroxidation, J. Biol. Chem. 292 (2017) 8223–8235.
[2] A. Ayala, M.F. Munoz, S. Arguelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell Longev. 2014 (2014) 360438.
[3] S.H. Lee, I.A. Blair, Characterization of 4-oxo-2-nonenal as a novel product of lipid peroxidation, Chem. Res. Toxicol. 13 (2000) 698–702.
[4] K.A. Massey, A. Nicolaou, Lipidomics of oxidized polyunsaturated fatty acids, Free Radic. Biol. Med. 59 (2013) 45–55.
[5] G. Vistoli, D. De Maddis, A. Cipak, et al., Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation, Free Radic. Res. 47 (Suppl 1) (2013) 3–27.
[6] E. Miller, A. Morel, L. Saso, et al., Isoprostanes and neuroprostanes as biomarkers of oxidative stress in neurodegenerative diseases, Oxid. Med. Cell Longev. 2014 (2014) 572491.
[7] R. Pamplona, Advanced lipoxidation end-products, Chem. Biol. Interact. 192 (2011) 14–20.
[8] X. Wei, H. Yin, Covalent modification of DNA by alpha, beta-unsaturated aldehydes derived from lipid peroxidation: recent progress and challenges, Free Radic. Res. 49 (2015) 905–917.
[9] A. Naudi, M. Jove, V. Ayala, et al., Non-enzymatic modification of aminophospholipids by carbonyl-amine reactions, Int. J. Mol. Sci. 14 (2013) 3285–3313.
[10] R.M. Domingues, P. Domingues, T. Melo, et al., Lipoxidation adducts with peptides and proteins: deleterious modifications or signaling mechanisms? J. Proteom. 92 (2013) 110–131.
[11] S. Lee, K.G. Birukov, C.E. Romanoski, et al., Role of phospholipid oxidation products in atherosclerosis, Circ. Res. 111 (2012) 778–799.
[12] M.X. Fu, J.R. Requena, A.J. Jenkins, et al., The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions, J. Biol. Chem. 271 (1996) 9982–9986.
[13] J.P. Castro, T. Jung, T. Grune, et al., 4-Hydroxynonenal (HNE) modified proteins in metabolic diseases, Free Radic. Biol. Med. 111 (2017) 309–315.
[14] A. Hohn, T. Grune, Lipofuscin: formation, effects and role of macroautophagy, Redox Biol. 1 (2013) 140–144.
[15] R.J. Schaur, Basic aspects of the biochemical reactivity of 4-hydroxynonenal, Mol. Aspect. Med. 24 (2003) 149–159.
[16] W.H. Zhang, J. Liu, G. Xu, et al., Model studies on protein side chain modification by 4-oxo-2-nonenal, Chem. Res. Toxicol. 16 (2003) 512–523.
[17] T. Maeshima, K. Honda, M. Chikazawa, et al., Quantitative analysis of acrolein-specific adducts generated during lipid peroxidation-modification of proteins in vitro: identification of N(tau)-(3-propanal)histidine as the major adduct, Chem. Res. Toxicol. 25 (2012) 1384–1392.
[18] D. Del Rio, A.J. Stewart, N. Pellegrini, A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress, Nutr. Metabol. Cardiovasc. Dis. 15 (2005) 316–328.
[19] L.J. Roberts 2nd, G.L. Milne, Isoprostanes, J. Lipid Res. 50 (Suppl) (2009) S219–S223.
[20] J.D. Morrow, K.E. Hill, R.F. Burk, et al., A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 9383–9387.
[21] Z. Mallat, I. Philip, M. Lebret, et al., Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilatation and progression to heart failure, Circulation 97 (1998) 1536–1539.
[22] H.C. Yen, T.W. Chen, T.C. Yang, et al., Levels of F2-isoprostanes, F4-neuroprostanes, and total nitrate/nitrite in plasma and cerebrospinal fluid of patients with traumatic brain injury, Free Radic. Res. 49 (2015) 1419–1430.
[23] R. Bartova, D. Petrlenicova, K. Oresanska, et al., Changes in levels of oxidative stress markers and some neuronal enzyme activities in cerebrospinal fluid of multiple sclerosis patients, Neuroendocrinol. Lett. 37 (2016) 102–106.
[24] Y. Chen, J.D. Morrow, L.J. Roberts 2nd, Formation of reactive cyclopentenone compounds in vivo as products of the isoprostane pathway, J. Biol. Chem. 274 (1999) 10863–10868.
[25] G.L. Milne, G. Zanoni, A. Porta, et al., The cyclopentenone product of lipid peroxidation, 15-A2t-isoprostane, is efficiently metabolized by HepG2 cells via conjugation with glutathione, Chem. Res. Toxicol. 17 (2004) 17–25.
[26] J.M. Galano, J.C. Lee, C. Gladine, et al., Non-enzymatic cyclic oxygenated metabolites of adrenic, docosahexaenoic, eicosapentaenoic and alpha-linolenic acids; bioactivities and potential use as biomarkers, Biochim. Biophys. Acta 1851 (2015) 446–455.
[27] M. VanRollins, R.L. Woltjer, H. Yin, et al., F2-dihomo-isoprostanes arise from free radical attack on adrenic acid, J. Lipid Res. 49 (2008) 995–1005.
[28] R.G. Salomon, E. Batyreva, K. Kaur, et al., Isolevuglandin-protein adducts in humans: products of free radical-induced lipid oxidation through the isoprostane pathway, Biochim. Biophys. Acta 1485 (2000) 225–235.
[29] H. Yin, N.A. Porter, New insights regarding the autoxidation of polyunsaturated fatty acids, Antioxidants Redox Signal. 7 (2005) 170–184.
[30] J.P. Fessel, L. Jackson Roberts, Isofurans: novel products of lipid peroxidation that define the occurrence of oxidant injury in settings of elevated oxygen tension, Antioxidants Redox Signal. 7 (2005) 202–209.
[31] G.O. Fruhwirth, A. Loidl, A. Hermetter, Oxidized phospholipids: from molecular properties to disease, Biochim. Biophys. Acta 1772 (2007) 718–736.
[32] S. Parthasarathy, D. Steinberg, J.L. Witztum, The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis, Annu. Rev. Med. 43 (1992) 219–225.
[33] M. Khazaei, F. Moien-Afshari, I. Laher, Vascular endothelial function in health and diseases, Pathophysiology 15 (2008) 49–67.
[34] A. Sandoo, J.J. van Zanten, G.S. Metsios, et al., The endothelium and its role in regulating vascular tone, Open Cardiovasc. Med. J. 4 (2010) 302–312.
[35] K.H. Park, W.J. Park, Endothelial dysfunction: clinical implications in cardiovascular disease and therapeutic approaches, J. Kor. Med. Sci. 30 (2015) 1213–1225.
[36] P.O. Bonetti, L.O. Lerman, A. Lerman, Endothelial dysfunction: a marker of atherosclerotic risk, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 168–175.
[37] S. Xu, S. Ogura, J. Chen, et al., LOX-1 in atherosclerosis: biological functions and pharmacological modifiers, Cell. Mol. Life Sci. 70 (2013) 2859–2872.
[38] R. Yoshimoto, Y. Fujita, A. Kakino, et al., The discovery of LOX-1, its ligands and clinical significance, Cardiovasc. Drugs Ther. 25 (2011) 379–391.
[39] A.J. Murphy, K.J. Woollard, High-density lipoprotein: a potent inhibitor of inflammation, Clin. Exp. Pharmacol. Physiol. 37 (2010) 710–718.
[40] B.A. Kingwell, M.J. Chapman, A. Kontush, et al., HDL-targeted therapies: progress, failures and future, Nat. Rev. Drug Discov. 13 (2014) 445–464.
[41] J.L. Martin-Ventura, R. Rodrigues-Diez, D. Martinez-Lopez, et al., Oxidative stress in human atherothrombosis: sources, markers and therapeutic targets, Int. J. Mol. Sci. 18 (2017) pii: E2315.
[42] E. Malle, G. Marsche, J. Arnhold, et al., Modification of low-density lipoprotein by myeloperoxidase-derived oxidants and reagent hypochlorous acid, Biochim. Biophys. Acta 1761 (2006) 392–415.
[43] A.M. Fogelman, I. Shechter, J. Seager, et al., Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophages, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 2214–2218.
[44] H.N. Yassine, A.M. Jackson, P.D. Reaven, et al., The application of multiple reaction monitoring to assess Apo A-I methionine oxidations in diabetes and cardiovascular disease, Transl. Proteom. 4–5 (2014) 18–24.
[45] S. Horkko, D.A. Bird, E. Miller, et al., Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins, J. Clin. Invest. 103 (1999) 117–128.
[46] X. Que, M.Y. Hung, C. Yeang, et al., Oxidized phospholipids are proinflammatory and proatherogenic in hypercholesterolaemic mice, Nature 558 (2018) 301–306.
[47] S.P. Forbes, L.J. Druhan, J.E. Guzman, et al., Mechanism of 4-HNE mediated inhibition of hDDAH-1: implications in no regulation, Biochemistry 47 (2008) 1819–1826.
[48] T. Okada, K. Morino, F. Nakagawa, et al., N-3 polyunsaturated fatty acids decrease the protein expression of soluble epoxide hydrolase via oxidative stress-induced P38 kinase in rat endothelial cells, Nutrients 9 (2017).
[49] Y.X. Wang, A. Ulu, L.N. Zhang, et al., Soluble epoxide hydrolase in atherosclerosis, Curr. Atheroscler. Rep. 12 (2010) 174–183.
[50] A. Ishikado, K. Morino, Y. Nishio, et al., 4-Hydroxy hexenal derived from docosahexaenoic acid protects endothelial cells via Nrf2 activation, PLoS One 8 (2013) e69415.
[51] G. Leonarduzzi, P. Gamba, S. Gargiulo, et al., Inflammation-related gene expression by lipid oxidation-derived products in the progression of atherosclerosis, Free Radic. Biol. Med. 52 (2012) 19–34.
[52] N. Shanmugam, J.L. Figarola, Y. Li, et al., Proinflammatory effects of advanced

lipoxidation end products in monocytes, Diabetes 57 (2008) 879–888.
[53] L.J. van Tits, R. Stienstra, P.L. van Lent, et al., Oxidized LDL enhances pro-inflammatory responses of alternatively activated M2 macrophages: a crucial role for Kruppel-like factor 2, Atherosclerosis 214 (2011) 345–349.
[54] C.E. Kim, S.J. Lee, K.W. Seo, et al., Acrolein increases 5-lipoxygenase expression in murine macrophages through activation of ERK pathway, Toxicol. Appl. Pharmacol. 245 (2010) 76–82.
[55] T.E. O'Toole, Y.T. Zheng, J. Hellmann, et al., Acrolein activates matrix metalloproteinases by increasing reactive oxygen species in macrophages, Toxicol. Appl. Pharmacol. 236 (2009) 194–201.
[56] A. Moghe, S. Ghare, B. Lamoreau, et al., Molecular mechanisms of acrolein toxicity: relevance to human disease, Toxicol. Sci. 143 (2015) 242–255.
[57] A.C. Schrimpe-Rutledge, K.Y. Fong, D.W. Wright, Impact of 4-hydroxynonenal on matrix metalloproteinase-9 regulation in lipopolysaccharide-stimulated RAW 264.7 cells, Cell Biochem. Funct. 33 (2015) 59–66.
[58] S.J. Lee, K.W. Seo, M.R. Yun, et al., 4-Hydroxynonenal enhances MMP-2 production in vascular smooth muscle cells via mitochondrial ROS-mediated activation of the Akt/NF-kappaB signaling pathways, Free Radic. Biol. Med. 45 (2008) 1487–1492.
[59] S. Akiba, S. Kumazawa, H. Yamaguchi, et al., Acceleration of matrix metalloproteinase-1 production and activation of platelet-derived growth factor receptor beta in human coronary smooth muscle cells by oxidized LDL and 4-hydroxynonenal, Biochim. Biophys. Acta 1763 (2006) 797–804.
[60] R. Vatsyayan, H. Kothari, U.R. Pendurthi, et al., 4-Hydroxy-2-nonenal enhances tissue factor activity in human monocytic cells via p38 mitogen-activated protein kinase activation-dependent phosphatidylserine exposure, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1601–1611.
[61] T.J. Lee, J.T. Lee, S.K. Moon, et al., Age-related differential growth rate and response to 4-hydroxynonenal in mouse aortic smooth muscle cells, Int. J. Mol. Med. 17 (2006) 29–35.
[62] T. Munzel, G.G. Camici, C. Maack, et al., Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series, J. Am. Coll. Cardiol. 70 (2017) 212–229.
[63] T.J. Guzik, J. Sadowski, B. Kapelak, et al., Systemic regulation of vascular NAD(P)H oxidase activity and nox isoform expression in human arteries and veins, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1614–1620.
[64] R. Stocker, J.F. Keaney Jr., New insights on oxidative stress in the artery wall, J. Thromb. Haemostasis 3 (2005) 1825–1834.
[65] K. Zarkovic, P. Larroque-Cardoso, M. Pucelle, et al., Elastin aging and lipid oxidation products in human aorta, Redox Biol. 4 (2015) 109–117.
[66] V.M. Subbotin, Neovascularization of coronary tunica intima (DIT) is the cause of coronary atherosclerosis. Lipoproteins invade coronary intima via neovascularization from adventitial vasa vasorum, but not from the arterial lumen: a hypothesis, Theor. Biol. Med. Model. 9 (2012) 11.
[67] P. Larroque-Cardoso, E. Mucher, M.H. Grazide, et al., 4-Hydroxynonenal impairs transforming growth factor-beta1-induced elastin synthesis via epidermal growth factor receptor activation in human and murine fibroblasts, Free Radic. Biol. Med. 71 (2014) 427–436.
[68] S.J. Lee, C.E. Kim, M.R. Yun, et al., 4-Hydroxynonenal enhances MMP-9 production in murine macrophages via 5-lipoxygenase-mediated activation of ERK and p38 MAPK, Toxicol. Appl. Pharmacol. 242 (2010) 191–198.
[69] S.C. Kolwicz Jr., S. Purohit, R. Tian, Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes, Circ. Res. 113 (2013) 603–616.
[70] G.D. Lopaschuk, J.R. Ussher, C.D. Folmes, et al., Myocardial fatty acid metabolism in health and disease, Physiol. Rev. 90 (2010) 207–258.
[71] N. Fillmore, J. Mori, G.D. Lopaschuk, Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy, Br. J. Pharmacol. 171 (2014) 2080–2090.
[72] H.C. Chiu, A. Kovacs, R.M. Blanton, et al., Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy, Circ. Res. 96 (2005) 225–233.
[73] B.N. Finck, J.J. Lehman, T.C. Leone, et al., The cardiac phenotype induced by PPARalpha overexpression mimics that caused by diabetes mellitus, J. Clin. Invest. 109 (2002) 121–130.
[74] K. Watanabe, H. Fujii, T. Takahashi, et al., Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor alpha associated with age-dependent cardiac toxicity, J. Biol. Chem. 275 (2000) 22293–22299.
[75] E.M. Burkart, N. Sambandam, X. Han, et al., Nuclear receptors PPARbeta/delta and PPARalpha direct distinct metabolic regulatory programs in the mouse heart, J. Clin. Invest. 117 (2007) 3930–3939.
[76] T. Shiraki, N. Kamiya, S. Shiki, et al., Alpha,beta-unsaturated ketone is a core moiety of natural ligands for covalent binding to peroxisome proliferator-activated receptor gamma, J. Biol. Chem. 280 (2005) 14145–14153.
[77] T. Itoh, L. Fairall, K. Amin, et al., Structural basis for the activation of PPARgamma by oxidized fatty acids, Nat. Struct. Mol. Biol. 15 (2008) 924–931.
[78] V. Benedusi, F. Martorana, L. Brambilla, et al., The peroxisome proliferator-activated receptor gamma (PPARgamma) controls natural protective mechanisms against lipid peroxidation in amyotrophic lateral sclerosis, J. Biol. Chem. 287 (2012) 35899–35911.
[79] B.N. Finck, D.P. Kelly, Peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) regulatory cascade in cardiac physiology and disease, Circulation 115 (2007) 2540–2548.
[80] B.Y. Nguyen, A. Ruiz-Velasco, T. Bui, et al., Mitochondrial function in the heart: the insight into mechanisms and therapeutic potentials, Br. J. Pharmacol. (2018) PMID: 29968316.
[81] E.J. Anderson, L.A. Katunga, M.S. Willis, Mitochondria as a source and target of lipid peroxidation products in healthy and diseased heart, Clin. Exp. Pharmacol. Physiol. 39 (2012) 179–193.
[82] A.J. Chicco, G.C. Sparagna, Role of cardiolipin alterations in mitochondrial dysfunction and disease, Am. J. Physiol. Cell Physiol. 292 (2007) C33–C44.
[83] J. Luo, B.G. Hill, Y. Gu, et al., Mechanisms of acrolein-induced myocardial dysfunction: implications for environmental and endogenous aldehyde exposure, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H3673–H3684.
[84] Z. Wu, E.Y. He, G.I. Scott, et al., alpha,beta-Unsaturated aldehyde pollutant acrolein suppresses cardiomyocyte contractile function: role of TRPV1 and oxidative stress, Environ. Toxicol. 30 (2015) 638–647.
[85] H.H. Refsgaard, L. Tsai, E.R. Stadtman, Modifications of proteins by polyunsaturated fatty acid peroxidation products, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 611–616.
[86] M. Glei, A. Schaeferhenrich, U. Claussen, et al., Comet fluorescence in situ hybridization analysis for oxidative stress-induced DNA damage in colon cancer relevant genes, Toxicol. Sci. 96 (2007) 279–284.
[87] A.G. Baker, D. Wiesler, M.V. Novotny, Tandem mass spectrometry of model peptides modified with trans-2-hexenal, a product of lipid peroxidation, J. Am. Soc. Mass Spectrom. 10 (1999) 613–624.
[88] P. Ping, C.P. Baines, Y. Gu, et al., Cardiac toxic effects of trans-2-hexenal are mediated by induction of cardiomyocyte apoptotic pathways, Cardiovasc. Toxicol. 3 (2003) 341–351.
[89] K. Uchida, 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress, Prog. Lipid Res. 42 (2003) 318–343.
[90] S.J. Chapple, X. Cheng, G.E. Mann, Effects of 4-hydroxynonenal on vascular endothelial and smooth muscle cell redox signaling and function in health and disease, Redox Biol. 1 (2013) 319–331.
[91] Y. Zhang, M. Sano, K. Shinmura, et al., 4-hydroxy-2-nonenal protects against cardiac ischemia-reperfusion injury via the Nrf2-dependent pathway, J. Mol. Cell. Cardiol. 49 (2010) 576–586.
[92] E. Lopez-Bernardo, A. Anedda, P. Sanchez-Perez, et al., 4-Hydroxynonenal induces Nrf2-mediated UCP3 upregulation in mouse cardiomyocytes, Free Radic. Biol. Med. 88 (2015) 427–438.
[93] E. Nagy, A.L. Jermendy, B. Merkely, et al., Clinical importance of epicardial adipose tissue, Arch. Med. Sci. 13 (2017) 864–874.
[94] R. Li, W.Q. Wang, H. Zhang, et al., Adiponectin improves endothelial function in hyperlipidemic rats by reducing oxidative/nitrative stress and differential regulation of eNOS/iNOS activity, Am. J. Physiol. Endocrinol. Metab. 293 (2007) E1703–E1708.
[95] H.S. Sacks, J.N. Fain, Human epicardial adipose tissue: a review, Am. Heart J. 153 (2007) 907–917.
[96] A. Salgado-Somoza, E. Teijeira-Fernandez, A.L. Fernandez, et al., Proteomic analysis of epicardial and subcutaneous adipose tissue reveals differences in proteins involved in oxidative stress, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H202–H209.
[97] B. Demir, E. Demir, G. Aciksari, et al., The association between the epicardial adipose tissue thickness and oxidative stress parameters in isolated metabolic syndrome patients: a multimarker approach, Int. J. Endocrinol. 2014 (2014) 954045.
[98] H.E. Bays, Adiposopathy is "sick fat" a cardiovascular disease? J. Am. Coll. Cardiol. 57 (2011) 2461–2473.
[99] W. Shen, Z. Wang, M. Punyanita, et al., Adipose tissue quantification by imaging methods: a proposed classification, Obes. Res. 11 (2003) 5–16.
[100] S.B. Heymsfield, T.A. Wadden, Mechanisms, pathophysiology, and management of obesity, N. Engl. J. Med. 376 (2017) 1492.
[101] L.F. Van Gaal, I.L. Mertens, C.E. De Block, Mechanisms linking obesity with cardiovascular disease, Nature 444 (2006) 875–880.
[102] S.A. Ritchie, J.M. Connell, The link between abdominal obesity, metabolic syndrome and cardiovascular disease, Nutr. Metabol. Cardiovasc. Dis. 17 (2007) 319–326.
[103] S. Furukawa, T. Fujita, M. Shimabukuro, et al., Increased oxidative stress in obesity and its impact on metabolic syndrome, J. Clin. Invest. 114 (2004) 1752–1761.
[104] A.F. Soares, M. Guichardant, D. Cozzone, et al., Effects of oxidative stress on adiponectin secretion and lactate production in 3T3-L1 adipocytes, Free Radic. Biol. Med. 38 (2005) 882–889.
[105] G. Murdolo, M. Piroddi, F. Luchetti, et al., Oxidative stress and lipid peroxidation by-products at the crossroad between adipose organ dysregulation and obesity-linked insulin resistance, Biochimie 95 (2013) 585–594.
[106] S.P. Singh, M. Niemczyk, D. Saini, et al., Role of the electrophilic lipid peroxidation product 4-hydroxynonenal in the development and maintenance of obesity in mice, Biochemistry 47 (2008) 3900–3911.
[107] P.A. Grimsrud, M.J. Picklo, Sr, T.J. Griffin, et al., Carbonylation of adipose proteins in obesity and insulin resistance: identification of adipocyte fatty acid-binding protein as a cellular target of 4-hydroxynonenal, Mol. Cell. Proteomics 6 (2007) 624–637.
[108] F. Bost, M. Aouadi, L. Caron, et al., The role of MAPKs in adipocyte differentiation and obesity, Biochimie 87 (2005) 51–56.
[109] M.A. Elrayess, S. Almuraikhy, W. Kafienah, et al., 4-hydroxynonenal causes impairment of human subcutaneous adipogenesis and induction of adipocyte insulin resistance, Free Radic. Biol. Med. 104 (2017) 129–137.
[110] K.S. Echtay, T.C. Esteves, J.L. Pakay, et al., A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling, EMBO J. 22 (2003) 4103–4110.
[111] M. Csala, T. Kardon, B. Legeza, et al., On the role of 4-hydroxynonenal in health

and disease, Biochim. Biophys. Acta 1852 (2015) 826–838.
[112] A. Negre-Salvayre, C. Coatrieux, C. Ingueneau, et al., Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors, Br. J. Pharmacol. 153 (2008) 6–20.
[113] Y.C. Awasthi, R. Sharma, A. Sharma, et al., Self-regulatory role of 4-hydroxynonenal in signaling for stress-induced programmed cell death, Free Radic. Biol. Med. 45 (2008) 111–118.
[114] C.M. Spickett, A.R. Pitt, Oxidative lipidomics coming of age: advances in analysis of oxidized phospholipids in physiology and pathology, Antioxidants Redox Signal. 22 (2015) 1646–1666.
[115] V. Serbulea, D. DeWeese, N. Leitinger, The effect of oxidized phospholipids on phenotypic polarization and function of macrophages, Free Radic. Biol. Med. 111 (2017) 156–168.
[116] L. Gros, A.A. Ishchenko, M. Saparbaev, Enzymology of repair of etheno-adducts, Mutat. Res. 531 (2003) 219–229.
[117] S.H. Lee, I.A. Blair, Oxidative DNA damage and cardiovascular disease, Trends Cardiovasc. Med. 11 (2001) 148–155.
[118] J. Nair, S. De Flora, A. Izzotti, et al., Lipid peroxidation-derived etheno-DNA adducts in human atherosclerotic lesions, Mutat. Res. 621 (2007) 95–105.
[119] R.J. Schaur, W. Siems, N. Bresgen, et al., 4-Hydroxy-nonenal-A bioactive lipid peroxidation product, Biomolecules 5 (2015) 2247–2337.
[120] M. Navab, S.T. Reddy, B.J. Van Lenten, et al., HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms, Nat. Rev. Cardiol. 8 (2011) 222–232.
[121] A. Rossi, P. Kapahi, G. Natoli, et al., Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase, Nature 403 (2000) 103–108.
[122] D.S. Straus, G. Pascual, M. Li, et al., 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4844–4849.
[123] E. Cernuda-Morollon, E. Pineda-Molina, F.J. Canada, et al., 15-Deoxy-Delta 12,14-prostaglandin J2 inhibition of NF-kappaB-DNA binding through covalent modification of the p50 subunit, J. Biol. Chem. 276 (2001) 35530–35536.
[124] T. Shiraki, N. Kamiya, H. Jingami, [Activation mechanism of PPARgamma by its endogenous ligands], Tanpakushitsu Kakusan Koso 50 (2005) 1660–1665.
[125] F. Lopez-Jimenez, M. Cortes-Bergoderi, Update: systemic diseases and the cardiovascular system (i): obesity and the heart, Rev. Esp. Cardiol. 64 (2011) 140–149.
[126] S. Pennathur, J.W. Heinecke, Mechanisms for oxidative stress in diabetic cardiovascular disease, Antioxidants Redox Signal. 9 (2007) 955–969.
[127] M.P. Mattson, Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders, Exp. Gerontol. 44 (2009) 625–633.
[128] H.K. Vincent, S.K. Powers, A.J. Dirks, et al., Mechanism for obesity-induced increase in myocardial lipid peroxidation, Int. J. Obes. Relat. Metab. Disord. 25 (2001) 378–388.
[129] X. Zhang, Z. Wang, J. Li, et al., Increased 4-hydroxynonenal formation contributes to obesity-related lipolytic activation in adipocytes, PLoS One 8 (2013) e70663.
[130] A.L. Kennedy, T.J. Lyons, Glycation, oxidation, and lipoxidation in the development of diabetic complications, Metabolism 46 (1997) 14–21.
[131] M. Brownlee, H. Vlassara, A. Cerami, Nonenzymatic glycosylation products on collagen covalently trap low-density lipoprotein, Diabetes 34 (1985) 938–941.
[132] A. Mezzetti, F. Cipollone, F. Cuccurullo, Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm, Cardiovasc. Res. 47 (2000) 475–488.
[133] R. Bucala, Z. Makita, G. Vega, et al., Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 9441–9445.
[134] C.B. Afonso, B.C. Sousa, A.R. Pitt, et al., A mass spectrometry approach for the identification and localization of small aldehyde modifications of proteins, Arch. Biochem. Biophys. 646 (2018) 38–45.
[135] D. Tsikas, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges, Anal. Biochem. 524 (2017) 13–30.
[136] C.M. Spickett, The lipid peroxidation product 4-hydroxy-2-nonenal: advances in chemistry and analysis, Redox Biol. 1 (2013) 145–152.
[137] N. Vidal, J.P. Cavaille, F. Graziani, et al., High throughput assay for evaluation of reactive carbonyl scavenging capacity, Redox Biol. 2 (2014) 590–598.
[138] L.J. Yan, M.J. Forster, Chemical probes for analysis of carbonylated proteins: a review, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 879 (2011) 1308–1315.
[139] S. Zelzer, H. Mangge, R. Oberreither, et al., Oxidative stress: determination of 4-hydroxy-2-nonenal by gas chromatography/mass spectrometry in human and rat plasma, Free Radic. Res. 49 (2015) 1233–1238.
[140] S. Rauli, M.D. Puppo, F. Magni, et al., Validation of malondialdehyde and 4-hydroxy-2-trans-nonenal measurement in plasma by NICI-GC-MS, J. Biochem. 123 (1998) 918–923.
[141] D. Spies-Martin, O. Sommerburg, C.D. Langhans, et al., Measurement of 4-hydroxynonenal in small volume blood plasma samples: modification of a gas chromatographic-mass spectrometric method for clinical settings, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 774 (2002) 231–239.
[142] M. Golizeh, T. Geib, L. Sleno, Identification of 4-hydroxynonenal protein targets in rat, mouse and human liver microsomes by two-dimensional liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 30 (2016) 1488–1494.
[143] A.M. Gioacchini, N. Calonghi, C. Boga, et al., Determination of 4-hydroxy-2-nonenal at cellular levels by means of electrospray mass spectrometry, Rapid Commun. Mass Spectrom. 13 (1999) 1573–1579.
[144] M. Colzani, G. Aldini, M. Carini, Mass spectrometric approaches for the identification and quantification of reactive carbonyl species protein adducts, J. Proteom. 92 (2013) 28–50.
[145] M. Carini, G. Aldini, R.M. Facino, Mass spectrometry for detection of 4-hydroxy-trans-2-nonenal (HNE) adducts with peptides and proteins, Mass Spectrom. Rev. 23 (2004) 281–305.
[146] K. Zarkovic, A. Jakovcevic, N. Zarkovic, Contribution of the HNE-immunohistochemistry to modern pathological concepts of major human diseases, Free Radic. Biol. Med. 111 (2017) 110–126.
[147] S. Borovic, F. Rabuzin, G. Waeg, et al., Enzyme-linked immunosorbent assay for 4-hydroxynonenal-histidine conjugates, Free Radic. Res. 40 (2006) 809–820.
[148] W. Palinski, S. Yla-Herttuala, M.E. Rosenfeld, et al., Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein, Arteriosclerosis 10 (1990) 325–335.
[149] Q. Chen, H. Esterbauer, G. Jurgens, Studies on epitopes on low-density lipoprotein modified by 4-hydroxynonenal. Biochemical characterization and determination, Biochem. J. 288 (Pt 1) (1992) 249–254.
[150] K. Uchida, K. Itakura, S. Kawakishi, et al., Characterization of epitopes recognized by 4-hydroxy-2-nonenal specific antibodies, Arch. Biochem. Biophys. 324 (1995) 241–248.
[151] K. Itakura, T. Oya-Ito, T. Osawa, et al., Detection of lipofuscin-like fluorophore in oxidized human low-density lipoprotein. 4-hydroxy-2-nonenal as a potential source of fluorescent chromophore, FEBS Lett. 473 (2000) 249–253.
[152] C. Wakita, K. Honda, T. Shibata, et al., A method for detection of 4-hydroxy-2-nonenal adducts in proteins, Free Radic. Biol. Med. 51 (2011) 1–4.
[153] S. Liu, W. Hou, H. Qin, et al., Oxidized LDL stimulates lipid peroxidation-derived DNA and protein adducts in human vascular endothelial and smooth muscle cells, J. Huazhong Univ. Sci. Technol. Med. Sci. 35 (2015) 200–205.
[154] A. Arcaro, M. Daga, G.P. Cetrangolo, et al., Generation of adducts of 4-Hydroxy-2-nonenal with heat shock 60 kDa protein 1 in human promyelocytic HL-60 and monocytic THP-1 cell lines, Oxid. Med. Cell Longev. 2015 (2015) 296146.
[155] K. Uchida, L.I. Szweda, H.Z. Chae, et al., Immunochemical detection of 4-hydroxynonenal protein adducts in oxidized hepatocytes, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 8742–8746.
[156] D. Weber, L. Milkovic, S.J. Bennett, et al., Measurement of HNE-protein adducts in human plasma and serum by ELISA-Comparison of two primary antibodies, Redox Biol. 1 (2013) 226–233.
[157] J.A. Doorn, D.R. Petersen, Covalent modification of amino acid nucleophiles by the lipid peroxidation products 4-hydroxy-2-nonenal and 4-oxo-2-nonenal, Chem. Res. Toxicol. 15 (2002) 1445–1450.
[158] A. Vila, K.A. Tallman, A.T. Jacobs, et al., Identification of protein targets of 4-hydroxynonenal using click chemistry for ex vivo biotinylation of azido and alkynyl derivatives, Chem. Res. Toxicol. 21 (2008) 432–444.
[159] N. Rauniyar, L. Prokai, Isotope-coded dimethyl tagging for differential quantification of posttranslational protein carbonylation by 4-hydroxy-2-nonenal, an end-product of lipid peroxidation, J. Mass Spectrom. 46 (2011) 976–985.
[160] J.D. Chavez, J. Wu, W. Bisson, et al., Site-specific proteomic analysis of lipoxidation adducts in cardiac mitochondria reveals chemical diversity of 2-alkenal adduction, J. Proteom. 74 (2011) 2417–2429.
[161] J. Wu, X. Luo, L.J. Yan, Two dimensional blue native/SDS-PAGE to identify mitochondrial complex I subunits modified by 4-hydroxynonenal (HNE), Front. Physiol. 6 (2015) 98.
[162] A. Pecorelli, C. Cervellati, A. Cortelazzo, et al., Proteomic analysis of 4-hydroxynonenal and nitrotyrosine modified proteins in RTT fibroblasts, Int. J. Biochem. Cell Biol. 81 (2016) 236–245.
[163] R.C. Gupta, V. Singh-Gupta, K. Zhang, et al., Abstract 12949: elamipretide (BendaviaTM) restores 4-hydroxy-2-nonenal protein adducts and aldehyde dehydrogenase-2 activity and mRNA expression in left ventricular myocardium of dogs with advanced heart failure, Circulation 134 (2018) A12949.
[164] K.M. Gomes, J.C. Campos, L.R. Bechara, et al., Aldehyde dehydrogenase 2 activation in heart failure restores mitochondrial function and improves ventricular function and remodelling, Cardiovasc. Res. 103 (2014) 498–508.
[165] K. Nakamura, K. Kusano, Y. Nakamura, et al., Carvedilol decreases elevated oxidative stress in human failing myocardium, Circulation 105 (2002) 2867–2871.
[166] S. Mak, D.C. Lehotay, M. Yazdanpanah, et al., Unsaturated aldehydes including 4-OH-nonenal are elevated in patients with congestive heart failure, J. Card. Fail. 6 (2000) 108–114.
[167] M. Giera, H. Lingeman, W.M. Niessen, Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): a brief overview, Chromatographia 75 (2012) 433–440.
[168] K. Yagi, A simple fluorometric assay for lipoperoxide in blood plasma, Biochem. Med. 15 (1976) 212–216.
[169] J.A. Knight, R.K. Pieper, L. McClellan, Specificity of the thiobarbituric acid reaction: its use in studies of lipid peroxidation, Clin. Chem. 34 (1988) 2433–2438.
[170] J.C. Cooley, C.E. Lunte, Detection of malondialdehyde in vivo using microdialysis sampling with CE-fluorescence, Electrophoresis 32 (2011) 2994–2999.
[171] R. Agarwal, S.D. Chase, Rapid, fluorimetric-liquid chromatographic determination of malondialdehyde in biological samples, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 775 (2002) 121–126.
[172] D. Del Rio, N. Pellegrini, B. Colombi, et al., Rapid fluorimetric method to detect total plasma malondialdehyde with mild derivatization conditions, Clin. Chem. 49 (2003) 690–692.
[173] J.Y. Sheu, H.P. Ku, W.C. Tseng, et al., Determination of thiobarbituric acid adduct of malondialdehyde using on-line microdialysis coupled with high-performance liquid chromatography, Anal. Sci. 19 (2003) 621–624.
[174] G.W. Cheng, H.L. Wu, Y.L. Huang, Automated on-line microdialysis sampling

coupled with high-performance liquid chromatography for simultaneous determination of malondialdehyde and ofloxacin in whole blood, Talanta 79 (2009) 1071–1075.
[175] D. Tsikas, S. Rothmann, J.Y. Schneider, et al., Development, validation and biomedical applications of stable-isotope dilution GC-MS and GC-MS/MS techniques for circulating malondialdehyde (MDA) after pentafluorobenzyl bromide derivatization: MDA as a biomarker of oxidative stress and its relation to 15(S)-8-iso-prostaglandin F2alpha and nitric oxide (NO), J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 1019 (2016) 95–111.
[176] J. Pilz, I. Meineke, C.H. Gleiter, Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative, J. Chromatogr. B Biomed. Sci. Appl. 742 (2000) 315–325.
[177] O. Korchazhkina, C. Exley, S. Andrew Spencer, Measurement by reversed-phase high-performance liquid chromatography of malondialdehyde in normal human urine following derivatisation with 2,4-dinitrophenylhydrazine, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 794 (2003) 353–362.
[178] S. Zelzer, R. Oberreither, C. Bernecker, et al., Measurement of total and free malondialdehyde by gas-chromatography mass spectrometry–comparison with high-performance liquid chromatography methology, Free Radic. Res. 47 (2013) 651–656.
[179] R. Malaei, A.M. Ramezani, G. Absalan, Analysis of malondialdehyde in human plasma samples through derivatization with 2,4-dinitrophenylhydrazine by ultrasound-assisted dispersive liquid-liquid microextraction-GC-FID approach, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 1089 (2018) 60–69.
[180] J. Mao, H. Zhang, J. Luo, et al., New method for HPLC separation and fluorescence detection of malonaldehyde in normal human plasma, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 832 (2006) 103–108.
[181] H.L. Lord, J. Rosenfeld, V. Volovich, et al., Determination of malondialdehyde in human plasma by fully automated solid phase analytical derivatization, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 877 (2009) 1292–1298.
[182] D. Gerard-Monnier, I. Erdelmeier, K. Regnard, et al., Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation, Chem. Res. Toxicol. 11 (1998) 1176–1183.
[183] Y.H. Siddique, G. Ara, M. Afzal, Estimation of lipid peroxidation induced by hydrogen peroxide in cultured human lymphocytes, Dose Response 10 (2012) 1–10.
[184] J.P. Steghens, A.L. van Kappel, I. Denis, et al., Diaminonaphtalene, a new highly specific reagent for HPLC-UV measurement of total and free malondialdehyde in human plasma or serum, Free Radic. Biol. Med. 31 (2001) 242–249.
[185] M. Giera, D.P. Kloos, A. Raaphorst, et al., Mild and selective labeling of malondialdehyde with 2-aminoacridone: assessment of urinary malondialdehyde levels, Analyst 136 (2011) 2763–2769.
[186] P. Manini, R. Andreoli, S. Sforza, et al., Evaluation of Alternate Isotope-Coded Derivatization Assay (AIDA) in the LC-MS/MS analysis of aldehydes in exhaled breath condensate, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 878 (2010) 2616–2622.
[187] M. Eggink, S. Charret, M. Wijtmans, et al., Development of an on-line weak-cation exchange liquid chromatography-tandem mass spectrometric method for screening aldehyde products in biological matrices, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 877 (2009) 3937–3945.
[188] L. Yu, P. Liu, Y.L. Wang, et al., Profiling of aldehyde-containing compounds by stable isotope labelling-assisted mass spectrometry analysis, Analyst 140 (2015) 5276–5286.
[189] L. Sangalli, L.M. Chiesa, E. Passero, et al., Improved procedure for the determination of malonaldehyde by gas-chromatography with electron-capture detection as 2,4,6-trichlorophenylhydrazine derivative, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 796 (2003) 201–207.
[190] H.S. Shin, Determination of malondialdehyde in human blood by headspace-solid phase micro-extraction gas chromatography-mass spectrometry after derivatization with 2,2,2-trifluoroethylhydrazine, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 877 (2009) 3707–3711.
[191] U. Dreissigacker, M.T. Suchy, N. Maassen, et al., Human plasma concentrations of malondialdehyde (MDA) and the F2-isoprostane 15(S)-8-iso-PGF(2alpha) may be markedly compromised by hemolysis: evidence by GC-MS/MS and potential analytical and biological ramifications, Clin. Biochem. 43 (2010) 159–167.
[192] F. Karatas, M. Karatepe, A. Baysar, Determination of free malondialdehyde in human serum by high-performance liquid chromatography, Anal. Biochem. 311 (2002) 76–79.
[193] A. Zinellu, S. Sotgia, L. Deiana, et al., Field-amplified online sample stacking capillary electrophoresis UV detection for plasma malondialdehyde measurement, Electrophoresis 32 (2011) 1893–1897.
[194] K. Syslova, P. Kacer, M. Kuzma, et al., Rapid and easy method for monitoring oxidative stress markers in body fluids of patients with asbestos or silica-induced lung diseases, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 877 (2009) 2477–2486.
[195] C.A. Sobsey, J. Han, K. Lin, et al., Development and evaluation of a liquid chromatography-mass spectrometry method for rapid, accurate quantitation of malondialdehyde in human plasma, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 1029–1030 (2016) 205–212.
[196] D. Tsikas, S. Rothmann, J.Y. Schneider, et al., Simultaneous GC-MS/MS measurement of malondialdehyde and 4-hydroxy-2-nonenal in human plasma: effects of long-term L-arginine administration, Anal. Biochem. 524 (2017) 31–44.
[197] D.V. Folden, A. Gupta, A.C. Sharma, et al., Malondialdehyde inhibits cardiac contractile function in ventricular myocytes via a p38 mitogen-activated protein kinase-dependent mechanism, Br. J. Pharmacol. 139 (2003) 1310–1316.
[198] C.R. Diaz-Velez, S. Garcia-Castineiras, E. Mendoza-Ramos, et al., Increased malondialdehyde in peripheral blood of patients with congestive heart failure, Am. Heart J. 131 (1996) 146–152.
[199] J.J. Belch, A.B. Bridges, N. Scott, et al., Oxygen free radicals and congestive heart failure, Br. Heart J. 65 (1991) 245–248.
[200] A. Serdar, D. Yesilbursa, Z. Serdar, et al., Relation of functional capacity with the oxidative stress and antioxidants in chronic heart failure, Congest. Heart Fail. 7 (2001) 309–311.
[201] S. Radovanovic, A. Savic-Radojevic, M. Pljesa-Ercegovac, et al., Markers of oxidative damage and antioxidant enzyme activities as predictors of morbidity and mortality in patients with chronic heart failure, J. Card. Fail. 18 (2012) 493–501.
[202] R.A. Alarcon, Fluorometric determination of acrolein and related compounds with m-aminophenol, Anal. Chem. 40 (1968) 1704–1708.
[203] F. Bohnenstengel, M. Eichelbaum, E. Golbs, et al., High-performance liquid chromatographic determination of acrolein as a marker for cyclophosphamide bioactivation in human liver microsomes, J. Chromatogr. B Biomed. Sci. Appl. 692 (1997) 163–168.
[204] M. Togashi, Y. Urano, H. Kojima, et al., Sensitive detection of acrolein in serum using time-resolved luminescence, Org. Lett. 12 (2010) 1704–1707.
[205] M. Togashi, T. Terai, H. Kojima, et al., Practical fluorescence detection of acrolein in human plasma via a two-step tethering approach, Chem Commun (Camb). 50 (2014) 14946–14948.
[206] M.H. El-Maghrabey, N. Kishikawa, K. Ohyama, et al., Analytical method for lipoperoxidation relevant reactive aldehydes in human sera by high-performance liquid chromatography-fluorescence detection, Anal. Biochem. 464 (2014) 36–42.
[207] A. Paci, A. Rieutord, D. Guillaume, et al., Quantitative high-performance liquid chromatographic determination of acrolein in plasma after derivatization with Luminarin 3, J. Chromatogr. B Biomed. Sci. Appl. 739 (2000) 239–246.
[208] T. Imazato, M. Kanematsu, N. Kishikawa, et al., Determination of acrolein in serum by high-performance liquid chromatography with fluorescence detection after pre-column fluorogenic derivatization using 1,2-diamino-4,5-dimethoxybenzene, Biomed. Chromatogr. 29 (2015) 1304–1308.
[209] K. Uchida, M. Kanematsu, Y. Morimitsu, et al., Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins, J. Biol. Chem. 273 (1998) 16058–16066.
[210] J. Pan, B. Awoyemi, Z. Xuan, et al., Detection of acrolein-derived cyclic DNA adducts in human cells by monoclonal antibodies, Chem. Res. Toxicol. 25 (2012) 2788–2795.
[211] H. Li, J. Wang, B. Kaphalia, et al., Quantitation of acrolein-protein adducts: potential biomarker of acrolein exposure, J. Toxicol. Environ. Health 67 (2004) 513–524.
[212] M.A. Bradley, W.R. Markesbery, M.A. Lovell, Increased levels of 4-hydroxynonenal and acrolein in the brain in preclinical Alzheimer disease, Free Radic. Biol. Med. 48 (2010) 1570–1576.
[213] R. Andreoli, P. Manini, M. Corradi, et al., Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom. 17 (2003) 637–645.
[214] S. Uchiyama, Y. Inaba, N. Kunugita, Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, J. Chromatogr. A 1217 (2010) 4383–4388.
[215] I.G. Casella, M. Contursi, Determination of aliphatic aldehydes by liquid chromatography with pulsed amperometric detection, J. Chromatogr. A 1063 (2005) 129–135.
[216] J. Wu, J.F. Stevens, C.S. Maier, Mass spectrometry-based quantification of myocardial protein adducts with acrolein in an in vivo model of oxidative stress, Mol. Nutr. Food Res. 55 (2011) 1401–1410.
[217] T.P. Devasagayam, K.K. Boloor, T. Ramasarma, Methods for estimating lipid peroxidation: an analysis of merits and demerits, Indian J. Biochem. Biophys. 40 (2003) 300–308.
[218] J. Klawitter, M. Haschke, T. Shokati, et al., Quantification of 15-F2t-isoprostane in human plasma and urine: results from enzyme-linked immunoassay and liquid chromatography/tandem mass spectrometry cannot be compared, Rapid Commun. Mass Spectrom. 25 (2011) 463–468.
[219] D.R. Briskey, G.R. Wilson, R.G. Fassett, et al., Optimized method for quantification of total F(2)-isoprostanes using gas chromatography-tandem mass spectrometry, J. Pharmaceut. Biomed. Anal. 90 (2014) 161–166.
[220] G.L. Milne, Q. Dai, L.J. Roberts 2nd, The isoprostanes–25 years later, Biochim. Biophys. Acta 1851 (2015) 433–445.
[221] A.E. Barden, E. Mas, K.D. Croft, et al., Minimizing artifactual elevation of lipid peroxidation products (F2-isoprostanes) in plasma during collection and storage, Anal. Biochem. 449 (2014) 129–131.
[222] B. Halliwell, C.Y. Lee, Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues, Antioxidants Redox Signal. 13 (2010) 145–156.
[223] A. Pekala-Wojciechowska, M. Poznanski, K. Szyszow, et al., Concentration of 8-isoprostanes in the exhaled breath condensate as a marker of oxidative stress in patients with type 1 diabetes, Adv. Respir. Med. 86 (2018) 3–6.
[224] S. Carrillo-Ibarra, J.I. Cerrillos-Gutierrez, A. Escalante-Nunez, et al., The oxidative and inflammatory state in patients with acute renal graft dysfunction treated with tacrolimus, Oxid. Med. Cell Longev. 2016 (2016) 5405847.
[225] X. Chu, Y. Ageishi, K. Nishimura, et al., Development of enzyme-linked immunosorbent assay for 8-iso-prostaglandin F2alpha, a biomarker of oxidative stress in vivo, and its application to the quantification in aged rats, J. Pharmaceut. Biomed. Anal. 50 (2009) 911–916.
[226] I.F. Wiswedel, (2)-isoprostanes: sensitive biomarkers of oxidative stress in vitro

and in vivo: a gas chromatography-mass spectrometric approach, Methods Mol. Biol. 580 (2009) 3–16.
[227] G.L. Milne, B. Gao, E.S. Terry, et al., Measurement of F2- isoprostanes and isofurans using gas chromatography-mass spectrometry, Free Radic. Biol. Med. 59 (2013) 36–44.
[228] V. Cavalca, F. Minardi, S. Scurati, et al., Simultaneous quantification of 8-iso-prostaglandin-F(2alpha) and 11-dehydro thromboxane B(2) in human urine by liquid chromatography-tandem mass spectrometry, Anal. Biochem. 397 (2010) 168–174.
[229] D. Tsikas, M.T. Suchy, Assessment of urinary F(2)-isoprostanes in experimental and clinical studies: mass spectrometry versus ELISA, Hypertension 60 (2012) e14.
[230] M.L. Langhorst, M.J. Hastings, W.H. Yokoyama, et al., Determination of F2-isoprostanes in urine by online solid phase extraction coupled to liquid chromatography with tandem mass spectrometry, J. Agric. Food Chem. 58 (2010) 6614–6620.
[231] S.A. Brose, A.G. Baker, M.Y. Golovko, A fast one-step extraction and UPLC-MS/MS analysis for E2/D 2 series prostaglandins and isoprostanes, Lipids 48 (2013) 411–419.
[232] D. Tsikas, M.T. Suchy, Protocols for the measurement of the F2-isoprostane, 15(S)-8-iso-prostaglandin F2alpha, in biological samples by GC-MS or GC-MS/MS coupled with immunoaffinity column chromatography, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 1019 (2016) 191–201.
[233] W. Liu, J.D. Morrow, H. Yin, Quantification of F2-isoprostanes as a reliable index of oxidative stress in vivo using gas chromatography-mass spectrometry (GC-MS) method, Free Radic. Biol. Med. 47 (2009) 1101–1107.
[234] V.B. Ritov, D.E. Kelley, V.E. Kagan, Derivatization of F2-isoprostanes with 1-pyrenyldiazomethane and their subsequent determination by fluorescence high-performance liquid chromatography, Anal. Biochem. 311 (2002) 10–18.
[235] X. Liu, P.D. Whitefield, Y. Ma, Quantification of F(2)-isoprostane isomers in cultured human lung epithelial cells after silica oxide and metal oxide nanoparticle treatment by liquid chromatography/tandem mass spectrometry, Talanta 81 (2010) 1599–1606.
[236] A.W. Taylor, M.G. Traber, Quantitation of plasma total 15-series F(2)-isoprostanes by sequential solid phase and liquid-liquid extraction, Anal. Biochem. 396 (2010) 319–321.
[237] Y.Y. Lee, J.M. Galano, C. Oger, et al., Assessment of isoprostanes in human plasma: technical considerations and the use of mass spectrometry, Lipids 51 (2016) 1217–1229.
[238] A.W. Taylor, R.S. Bruno, M.G. Traber, Women and smokers have elevated urinary F(2)-isoprostane metabolites: a novel extraction and LC-MS methodology, Lipids 43 (2008) 925–936.
[239] T. Petrosino, M. Serafini, Matrix effect in F(2)-isoprostanes quantification by HPLC-MS/MS: a validated method for analysis of iPF(2)alpha-III and iPF(2)alpha-VI in human urine, J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 965 (2014) 100–106.
[240] G. Rodríguez Patiño, M.A. Castillo Rodríguez, J.E. Ramírez Bribiesca, et al., Development of a method for the determination of 8-iso-PGF2alpha in sheep and goat plasma using solid phase micro-extraction and UPLC-MS/MS, Rapid Commun. Mass Spectrom. 32 (19) (2018) 1675–1682.
[241] P. Purwaha, Y. Gu, U. Kelavkar, et al., LC/ESR/MS study of pH-dependent radical generation from 15-LOX-catalyzed DPA peroxidation, Free Radic. Biol. Med. 51 (2011) 1461–1470.
[242] Y. Okimotoa, A. Watanabea, E. Nikia, et al., A novel fluorescent probe diphenyl-1-pyrenylphosphine to follow lipid peroxidation in cell membranes, FEBS Lett. 474 (2000) 137–140.
[243] I. Matot, Y. Manevich, A.B. Al-Mehdi, et al., Fluorescence imaging of lipid peroxidation in isolated rat lungs during nonhypoxic lung ischemia, Free Radic. Biol. Med. 34 (2003) 785–790.
[244] G. Stubiger, O. Belgacem, P. Rehulka, et al., Analysis of oxidized phospholipids by MALDI mass spectrometry using 6-aza-2-thiothymine together with matrix additives and disposable target surfaces, Anal. Chem. 82 (2010) 5502–5510.
[245] L.J. Sparvero, A.A. Amoscato, C.E. Dixon, et al., Mapping of phospholipids by MALDI imaging (MALDI-MSI): realities and expectations, Chem. Phys. Lipids 165 (2012) 545–562.
[246] G. Stubiger, E. Aldover-Macasaet, W. Bicker, et al., Targeted profiling of atherogenic phospholipids in human plasma and lipoproteins of hyperlipidemic patients using MALDI-QIT-TOF-MS/MS, Atherosclerosis 224 (2012) 177–186.
[247] D. Gao, E.A. Podrez, Characterization of covalent modifications of HDL apoproteins by endogenous oxidized phospholipids, Free Radic. Biol. Med. 115 (2018) 57–67.
[248] V. Bochkov, B. Gesslbauer, C. Mauerhofer, et al., Pleiotropic effects of oxidized phospholipids, Free Radic. Biol. Med. 111 (2017) 6–24.
[249] Z. Ni, G. Angelidou, R. Hoffmann, et al., LPPtiger software for lipidome-specific prediction and identification of oxidized phospholipids from LC-MS datasets, Sci. Rep. 7 (2017) 15138.
[250] H. Hinterwirth, G. Stubiger, W. Lindner, et al., Gold nanoparticle-conjugated anti-oxidized low-density lipoprotein antibodies for targeted lipidomics of oxidative stress biomarkers, Anal. Chem. 85 (2013) 8376–8384.
[251] K.A. Zemski Berry, W.W. Turner, M.S. VanNieuwenhze, et al., Characterization of oxidized phosphatidylethanolamine derived from RAW 264.7 cells using 4-(dimethylamino)benzoic acid derivatives, Eur. J. Mass Spectrom. 16 (2010) 463–470.
[252] G. Aldini, G. Vistoli, M. Stefek, et al., Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products, Free Radic. Res. 47 (Suppl 1) (2013) 93–137.
[253] L. Horakova, S. Stolc, Antioxidant and pharmacodynamic effects of pyridoindole stobadine, Gen. Pharmacol. 30 (1998) 627–638.
[254] Z. Kyselova, A. Gajdosik, A. Gajdosikova, et al., Effect of the pyridoindole antioxidant stobadine on development of experimental diabetic cataract and on lens protein oxidation in rats: comparison with vitamin E and BHT, Mol. Vis. 11 (2005) 56–65.
[255] L. Horakova, A. Giessauf, G. Raber, et al., Effect of stobadine on Cu(++)-mediated oxidation of low-density lipoprotein, Biochem. Pharmacol. 51 (1996) 1277–1282.
[256] B. Pekiner, N.N. Ulusu, N. Das-Evcimen, et al., In vivo treatment with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca2+ -ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E, Biochim. Biophys. Acta 1588 (2002) 71–78.
[257] S. Steenken, A.R. Sundquist, S.V. Jovanovic, et al., Antioxidant activity of the pyridoindole stobadine. Pulse radiolytic characterization of one-electron-oxidized stobadine and quenching of singlet molecular oxygen, Chem. Res. Toxicol. 5 (1992) 355–360.
[258] R. Nagai, D.B. Murray, T.O. Metz, et al., Chelation: a fundamental mechanism of action of AGE inhibitors, AGE breakers, and other inhibitors of diabetes complications, Diabetes 61 (2012) 549–559.
[259] D.L. Price, P.M. Rhett, S.R. Thorpe, et al., Chelating activity of advanced glycation end-product inhibitors, J. Biol. Chem. 276 (2001) 48967–48972.
[260] I.A. Blair, Endogenous glutathione adducts, Curr. Drug Metabol. 7 (2006) 853–872.
[261] G. Aldini, A. Altomare, G. Baron, et al., N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why, Free Radic. Res. 52 (2018) 751–762.
[262] A.L. Furfaro, S. Menini, S. Patriarca, et al., HNE-dependent molecular damage in diabetic nephropathy and its possible prevention by N-acetyl-cysteine and oxerutin, Biofactors 24 (2005) 291–298.
[263] J. Sochman, N-acetylcysteine in acute cardiology: 10 years later: what do we know and what would we like to know?!, J. Am. Coll. Cardiol. 39 (2002) 1422–1428.
[264] P.V. Dludla, S.C. Dias, N. Obonye, et al., A systematic review on the protective effect of N-acetyl cysteine against diabetes-associated cardiovascular complications, Am. J. Cardiovasc. Drugs 18 (2018) 283–298.
[265] D.R.A. Reyes, M.J. Gomes, C.M. Rosa, et al., N-acetylcysteine influence on oxidative stress and cardiac remodeling in rats during transition from compensated left ventricular hypertrophy to heart failure, Cell. Physiol. Biochem. 44 (2017) 2310–2321.
[266] B. Giam, P.Y. Chu, S. Kuruppu, et al., N-acetylcysteine attenuates the development of cardiac fibrosis and remodeling in a mouse model of heart failure, Phys. Rep. 4 (2016).
[267] M. Sanson, N. Auge, C. Vindis, et al., Oxidized low-density lipoproteins trigger endoplasmic reticulum stress in vascular cells: prevention by oxygen-regulated protein 150 expression, Circ. Res. 104 (2009) 328–336.
[268] Y. Cui, C.A. Narasimhulu, L. Liu, et al., N-acetylcysteine inhibits in vivo oxidation of native low-density lipoprotein, Sci. Rep. 5 (2015) 16339.
[269] Y. Lu, W. Qin, T. Shen, et al., The antioxidant N-acetylcysteine promotes atherosclerotic plaque stabilization through suppression of RAGE, MMPs and NF-kappaB in ApoE-deficient mice, J. Atherosclerosis Thromb. 18 (2011) 998–1008.
[270] B. Bouguerne, N. Belkheiri, F. Bedos-Belval, et al., Antiatherogenic effect of bis-vanillyl-hydralazone, a new hydralazine derivative with antioxidant, carbonyl scavenger, and antiapoptotic properties, Antioxidants Redox Signal. 14 (2011) 2093–2106.
[271] S. Galvani, C. Coatrieux, M. Elbaz, et al., Carbonyl scavenger and antiatherogenic effects of hydrazine derivatives, Free Radic. Biol. Med. 45 (2008) 1457–1467.
[272] C. Vindis, I. Escargueil-Blanc, M. Elbaz, et al., Desensitization of platelet-derived growth factor receptor-beta by oxidized lipids in vascular cells and atherosclerotic lesions: prevention by aldehyde scavengers, Circ. Res. 98 (2006) 785–792.
[273] S. Picard, S. Parthasarathy, J. Fruebis, et al., Aminoguanidine inhibits oxidative modification of low density lipoprotein protein and the subsequent increase in uptake by macrophage scavenger receptors, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6876–6880.
[274] Z. Kang, H. Li, G. Li, et al., Reaction of pyridoxamine with malondialdehyde: mechanism of inhibition of formation of advanced lipoxidation end-products, Amino Acids 30 (2006) 55–61.
[275] J.M. Onorato, A.J. Jenkins, S.R. Thorpe, et al., Pyridoxamine, an inhibitor of advanced glycation reactions, also inhibits advanced lipoxidation reactions. Mechanism of action of pyridoxamine, J. Biol. Chem. 275 (2000) 21177–21184.
[276] T.O. Metz, N.L. Alderson, M.E. Chachich, et al., Pyridoxamine traps intermediates in lipid peroxidation reactions in vivo: evidence on the role of lipids in chemical modification of protein and development of diabetic complications, J. Biol. Chem. 278 (2003) 42012–42019.
[277] S.H. Lee, A. Matsunaga, T. Oe, Inhibition effect of pyridoxamine on lipid hydroperoxide-derived modifications to human serum albumin, PLoS One 13 (2018) e0196050.
[278] G.M. Cahuana, J.R. Tejedo, J. Jimenez, et al., Involvement of advanced lipoxidation end products (ALEs) and protein oxidation in the apoptotic actions of nitric oxide in insulin secreting RINm5F cells, Biochem. Pharmacol. 66 (2003) 1963–1971.
[279] T.P. Degenhardt, N.L. Alderson, D.D. Arrington, et al., Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat, Kidney Int. 61 (2002) 939–950.
[280] P.A. Voziyan, B.G. Hudson, Pyridoxamine as a multifunctional pharmaceutical: targeting pathogenic glycation and oxidative damage, Cell. Mol. Life Sci. 62 (2005) 1671–1681.
[281] A.A. Boldyrev, G. Aldini, W. Derave, Physiology and pathophysiology of carnosine, Physiol. Rev. 93 (2013) 1803–1845.
[282] A.R. Hipkiss, Carnosine and its possible roles in nutrition and health, Adv. Food

Nutr. Res. 57 (2009) 87–154.
[283] S. Zhou, E.A. Decker, Ability of carnosine and other skeletal muscle components to quench unsaturated aldehydic lipid oxidation products, J. Agric. Food Chem. 47 (1999) 51–55.
[284] M. Carini, G. Aldini, G. Beretta, et al., Acrolein-sequestering ability of endogenous dipeptides: characterization of carnosine and homocarnosine/acrolein adducts by electrospray ionization tandem mass spectrometry, J. Mass Spectrom. 38 (2003) 996–1006.
[285] G. Vistoli, M. Colzani, A. Mazzolari, et al., Quenching activity of carnosine derivatives towards reactive carbonyl species: focus on alpha-(methylglyoxal) and beta-(malondialdehyde) dicarbonyls, Biochem. Biophys. Res. Commun. 492 (2017) 487–492.
[286] M. Orioli, G. Aldini, M.C. Benfatto, et al., HNE Michael adducts to histidine and histidine-containing peptides as biomarkers of lipid-derived carbonyl stress in urines: LC-MS/MS profiling in Zucker obese rats, Anal. Chem. 79 (2007) 9174–9184.
[287] E.J. Anderson, G. Vistoli, L.A. Katunga, et al., A carnosine analog mitigates metabolic disorders of obesity by reducing carbonyl stress, J. Clin. Invest. 128 (2018) 5280–5293.
[288] S. Menini, C. Iacobini, C. Ricci, et al., D-Carnosine octylester attenuates atherosclerosis and renal disease in ApoE null mice fed a Western diet through reduction of carbonyl stress and inflammation, Br. J. Pharmacol. 166 (2012) 1344–1356.
[289] T. Grune, K.J. Davies, The proteasomal system and HNE-modified proteins, Mol. Aspect. Med. 24 (2003) 195–204.
[290] A. Gonen, L.F. Hansen, W.W. Turner, et al., Atheroprotective immunization with malondialdehyde-modified LDL is hapten specific and dependent on advanced MDA adducts: implications for development of an atheroprotective vaccine, J. Lipid Res. 55 (2014) 2137–2155.
[291] E. Ho, K. Karimi Galougahi, C.C. Liu, et al., Biological markers of oxidative stress: applications to cardiovascular research and practice, Redox Biol. 1 (2013) 483–491.
[292] K.K. Griendling, R.M. Touyz, J.L. Zweier, et al., Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the American heart association, Circ. Res. 119 (2016) e39–75.
[293] I. Marrocco, F. Altieri, I. Peluso, Measurement and clinical significance of biomarkers of oxidative stress in humans, Oxid. Med. Cell Longev. 2017 (2017) 6501046.
[294] M.F. Walter, R.F. Jacob, R.E. Bjork, et al., Circulating lipid hydroperoxides predict cardiovascular events in patients with stable coronary artery disease: the PREVENT study, J. Am. Coll. Cardiol. 51 (2008) 1196–1202.
[295] C. Asselin, A. Ducharme, T. Ntimbane, et al., Circulating levels of linoleic acid and HDL-cholesterol are major determinants of 4-hydroxynonenal protein adducts in patients with heart failure, Redox Biol. 2 (2014) 148–155.
[296] R. Belardinelli, M. Solenghi, L. Volpe, et al., Trimetazidine improves endothelial dysfunction in chronic heart failure: an antioxidant effect, Eur. Heart J. 28 (2007) 1102–1108.
[297] I. Basarici, R.E. Altekin, I. Demir, et al., Urinary 8-isoprostane levels can indicate the presence, severity and extent of angiographic coronary artery disease, Acta Cardiol. 63 (2008) 415–422.
[298] B. Wang, J. Pan, L. Wang, et al., Associations of plasma 8-isoprostane levels with the presence and extent of coronary stenosis in patients with coronary artery disease, Atherosclerosis 184 (2006) 425–430.
[299] M.H. Shishehbor, R. Zhang, H. Medina, et al., Systemic elevations of free radical oxidation products of arachidonic acid are associated with angiographic evidence of coronary artery disease, Free Radic. Biol. Med. 41 (2006) 1678–1683.
[300] J.Y. Kim, Y.J. Hyun, Y. Jang, et al., Lipoprotein-associated phospholipase A2 activity is associated with coronary artery disease and markers of oxidative stress: a case-control study, Am. J. Clin. Nutr. 88 (2008) 630–637.
[301] F. Veglia, V. Cavalca, E. Tremoli, OXY-SCORE: a global index to improve evaluation of oxidative stress by combining pro- and antioxidant markers, Methods Mol. Biol. 594 (2010) 197–213.
[302] C. Vassalle, An easy and reliable automated method to estimate oxidative stress in the clinical setting, Methods Mol. Biol. 477 (2008) 31–39.
[303] F. Veglia, G. Cighetti, M. De Franceschi, et al., Age- and gender-related oxidative status determined in healthy subjects by means of OXY-SCORE, a potential new comprehensive index, Biomarkers 11 (2006) 562–573.
[304] K. Satoh, S. Yamada, Y. Koike, et al., A 1-hour enzyme-linked immunosorbent assay for quantitation of acrolein- and hydroxynonenal-modified proteins by epitope-bound casein matrix method, Anal. Biochem. 270 (1999) 323–328.
[305] X. Tang, L.M. Sayre, G.P. Tochtrop, A mass spectrometric analysis of 4-hydroxy-2-(E)-nonenal modification of cytochrome c, J. Mass Spectrom. 46 (2011) 290–297.
[306] A. Renner, M.R. Sagstetter, H. Harms, et al., Formation of 4-hydroxy-2-nonenal protein adducts in the ischemic rat heart after transplantation, J. Heart Lung Transplant. 24 (2005) 730–736.
[307] H. Ma, R. Guo, L. Yu, et al., Aldehyde dehydrogenase 2 (ALDH2) rescues myocardial ischaemia/reperfusion injury: role of autophagy paradox and toxic aldehyde, Eur. Heart J. 32 (2011) 1025–1038.
[308] Q. Gao, A. Sarkar, Y. Chen, et al., Overexpression of heart-type fatty acid binding protein enhances fatty acid-induced podocyte injury, Exp. Ther. Med. 15 (2018) 2054–2061.
[309] T.D. Calamaras, C. Lee, F. Lan, et al., Post-translational modification of serine/threonine kinase LKB1 via Adduction of the Reactive Lipid Species 4-Hydroxy-trans-2-nonenal (HNE) at lysine residue 97 directly inhibits kinase activity, J. Biol. Chem. 287 (2012) 42400–42406.
[310] S.W. Choi, Y.K. Jeon, S.H. Moon, et al., Suppression of hERG K(+) current and cardiac action potential prolongation by 4-hydroxynonenal via dual mechanisms, Redox Biol. 19 (2018) 190–199.
[311] M.F. Walter, R.F. Jacob, B. Jeffers, et al., Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study, J. Am. Coll. Cardiol. 44 (2004) 1996–2002.
[312] M. White, A. Ducharme, R. Ibrahim, et al., Increased systemic inflammation and oxidative stress in patients with worsening congestive heart failure: improvement after short-term inotropic support, Clin. Sci. (Lond.) 110 (2006) 483–489.
[313] B. Shao, S. Pennathur, I. Pagani, et al., Modifying apolipoprotein A-I by malondialdehyde, but not by an array of other reactive carbonyls, blocks cholesterol efflux by the ABCA1 pathway, J. Biol. Chem. 285 (2010) 18473–18484.
[314] C.S. Yarian, I. Rebrin, R.S. Sohal, Aconitase and ATP synthase are targets of malondialdehyde modification and undergo an age-related decrease in activity in mouse heart mitochondria, Biochem. Biophys. Res. Commun. 330 (2005) 151–156.
[315] T. Obama, R. Kato, Y. Masuda, et al., Analysis of modified apolipoprotein B-100 structures formed in oxidized low-density lipoprotein using LC-MS/MS, Proteomics 7 (2007) 2132–2141.
[316] C. Gniwotta, J.D. Morrow, L.J. Roberts 2ndet al., Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesions, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 3236–3241.
[317] E. Schwedhelm, A. Bartling, H. Lenzen, et al., Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study, Circulation 109 (2004) 843–848.
[318] A. Nishibe, Y. Kijima, M. Fukunaga, et al., Increased isoprostane content in coronary plaques obtained from vulnerable patients, Prostaglandins Leukot. Essent. Fatty Acids 78 (2008) 257–263.
[319] S.S. Davies, L.J. Roberts 2nd, F2-isoprostanes as an indicator and risk factor for coronary heart disease, Free Radic. Biol. Med. 50 (2011) 559–566.
[320] R.M. LeLeiko, C.S. Vaccari, S. Sola, et al., Usefulness of elevations in serum choline and free F2)-isoprostane to predict 30-day cardiovascular outcomes in patients with acute coronary syndrome, Am. J. Cardiol. 104 (2009) 638–643.
[321] R. De Cristofaro, B. Rocca, E. Vitacolonna, et al., Lipid and protein oxidation contribute to a prothrombotic state in patients with type 2 diabetes mellitus, J. Thromb. Haemostasis 1 (2003) 250–256.
[322] J. Bauer, A. Ripperger, S. Frantz, et al., Pathophysiology of isoprostanes in the cardiovascular system: implications of isoprostane-mediated thromboxane A2 receptor activation, Br. J. Pharmacol. 171 (2014) 3115–3131.