Biochimica et Biophysica Acta 1840 (2014) 809–817

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Biomarkers of lipid peroxidation in clinical material☆

Etsuo Niki *

Health Research Institute, National Institute of Advanced Industrial Science & Technology, Ikeda, Osaka, 563-8577, Japan

ARTICLE INFO

Article history:
Received 2 February 2013
Received in revised form 14 March 2013
Accepted 17 March 2013
Available online 27 March 2013

Keywords:
Biomarker
Hydroxycholesterol
Hydroxyeicosatetraenoic acid (HETE)
Hydroxyoctadecadienoic acid (HODE)
Isoprostane
Lipid peroxidation

ABSTRACT

Background: Free radical-mediated lipid peroxidation has been implicated in a number of human diseases. Diverse methods have been developed and applied to measure lipid peroxidation products as potential biomarkers to assess oxidative stress status in vivo, discover early indication of disease, diagnose progression of disease, and evaluate the effectiveness of drugs and antioxidants for treatment of disease and maintenance of health, respectively. However, standardized methods are not yet established.
Scope of review: Characteristics of various lipid peroxidation products as biomarkers are reviewed on the basis of mechanisms and dynamics of their formation and metabolism and also on the methods of measurement, with an emphasis on the advantages and limitations.
Major conclusions: Lipid hydroxides such as hydroxyoctadecadienoic acids (HODE), hydroxyeicosatetraenoic acids (HETE), and hydroxycholesterols may be recommended as reliable biomarkers. Notably, the four HODEs, 9-*cis*,*trans*, 9-*trans*,*trans*, 13-*cis*,*trans*, and 13-*trans*,*trans*-HODE, can be measured separately by LC–MS/MS and the *trans*,*trans*-forms are specific marker of free radical mediated lipid peroxidation. Further, isoprostanes and neuroprostanes are useful biomarker of lipid peroxidation. It is important to examine the distribution and temporal change of these biomarkers.
General significance: Despite the fact that lipid peroxidation products are non-specific biomarkers, they will enable to assess oxidative stress status, disease state, and effects of drugs and antioxidants. This article is part of a Special Issue entitled Current methods to study reactive oxygen species - pros and cons and biophysics of membrane proteins. Guest Editor: Christine Winterbourn.

1. Introduction

Biomarkers are defined as measures that can be used as indicators of normal biological processes, pathological processes, or pharmacologic and/or biochemical responses to therapeutic/nutritional intervention. Biomarkers are used for health examination, diagnosis of pathologic processes, assessment of treatment response and prognosis, safe and efficient drug development, and evaluation of the effects of drugs, foods, beverages, and supplements. Lipid peroxidation is the major consequence of oxidative stress and cause of oxidative damage [1]. Various biomarkers for lipid peroxidation have been proposed, developed, and applied for biological samples from humans and experimental animals. Substantial evidence shows the association between the level of these biomarkers and development of many diseases. Accordingly, the lipid peroxidation products have received much attention as biomarkers of oxidative stress and diseases and also as indicators of antioxidant effects and the advantages and limitations of various biomarkers and methods of measurement have been the subject of extensive studies and arguments [1–10].

Lipids are vulnerable to oxidation by both enzymes and non-enzymatic oxidants. Especially, polyunsaturated fatty acids (PUFAs) possessing more than two *cis*-double bonds each separated by one methylene group and their esters are readily oxidized by free radical mediated chain oxidation, termed lipid peroxidation, which has been shown to impair membrane functions and inactivate proteins and enzymes, leading eventually to various disorders and diseases. Lipid peroxidation products have been accepted as toxic mediators, but now they are known to exert diverse biological effects [11–13].

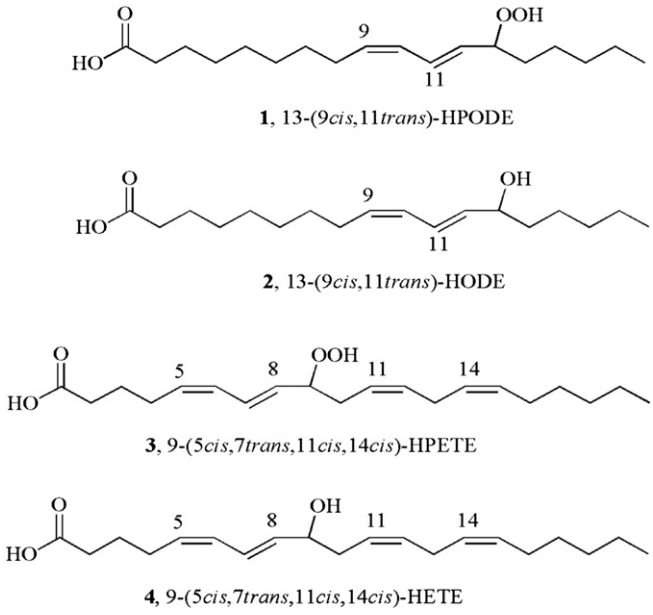
The mechanisms and products of lipid peroxidation have been studied extensively and are now fairly well understood [1,14]. One of the characteristics of lipid peroxidation is that it proceeds by non-specific fashion. PUFAs and their esters having bis-allylic hydrogens are very reactive toward oxygen radicals and readily oxidized to produce lipid hydroperoxides as major primary products (Scheme 1). The major PUFA in vivo is linoleic (18:2), linolenic (18:3), arachidonic (20:4), eicosapentaenoic acid (20:5, EPA), and docosahexaenoic acid (22:6, DHA), which have one, two, three, four, and five bis-allylic methylene

Abbreviations: CL, chemiluminescence; DHA, docosahexaenoic acid; DPPP, diphenylpyrenylphosphine; ELISA, enzyme linked immunosorbent assay; EPA, eicosapentaenoic acid; FL, fluorescence; GC, gas chromatography; H(P)ETE, hydro(pero)xyeicosatetraenoic acid; HPLC, high performance liquid chromatography; H(P)ODE, hydro(pero)xyoctadecadienoic acid; IsoP, isoprostane; MS, mass spectrometry; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid

☆ This article is part of a Special Issue entitled Current methods to study reactive oxygen species - pros and cons and biophysics of membrane proteins. Guest Editor: Christine Winterbourn

* 7-17-7 Kitakarasuyama, Setagaya, Tokyo 157-0061, Japan. Tel./Fax: +81 3 5313 2555.
E-mail address: etsuo-niki@aist.go.jp.

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.03.020



Scheme 1. Chemical structure of hydroperoxyoctadecadienoic acid (HPODE, **1**), hydroxyoctadecadienoic acid (HODE, **2**), hydroperoxyeicosatetraenoic acid (HPETE, **3**), and hydroxyeicosatetraenoic acid (HETE, **4**).

groups respectively. In contrast to lipoxygenase, peroxy radicals, chain carrying species of lipid peroxidation, attack these bis-allylic hydrogens non-selectively. For example, arachidonic acid has three bis-allylic methylene groups at carbon 7, 10, and 13 positions, which are equally attacked by peroxyl radicals and the resulting radicals may undergo several competing reactions to yield final products. Thus lipid peroxidation of PUFAs gives rise to diverse group of products with hundreds of regio-, stereo-, and enantio-isomers.

Cholesterol is also an important substrate of oxidation. It was accepted as an important component of cellular membranes and lipoproteins and also as a precursor to hormone synthesis, but it is oxidized in vivo substantially and its oxidation products play an important role as signaling messenger and biological mediators [15–18]. The oxidation products of cholesterol received attention as biomarkers of oxidative stress in vivo [19,20].

Lipids are oxidized not only by free radical pathways but also by non-radical oxidants and enzymes such as singlet oxygen, ozone, hypohalous acids, lipoxygenases, cyclooxygenases, and cytochrome P450. The same lipid oxidation products are formed by some of the free radical and non-radical oxidation. This point should be born in mind in the measurement and interpretation of data. It is important to understand the mechanisms of lipid oxidation by different oxidants in order to identify the responsible oxidant and assess the efficacy of antioxidant.

In the present article, the characteristics and measurement of lipid peroxidation products as a biomarker in clinical materials will be described, with special attention to the strengths and limitations as biomarkers on the basis of mechanisms and dynamics of their formation, metabolism, and excretion, rather than experimental details of protocols.

2. Lipid peroxidation products as biomarker: pros and cons

Various biomarkers of lipid peroxidation have been developed and applied to biological samples. Some representative biomarkers of lipid peroxidation are summarized in Table 1. The free radicals are too short-lived to be measured directly and stable products are measured. Early methods for determination of lipid peroxidation products depended largely on colorimetric assays involving the reaction of the lipid peroxidation products with chemical reagents that generated chromophores. For example, the iodometric assay is one of the oldest

Table 1
Biomarkers of lipid peroxidation [a].

Biomarker	Remarks/methods of measurement
Hydroperoxides	Primary product of lipid peroxidation, not stable/LC–UV, CL, FL, MS; DPPP
Hydroxides	Reduced form of hydroperoxides, HODE and HETE/LC–UV, MS; GC–MS; EIA
Isoprostanes	Free radical mediated oxidation product of arachidonic acid/LC–MS; GC–MS; EIA, RIA
Neuroprostanes	Free radical mediated oxidation product of DHA/LC–MS; GC–MS
TBARS, MDA	Thiobarbituric acid reactive substances measuring MDA and possibly others/spectrophotometry, HPLC
Conjugated diene	1,3-Diene of hydroperoxides and hydroxides/UV 234 nm
Ethane, pentane	Fragment product of hydroperoxides in exhaled gas/GC
Aldehydes, Ketones	Secondary products from hydroperoxides/LC; DNPH–UV/vis; EIA; RIA
LysoPC	Hydrolysis of PC by phospholipase A2/TLC, LC–MS/MS
7-Hydroxycholesterol	Reduction of 7-hydroperoxycholesterol, enzymatic oxidation/GC–MS
7- Ketocholesterol	Free radical oxidation of cholesterol/GC–MS
Oxidized LDL	Oxidatively modified LDL by multiple oxidants/EIA, RIA
LPO-modified proteins	Proteins modified by aldehydes/LC–MS; EIA; RIA
Lipofuscin	Fluorescence

[a] CL, chemiluminescence; DNPH, 2,4-dinitrophenylhydrazine; DPPP, diphenylpyrenylphosphine; EIA, enzyme immunoassay; FL, fluorescence; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; GC, gas chromatography; LC, liquid chromatography; LPO, lipid peroxidation; Lyso PC, lysophosphatidylcholine; MS, mass spectrometry; RIA, radio immunoassay; TLC, thin layer chromatography; UV/V, ultraviolet/visible spectrophotometry.

methods applied for measurement of lipid peroxidation products. In this method, iodine formed by the reaction of hydroperoxides and iodide is measured by titration [21]. This is a simple and convenient method, but due to a lack of specificity and sensitivity this method is not used today for clinical materials. With the advent of modern technologies such as chromatography, mass spectrometry, immunochemistry, and imaging technique, much more sophisticated methods are now available and applied for the identification, structure determination and quantification of lipid peroxidation products in biological samples. Nonetheless, there is still no method which is specific, accurate, sensitive, and quantitative enough to measure lipid peroxidation products in biological samples. The human samples contain low levels of lipid peroxidation products and their measurement may be interfered by other biological materials and also by artifactual oxidation during sampling, storage, and analysis. The characteristics of these biomarkers are described below.

2.1. Lipid hydroperoxides

Hydroperoxides are formed as the major primary product of lipid peroxidation of PUFA and cholesterol. Hydroperoxides are produced in the oxidation by singlet oxygen and lipoxygenase as well as free radical mediated lipid peroxidation [1]. Singlet oxygen oxidizes mono-olefins such as oleic acid and squalene as well as PUFA to give rise to hydroperoxides with concomitant migration of double bond. For example, the lipid peroxidation of linoleic acid by free radicals produces 9 and 13-hydroperoxyoctadecadienoic acid (HPODE), while the oxidation of linoleic acid by singlet oxygen gives four regio-isomers, 9, 10, 12, 13-HPODE, of which 10 and 12-HPODE do not contain conjugated diene. In contrast to lipoxygenases which attack specific position, peroxyl radicals attack bis-allylic hydrogens of PUFA equally; for example, peroxyl radicals abstract hydrogens at carbon 7, 10, and 13 positions of arachidonic acid at the same rate. The resulting carbon radicals react with molecular oxygen after rearrangement to yield thermochemically more stable 5, 8, 9, 11, 12, and 15-peroxyl radicals, which then abstract bis-allylic hydrogen to give the corresponding six regio-isomers of hydroperoxyeicosatetraenoic acid, HPETE. Since 8, 9, 11, and 12-peroxyl radicals, but not 5- and 15-peroxyl radicals, may

also undergo intramolecular cyclization to give prostaglandin type products, the yields of 5- and 15-HPODE are larger than 8-, 9-, 11-, and 12-HPODE.

In addition to PUFA hydroperoxides, cholesteryl hydroperoxide was detected in vivo. For example, 7-hydroperoxycholesterol was found in human atherosclerotic lesions [22]. The oxidation products of triglycerides are smaller than those of phospholipids and cholesterol and have received less attention.

Hydroperoxides are not stable end product but reduced in vivo by glutathione peroxidases and selelnoprotein P to the corresponding hydroxides. Hydroperoxides react with transition metal ions such as iron and copper to produce oxygen radicals and they are decomposed thermally and hence cannot be analyzed directly by gas chromatography (GC). Accordingly, hydroperoxides in biological samples are often reduced before analysis to convert hydroperoxides to the corresponding hydroxides.

Different kinds of lipid hydroperoxides are separated by HPLC and then quantified with chemiluminescence (CL), fluorescence (FL) or mass spectrometry (MS). HPLC-CL is specific and very sensitive [23–25], but care should be taken because chemiluminescence intensity depends on the type of lipid hydroperoxides and also analytical conditions including apparatus set up [26]. The observed levels of PC hydroperoxides in healthy human plasma measured by HPLC-CL vary considerably, ranging from nil to several hundred nM, the recent reported levels being often below 100 nM [27].

Recent advancement of lipidomics technique has made it possible to identify and characterize diverse molecular species of phospholipids and cholesteryl ester hydroperoxides in intact forms by LC–MS/MS [28]. The accurate quantification by MS requires deuterated samples of the same compounds, which are usually not commercially available except for several limited standards.

The above LC–CL, LC–FL, and LC–MS/MS methods are specific and capable of measuring different hydroperoxides separately, but they are neither simple nor rapid and they require skilled techniques and costly equipments. Simpler methods which measure total hydroperoxides have been developed also. One of such methods is the Fox assay in which ferric ion Fe^{3+} formed by the reaction of hydroperoxides and ferrous ion Fe^{2+} is measured spectrophotometrically at 560 nm after reaction with xylenol orange [29]. It should be pointed out that the reaction of hydroperoxides and ferrous ion produces ferric ion and at the same time alkoxyl radicals, which undoubtedly induce artifactual oxidation of lipids. In support of this notion, the reported levels of lipid hydroperoxides measured by Fox assay are much higher (often above 1 μM) [29] than those measured by other direct methods [1].

As an alternative method, diphenylpyrenylphosphine (DPPP) has been used to measure total lipid hydroperoxides in biological samples [30–32]. The reaction of lipid hydroperoixdes and DPPP gives lipid hydroxides and DPPP oxide stoichiometrically and the resulting DPPP oxide may be measured with HPLC-FL [30,33] or GC–MS [34]. The level of cholesteryl ester hydroperoxides in human plasma determined by this method was reported as 24.5 nM [30].

2.2. Lipid hydroxides, HODE, HETE and hydroxycholesterol.

Lipid hydroxides are also major lipid oxidation products found in biological samples. Especially, HODEs and HETEs are the most abundant lipid oxidation products found in human plasma, normally around 100 nM [1,35]. They are produced by reduction of hydroperoxides and also directly by oxidation with cytochrome P450. The lipid hydroxides derived from enzymatic oxidation and singlet oxygen oxidation are *cis,trans*-hydroxides, while free radical lipid peroxidation gives both *cis,trans* and *trasn,trans*-hydroxides. Therefore, *trans, trans*-hydroxides serve as specific biomarker for free radical lipid peroxidation. In terms of *chiral* specificity, enzymatic oxidation gives in general either *R* or *S* form product selectively, while free radical lipid peroxidation gives equal amount of *R* and *S* forms, that is, *racemic* products. This may be used to distinguish lipid peroxidation from non-radical oxidation. It may be noted, however, that the enzymatic lipid oxidation in membranes and lipoproteins by lipoxygenase may not be fully *enantio* specific [36].

As described above, hydroperoxides are chemically and thermally unstable and they are often analyzed as hydroxides after reduction with triphenylphosphine or sodium borohydride. It was found that sodium borohydride, a strong reducing agent for hydroperoxides and metal ions, induced ex vivo lipid oxidation during analytical procedures, which could be avoided if triphenylphosphine was used as the reducing agent [37]. Human plasma and tissue samples contain considerable iron.

In many cases, the biological samples are hydrolyzed to convert phospholipid hydroxides and cholesteryl ester hydroxides to the corresponding free fatty acid hydroxides and measured as total lipid hydroxides such as HODE, HETE and hydroxycholesterol. Lipid hydroxides are measured by the same method as hydroperoxides, although chemiluminescence cannot be applied. Tandem mass spectrometry is useful for analysis of geometrical isomers and LC–MS/MS has been used successfully as a potential tool.

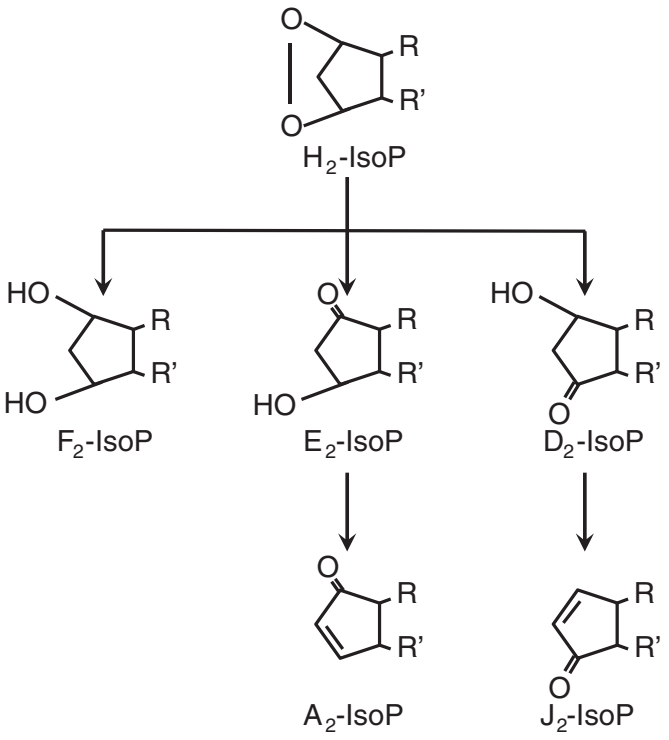
HODE is particularly a useful biomarker, since linoleates are the most abundant PUFA in vivo and oxidized by a straightforward mechanism to give only four kinds of regio- and stereo-isomers, which can be quantified separately with LC–MS. In contrast, free radical oxidation of arachidonates gives twelve kinds of regio- and stereo-isomeric HETEs and furthermore cyclic peroxides as well. The molar ratio of *cis,trans*-HODE/*trans,trans*-HODE is determined by the capacity of hydrogen donation at the reaction site and this ratio can be used as an indicator of in vivo antioxidant capacity and may be applied for the assessment of antioxidant efficacy in vivo [38].

Many studies observed the association between increase in HODE levels and progress of diseases. For example, it was found that human atherosclerotic lesions contained increased amounts of HODE than non-atherosclerotic vessel wall, the ratio of HODE/linoleic aicd being 5/1000 [39]. Furthermore, racemic 9- and 13-HODEs were significantly elevated in patients with nonalcoholic steatohepatitis (NASH) compared with patients with steatosis [44].

HETE has been measured also as a biomarker of lipid peroxidation [35]. The plasma level of HETE from healthy human subjects is similar to that of HODE, although the ratio of products to parent lipid HETE/arachidonates is higher than HODE/linoletes. It should be pointed out that the ratio of HETE to total oxidized arachidonates is lower than the ratio of HODE/oxidized linoleates, and importantly this ratio depends on the circumstances such as concentrations of substrates, antioxidants, and oxygen. Thus, HODE may be considered to be more suitable than HETE as a quantitative biomarker of lipid peroxidation.

2.3. Isoprostane and neuroprostane

Isoprostanes (IsoP) are a series of prostaglandin-like compounds produced by a free radical mediated lipid peroxidation of arachidonic acid independent of cyclooxygenase [40,41]. An important structural distinction between IsoP and cyclooxygenase-catalyzed PGs is that the former contains side chains that are predominantly oriented *cis* to the prostane ring, whereas the latter possess exclusively *trans* side chain [40]. Another difference between IsoPs and PGs is that IsoPs are formed primarily *in situ*, esterified to phosphplipids and subsequently released by a phospholipases, whereas PGs are generated only from free arachidonic acid. The 8, 9, 11, and 12-peroxyl radicals, but not 5 and 15-peroxyl radicals, derived from arachidonic acid undergo two consecutive intramolecular cyclizations, oxygen addition, and hydrogen abstraction to form PGG_2-like compounds, which are reduced to yield D_2, E_2, F_2, J_2, and A_2-IsoPs (Scheme 2). Among these, F_2-IsoPs containing F-type prostane ring have been studied most extensively. The oxidation of EPA and DHA by similar mechanisms gives F_3-isoprostanes and F_4-neuroprostanes respectively [42].



Scheme 2. Chemical structure of isoprostane.

Over the past two decades after discovery in 1990 [40], numerous studies have been carried out and it is now accepted that F_2-IsoPs are most reliable biomarker of oxidative stress in vivo [43]. In fact, elevation of IsoPs in human fluids and tissues has been observed in many human disorders. F_2-IsoPs are chemically and metabolically stable and nonreactive. Several methods have been developed to measure IsoPs including LC–MS, GC–MS, and enzyme and radio immunoassays [45]. ELISA-based assay is often less accurate than chromatography-based techniques due to cross-reactivity. It was reported that the correlation between IsoP levels obtained using enzyme immunoassay and GC–MS methods was poor, which may arise, at least in part, from the measurements of different isomers by the two methods [108].

It should be born in mind that there are many types and isomers of IsoPs. The most widely used F_2-IsoPs are composed of 5, 8, 12, and 15-series regio-isomers based on the carbon atom to which hydroxyl group is attached and each series contains 16 diastereomers, the total isomers being as many as 64 isomers [46]. In addition to F_2-IsoPs, D/E-type prostane rings (D_2/E_2-IsoPs) are formed depending on whether IsoP endoperoxide intermediates undergo reduction or isomerization [46,47]. Furthermore, the selectivity for production of IsoP from arachidonic acid is very small, being around 1%, which depends on the concentrations of oxygen and reactive hydrogens including lipids and antioxidants. IsoPs are present predominantly in ester forms in plasma and tissues, but in free acid forms in urine. Further, a significant proportion of IsoP in human urine are excreted as glucuronide conjugates [109].

2.4. Malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS)

MDA and TBARS have been used frequently as a standard biomarker of lipid peroxidation in vivo for many years due to simplicity and low cost [8,48]. Despite criticisms for lack of specificity and artifactual production during analytical processes, these biomarkers remain among the most commonly applied indices of oxidative damage. TBARS assay involves the reaction of MDA with thiobarbituric acid (TBA) under strong acidic condition and heating, leading to the formation of pink-colored products which can be measured by colorimetric or fluorometric methods. Originally it was accepted that TBARS measured malondialdehyde (MDA), but it actually measures other aldehydes and decomposition products from hydroperoxides as well. Actually many different aldehydes formed in the lipid peroxidation process can react with TBA and form complex with similar absorbance or emission wavelengths [49]. Further, many other biological materials may react with TBA, resulting in high TBARS levels.

In order to improve the specificity, a range of HPLC methods has been developed. Direct measurement of MDA by UV absorption with HPLC is possible but suffers from inferior sensitivity to MDA-TBA adduct. To overcome this problem, GC–MS [50] and high performance capillary electrophoresis methods [51] have been developed.

Numerous studies observed the association between an increase in TBARS values and diseases. In the Biomarkers of Oxidative Stress Study using acute CCl_4 poisoning as a rodent model for oxidative stress, the concentrations of lipid hydroperoxides, TBARS, MDA, IsoPs, protein carbonyls, methionine sulfoxidation, tyrosine products, 8-hydroxy-2′-deoxyguanosine (8-OHdG), leukocyte DNA–MDA adducts, and DNA-strand breaks were measured and it was concluded that MDA and IsoPs in plasma and urine as well as 8-OHdG in urine were potential candidates for general biomarkers of oxidative stress [43]. Overall, TBARS method is convenient, simple, and low cost, but the inherent problems of specificity and variability of data make it difficult to be a reliable quantitative biomarker of lipid peroxidation in vivo, although it may be used as a screening tool when measured with tightly controlled consistent protocol.

2.5. Aldehydes and ketones

Many kinds of carbonylated compounds such as aldehydes and ketones have been found in human fluids and tissues. Aldehydes are formed by the β-scission of alkoxyl radicals and by myeloperoxidase–hydrogen peroxide– chloride ion system [52–54]. Acrolein may be formed also by amine oxidase-mediated degradation of spermine and spermidine, which may constitute a significant source of acrolein in situations of oxidative stress and inflammation [55]. Notably, α,β-unsaturated aldehydes such as acrolein and 4-hydroxy-2-nonenal (HNE) have received much attention as biomarkers of lipid peroxidation in vivo and also as signaling and cytotoxic mediators [52,55,56].

Total carbonyl compounds were measured often from 2,4-dinitrophenylhydrazone formed by the reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine. Specific carbonyl compounds may be identified and measured with LC–MS/MS and GC/MS. Recently, carbonyl compounds were measured by ESI-MS in positive ion mode after derivatization with 7-(diethylamino)coumarin-3-carbohydrazide [57]. In the in vitro oxidation of PC vesicles containing docosahexaenoic (DHA, 22:6), arachidonic (20:4), linoleic (18:2) and oleic acids (18:1), the structures of 122 carbonylated compounds were identified with the tandem mass spectra of a single shotgun analysis after derivatization with 7-(diethylamino)coumarin-3-carbohydrazide [57].

HNE is metabolized by glutathione transferases [58] and mercapturic acid conjugates of HNE were found in urine as end metabolites [59]. α,β-Unsaturated aldehydes are highly reactive and readily react with thiol and amino groups of protein and with other biological molecules to form covalent bonds [54]. Aldehydes may also be oxidized to carboxylic acids. Since carbonyl compounds are highly reactive with biological molecules and readily metabolized by enzymes, they may not be an appropriate biomarker for lipid peroxidation and oxidative stress status in vivo.

2.6. Lysophosphatidylcholine (LysoPC)

LysoPC is formed by partial hydrolysis of PCs by the enzymatic action of phospholipase A2. It has been observed that the level of lysoPC increases in association with the level of lipid peroxidation and

considerable amount of lysoPC has been found in the oxidized LDL [60]. LysoPC is measured with column chromatography, TLC, and LC–MS/MS, but it should be born in mind that it is quickly metabolized by lysophospholipase and acyltransferase.

2.7. Hydroxycholesterols and 7-ketocholesterol

Cholesterol is also an important substrate for oxidation and oxidized by three mechanisms described above to give products collectively termed *oxysterols* [15,18,61]. The free radical mediated oxidation of cholesterol gives 7-hydroperoxycholesterol (7-OOHCh), 7-hydroxycholesterol (7-OHCh), 7-ketocholesterol (7-KCh), 5,6-epoxycholesterol (5,6-epoxyCh), and cholestane-3β,5α,6β-triol as major products. Both alpha and beta forms of 7-OOHCh, 7-OHCh, and 5,6-epoxyCh are formed.

Singlet oxygen and ozone oxidize cholesterol to yield products named secosterol-A and secosterol-B [62,63]. Singlet oxygen oxidation of cholesterol gives also 5-OOHCh. Further, cholesterol is oxidized by enzymes to OHCh at various positions of both cholesterol ring and side chain, primarily by cytochrome P450 enzymes which play central roles in cholesterol metabolism [64,65]. The side chain oxidation product of cholesterol such as 25-OHCh can be formed by both free radical oxidation [66] and the enzyme cholesterol 25-hydroxylase [67]. Chlorinated cholesterols may serve as markers of myeloperoxidase-dependent oxidation. It may be noted that the interconversion between oxysterols may take place in vivo.

It is assumed that 7β-OHCh and 7-KCh are the biomarker for free radical mediated oxidation of cholesterol. They are measured by GC–MS. The reported levels of oxysterols determined in human samples vary considerably: those of 7β-OHCh and 7-KCh in healthy human plasma being in the range of 3–20 and 12–30 nM, while 24-OHCh and 27-OHCh 60–200 and 72–400 nM, respectively [20,68–72]. Higher levels have been observed in many diseased patients. The oxysterols are found at substantially higher levels in the fatty streak and advanced plaques than in normal healthy tissue [18].

2.8. Oxidized LDL

Since the pioneering paper of Steinberg and his colleagues in 1989 [73] reporting the concept that oxidative modification of LDL is a critical initial event in the pathogenesis of atherosclerosis, overwhelming evidence has been accumulated showing that oxidized LDL exerts proinflammatory and proatherogenic effects. Circulating oxidized LDL has received much attention as a biomarker of atherosclerosis and cardiovascular disease. Despite extensive studies from numerous laboratories, the nature, composition, and biological effects of oxidized LDL have not been fully elucidated and are a matter of controversy [74]. This is not surprising considering the inherent heterogeneity of LDL itself and diverse pathways of oxidation by multiple oxidants.

The LDL particle contains on average 600 molecules of free cholesterol, 1600 molecules of cholesteryl ester, 700 molecules of phospholipids, 170 molecules of TG, and 1 molecule of ApoB, with varying amounts of antioxidants such as α- and γ-tocopherol, coenzyme Q10, and β-carotene [74]. Linoleic acid and arachidonic acid are the major PUFA in LDL, especially CE contains high proportion of linoleic acid. The fatty acid composition varies between LDLs and depends on diet. LDL is oxidized by both enzymes such as lipoxygenase, cytochrome p450, and myeloperoxidase and non-enzymatic oxidants, some free radicals while others non-radical oxidants. PUFA are the major target of free radicals and, as noted above, lipid peroxyl radicals act as chain-carrying radical in the lipid peroxidation independent of the type of radicals that initiate chain oxidation [75].

Literally hundreds of products have been identified in the oxidized LDL [76,77] and it is impossible to identify and quantify all of them. Considering the heterogeneity of LDL, it is difficult to define, characterize and quantify “oxidized LDL” and “minimally modified LDL.” In practice, “oxidized LDL” has been assessed by measuring some specific oxidation products contained in LDL particles.

Monoclonal antibodies were raised against LDL oxidized by copper or modified by aldehydes and applied to measure the level of oxidized LDL in circulating plasma with a sandwich ELISA [78–80]. It was reported that the levels of oxidized LDL in human plasma were 1.95 ng/5 μg LDL protein and 0.58 ng/5 μg LDL protein respectively for patients with acute myocardial infarction and healthy controls respectively [81]. This level is similar to the molar ratio of HODE to its parent fatty acid, linoleic acid [1].

It may be said that the plasma levels of oxidized LDL in patients of atherosclerosis and cardiovascular diseases are higher than those in healthy subjects [79,80,82], but it should be born in mind that the level of oxidized LDL and the above association is relative or at best semi-quantitative.

2.9. LPO-modified proteins

Numerous lipid peroxidation products are chemically reactive and react with biological molecules including proteins, peptides, and DNA. Notably, α,β-unsaturated carbonyl compounds are strong electrophiles and thus can readily react with nucleophilic side chains of proteins, resulting in the alteration of protein structure, function, and cellular distribution [8,52,54,55]. The structure of such adducts has been identified. Monoclonal antibody has been raised against several adducts and their structure has also been identified by LC–MS/MS analysis [83]. However, accurate quantitation of adducts from biological samples is difficult. Practically, immunohistochemistry for aldehyde-modified proteins has been carried out often for tissue samples to show their presence, in particular for the samples from experimental animals.

2.10. Other potential biomarkers

In addition to the lipid peroxidation products described above, several other lipid metabolites have been considered as biomarker. Nitro-fatty acids and *trans* fatty acids may serve as potential biomarker of oxidative stress. Notably, the levels and biological effects of 9-nitro-oleic acid and 10-nitro-oleic acid which are assumed to be formed in vivo by the reaction of nitrogen dioxide and oleic acid have been explored in many studies [reviewed in [84,85]]. Biological effects of *trans*-fatty acids have been the subject of extensive arguments [86]. They are contained in the diet but they may also be produced in vivo by reversible addition-elimination reaction of free radicals such as nitrogen dioxide and thiyl radicals to double bonds of unsaturated lipids. The levels of *trans* fatty acids found in human plasma are about two orders of magnitude higher than those of lipid peroxidation products such as HPODE and HODE.

Another biomarker of potential interest is the level and metabolites of antioxidants. It has been observed in many studies that smoking induces increase in oxidative stress biomarkers and that the plasma levels of vitamin C of smokers are lower than those of non-smokers [87]. The level of 5-nitro-γ-tocopherol was increased by smoking [88]. The total level of antioxidants in plasma termed TAC (total antioxidant capacity) or non-enzymatic antioxidant capacity (NEAC) [89] has been measured to estimate the level of oxidative stress or antioxidant capacity status in vivo, but it should be interpreted with caution [90,91]. On the other hand, specific oxidation products and metabolites of antioxidants such as α-tocopheryl quinone, 5-nitro-γ-tocopherol, biopyrrin, and allntoin may be a useful biomarker of oxidative stress and lipid peroxidation [91]. The ratio of reduced to oxidized forms of antioxidants such as GSH/GSSG and ubiquinol/ubiquinone is used as an index of oxidative stress.

The assessment of antioxidant capacity in vitro and in vivo has been the subject of extensive studies and arguments [reviewed in [91]]. The in vivo antioxidation capacity of antioxidant compounds, foods, beverages, and supplements may be assessed from the effects

of antioxidant administration to human subjects and experimental animals on the levels of lipid peroxidation products mentioned above.

3. Measurement of lipid peroxidation products: advantages and limitations

As described above, literally hundreds of products are formed by the lipid peroxidation in vivo, making it quite difficult to identify and quantify all of them. They are produced with different yields and metabolized and excreted at different rates. The physiological levels of lipid peroxidation products are determined by a balance between formation, metabolism, and excretion. Linoleates (18:2), arachidonates (20:4), docosahexanoates (22:6), and cholesterol are the major substrates for biological lipid peroxidation. PUFAs are present predominantly as esters of phospholipids, glycerol, and cholesterol. Their composition is dependent on the diet and varies between biological fluids and tissues. Some lipid peroxidation products are present in free forms, while others in ester forms. It has been observed that they are present predominantly as ester form in plasma, while in free form in urine. The rate of hydrolysis from cellular membranes may vary between the products and individuals [7].

In terms of measuring method, accuracy, sensitivity, specificity, reproducibility, simple and quick protocol, less consuming time, low cost, and less intensive labor are the important factors. The suitability of methods may also depend on the biomarker, sample, and objective. Lipids are vulnerable to air oxidation and therefore every care should be taken to avoid artifactual oxidation during sample collection, storage, and analytical processes.

It has been pointed out that accurate determination of biomarkers from biological samples is very difficult. In fact, it is often observed that the reported values span over one to two orders of magnitude even in control healthy subjects. 8-Oxo-7,8-dihydroguanine (8-oxoGua) is one of the most commonly measured marker of oxidative damage in vivo. The European Standards Committee on Oxidative DNA Damage (ESCODD) pointed out that the published estimates of the concentration of 8-oxoGua in DNA of normal human cells vary over a range of three orders of magnitude and that the measurements of the same samples at different laboratories gave inconsistent results [92]. The advantages and limitations of biomarkers and methods of measurement are considered below.

3.1. Sample preparation

Sample collection, storage, and handling are critically important for accurate determination of biomarkers. Two factors are decisive for accurate measurement of lipid peroxidation products, the artifactual generation and loss during sample treatment and the lack of selectivity, specificity, and sensitivity of the analytical method. The original biological samples contain very small amounts of products as compared to the large amounts of parent lipids. Therefore, a very small fraction of artifactual oxidation during sample handling and analysis may be considerable and exceed the basal level of the biomarker. It was pointed out that 7-oxygenated cholesterol, which is less reactive than PUFAs, was artificially formed by autoxidation of cholesterol during workup procedures and analysis of biological samples [93]. In general, antioxidants such as 2,6-di-*tert*-butyl-4-methylphenol and metal chelator are added to inhibit artifactual lipid oxidation during storage and handling. Fasting is required for sample collection from the subjects, because diets often contain considerable amounts of lipid peroxidation products [94].

The emerging lipidomics technology using mass spectrometry makes it possible to identify diverse lipid oxidation products in intact forms [14,95–97]. This may be useful for basic study but it is too sophisticated for practical and clinical use. In many cases, the samples are subjected to hydrolysis in alkaline solution to convert multiple esters to free fatty acid and free cholesterol forms. Since it is quite difficult to measure all of the lipid peroxidation products in intact forms, this is practically useful.

Hydroperoixdes and other peroxides are thermally unstable and may decompose during analytical processes, interfering accurate measurement. For example, in the analysis using GC, the temperature is raised often above 200 °C. Therefore, the samples are often reduced beforehand to convert labile hydroperoxides to the corresponding hydroxides. As described above, it was found that sodium borohydride induced artifactual lipid oxidation during analytical processes, probably by reducing ferric or cupric ions to the reactive ferrous and cuprous ions respectively [37]. Triphenylphosphine is recommended as a reducing agent for lipid hydroperoxides in biological samples.

3.2. Sample source

Various samples have been used to measure in vivo biomarkers, including plasma, serum, erythrocytes, urine, saliva, tear, exhaled gas, sweat, bile, celebrospinal fluid, synovial fluid, bronchoalveolar lavage fluid, and tissues. Some are less invasive, while others invasive. In contrast to plasma or serum, urine and saliva are not invasive but so variable that quantitative assessment is difficult, despite the fact the level in urine is normalized by creatinine. On the other hand, it was reported also that urine could be a better matrix than blood because it has a much lower organic as well as inorganic metal content making urine less liable for artificial increase of oxidative markers during sample collection, storage, preparation, and analysis and further that urinary levels of the biomarkers present intergraded indices over a longer period of time compared to blood levels [98].

3.3. Methods of measurement

Many methods of measurement have been proposed and developed, but no currently available method fulfills the requirements described above. Of the techniques available, high performance liquid chromatography (HPLC) based approaches offer the best sensitivity and specificity. The HPLC approach relies on separation of various products. Different post column detection systems can be applied, including UV/visible spectrophotometry, chemiluminescence (HPLC-CL), fluorescence (HPLC-FL), electrochemical-coulometry (HPLC-ECD), and mass spectrometry (LC–MS/MS) detection systems. The HPLC-CL method has high sensitivity and specificity for hydroperoxides.

The recent availability of high-sensitive liquid chromatography–mass spectrometry has provided a new approach for quantification that minimizes the sample size and the required preparation. The advantages of this method include minimal sample preparation, high sensitivity and elimination of the problem associated with thermal instability in gas chromatography analysis.

Mass spectrometry is an evolving technology and, with a combination of chromatography, facilitates the detection and quantification of scarce products in intact forms. The mass spectrometry-based methods are very sensitive and specific but necessitate a skilled technical stuff and laborious work. Further, lack of commercially available stable isotope-labeled analogs may be a limiting factor in mass spectrometry-based methods.

Immunoassays, although less specific and quantitative than LC–MS and GC–MS methods, have been found to be helpful tools for a huge sample-analyzing capacity at fairly low cost. Thus, a well-validated immunoassay technique could be a significant tool for evaluating free radical-mediated reactions in clinical research, where a large number of samples must be analyzed at an affordable cost. However, it has to be noted that the specific methods such as LC–MS or GC–MS and ELISA method applied for biological samples do not often give results in satisfactory agreement. Specific antibodies against lipid peroxidation products with high specificity and affinity are critically needed.

Imaging is a promising tool for study of oxidative stress in vivo. The emerging imaging mass-spectrometry represents an exciting new opportunity for correlating maps of lipid profiles and their oxidation products with structure and pathology [97,99]. Desorption electrospray ionization-mass spectrometry (DESI-MS) was used to monitor the changes in lipid classes, fatty acid distribution, and also oxidation products such as MDA [100], but probably higher sensitivity is required for imaging of lipid peroxidation products.

The antibodies have been used successfully for *in situ* localization by immunostaining in oxidative stress-injured tissues. For example, the aldehyde-modified proteins have been found in many tissues from experimental animals under oxidative stress [101].

The imaging technique has been applied in the study of many diseases such as atherosclerosis and cardiovascular diseases [102]. Imaging using specific antibody raised against oxidation products is a challenging approach. The antibody may be tagged with appropriate labels for use in nuclear scintigraphy, magnetic resonance, ultrasound imaging, or positron emission tomography (PET) [103]. The combined PET/CT scans using antibody against specific oxidation products may provide images that pinpoint the location of oxidative damage. The antibodies labeled with copper-64 and yttrium-90 may be used for non-invasing imaging and radio isotope therapy respectively.

3.4. Limitations of lipid peroxidation products as biomarker

The characteristic aspect of free radical oxidation is non-specificity, that is, free radicals attack biological molecules such as lipids, proteins, carbohydrates, and DNA randomly. The bisallylic hydrogens of PUFAs in different lipid classes and on different carbons are oxidized similarly by free radicals. An increase in free radical production and reaction in vivo will most likely result in the increase in the oxidized lipids, proteins and DNA bases simultaneously. The lack of specificity may be the inherent drawback of lipid peroxidation products as a biomarker. In contrast to oxidatively modified proteins such as amyloid β, phosphorylated tau, or oxidized DJ-1 which may link to specific disease, lipid peroxidation products are indices of free radical mediated oxidative stress in general but may not link to any specific disease. Furthermore, it is difficult to prove from the biomarkers of lipid peroxidation measured in blood or urine whether the lipid peroxidation is a cause or consequence of diseases and where the lipid peroxidation products come from. Abundant research over the past three decades has seldom demonstrated convincing link between increased lipid peroxidation and the development of disease or provided solid evidence that lipid peroxidation is fundamentally involved in pathogenesis. Furthermore, no disease has been treated with any antioxidant convincingly and successfully.

To be used as diagnostic tools, the biomarkers should correlate with disease severity. However, this has not been confirmed in many cases. The level of biomarker may not necessarily increase in accordance with the progress of disease, but may increase, reach plateau, or decline. For example, the oxidative stress was suggested to be important at the early stage of Parkinson disease [104,105].

In order to overcome such drawbacks, it is recommended to measure multiple biomarkers at different time points. For example, the fatty acid distribution depends markedly on tissues and lipid classes. The relative levels of HODE, HETE, IsoP, neuroprostane, and 7-OHCh may give a hint for the origin of tissues. Simultaneous measurements of oxidized proteins are undoubtedly useful. It is important to analyze the temporal change of multiple biomarkers and inter- and intra-individual variation.

One special exception is the oxidation product of 7-dehydrocholesterol (7-DHC). The level of 7-DHC is elevated in tissues and fluids of Smith–Lemli–Opitz syndrome (SLOS) patients due to defective 7-DHC reductase. 7-DHC is very reactive toward peroxyl radical and the oxidation product of 7-DHC, 3β,5α-dihydroxycholest-7-en-6-one, may be used as a specific biomarker of SLOS [106]. Another example is squalene hydroperoxides and metabolites, which may be used as biomarker of oxidative stress in human skin [107].

4. Concluding remarks

Biomarkers are needed to assess oxidative stress status in vivo, discover early indication of disease, diagnose progression of disease, and evaluate the effectiveness of treatment with drugs or antioxidants. To date, there is no established biomarker available for regular clinical use. Despite the limitations described above, lipid peroxidation products may serve as useful biomarkers, since lipid peroxidation is the major reaction taking place under oxidative stress and assumed to play an important role in the pathogenesis and progression of many diseases. Furthermore, lipid peroxidation products can be measured more quantitatively than the oxidatively modified proteins and DNA.

Considering chemical stability and consistency of the reported levels in human fluids and tissues, lipid hydroxides are considered to be more suitable than hydroperoxides. Specifically, HODEs, HETEs, and hydroxycholesterols may be recommended as reliable biomarkers. Notably, the four HODEs, 9-*cis*,*trans*, 9-*trans*,*trans*, 13-*cis*,*trans*, and 13-*trans*,*trans*-HODE, can be measured separately by LC–MS/MS and the *trans*,*trans*-forms are specific products of free radical mediated lipid peroxidation. Likewise, 7β-hydoxycholesterol and 7-KCh are considered to be a specific product of free radical oxidation. Further, F_2-IsoP and neuroprostanes are accepted biomarker of lipid peroxidation. As stated above, it is important to examine the distribution of these products and their temporal change. The potential usefulness as biomarker for health and disease should be validated in the future studies.

References

[1] E. Niki, Lipid peroxidation: physiological levels and dual biological effects, Free Radic. Biol. Med. 47 (2009) 469–484.
[2] W.A. Pryor, S.S. Godber, Noninvasive measured of oxidative stress status in humans, Free Radic. Biol. Med. 10 (1991) 177–184.
[3] I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini, A. Milzani, Biomarkers of oxidative damage in human diseases, Clin. Chem. 52 (2006) 601–623.
[4] E. Niki, Lipid peroxidation products as oxidative stress biomarkers, Biofactors 34 (2008) 171–180.
[5] B. Halliwell, M. Whiteman, Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142 (2004) 231–255.
[6] D. Giustarini, I. Dalle-Donne, D. Tsikas, R. Rossi, Oxidative stress and human diseases: origin, link, measurement, mechanisms, and biomarker, Crit. Rev. Clin. Lab. Sci. 46 (2009) 241–281.
[7] B. Halliwell, C.Y. Lee, Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues, Antioxid. Redox Signal. 13 (2010) 145–156.
[8] C.M. Spickett, I. Wiswedel, W. Siems, K. Zarkovic, N. Zarkovic, Advances in methods for the determination of biologically relevant lipid peroxidation products, Free Radic. Res. 44 (2010) 1172–1202.
[9] S.S. Davies, L.J. Roberts, F2-isoprostanes as an indicator and risk factor for coronary heart disease, Free Radic. Biol. Med. 50 (2011) 559–566.
[10] R. Lee, M. Margaritis, K.M. Channon, C. Antoniades, Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations, Curr. Med. Chem. 19 (2012) 2504–2520.
[11] E. Niki, Do antioxidants impair signaling by reactive oxygen species and lipid oxidation products? FEBS Lett. 586 (2012) 3767–3770.
[12] G. Leonarduzzi, P. Gamba, S. Gargiulo, F. Biasi, G. Poli, Inflammation-related gene expression by lipid oxidation-derived products in the progression of atherosclerosis, Free Radic. Biol. Med. 52 (2012) 19–34.
[13] A. Higdon, A.R. Diers, J.Y. Oh, A. Landar, V.M. Darley-Usmar, Cell signalling by reactive lipid species: new concepts and molecular mechanisms, Biochem. J. 442 (2012) 453–464.
[14] H. Yin, L. Xu, N.A. Porter, Free radical lipid peroxidation: mechanisms and analysis, Chem. Rev. 111 (2011) 5944–5972.
[15] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, Chem. Phys. Lipids 164 (2011) 457–468.
[16] U. Diczfalusy, Analysis of cholesterol oxidation products in biological samples, J. AOAC Int. 87 (2004) 467–473.
[17] D.M. van Reyk, A.J. Brown, L.M. Hult'en, R.T. Dean, W. Jessup, Oxysterols in biological systems: sources, metabolism and pathophysiological relevance, Redox Rep. 11 (2006) 255–262.
[18] R.C. Murphy, K.M. Johnson, Cholesterol, reactive oxygen species, and the formation of biologically active metabolites, J. Biol. Chem. 283 (2008) 15521–15525.
[19] K. Ishii, J. Adachi, M. Tomita, M. Kurosaka, Y. Ueno, Oxysterols as indices of oxidative stress in man after paraquat ingestion, Free Radic. Res. 36 (2002) 163–168.

[20] L. Iuliano, F. Micheletta, S. Natoli, S. Ginanni Corradini, M. Iappelli, W. Elisei, L. Giovannelli, F. Violi, U. Diczfalusy, Measurement of oxysterols and alpha-tocopherol in plasma and tissue samples as indices of oxidant stress status, Anal. Biochem. 312 (2003) 217–223.
[21] W. Jessup, R. Dean, J. Gebicki, Iodometric determination of hydroperoxides in lipids and proteins, Meth. Enzymol. 233 (1994) 289–303.
[22] G.M. Chisolm, G. Ma, K.C. Irwin, L.L. Martin, K.G. Gunderson, L.F. Linberg, D.W. Morel, P.E. DiCorleto, 7 beta-hydroperoxycholest-5-en-3 beta-ol, a component of human atherosclerotic lesions, is the primary cytotoxin of oxidized human low density lipoprotein, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 11452–11456.
[23] T. Iwaoka, F. Tabata, T. Takahashi, Lipid peroxidation and lipid peroxide detected by chemiluminescence, Free Radic. Biol. Med. 3 (1987) 329–333.
[24] Y. Yamamoto, M.H. Brodsky, J.C. Baker, B.N. Ames, Detection and characterization of lipid hydroperoxides at picomole levels by high-performance liquid chromatography, Anal. Biochem. 160 (1987) 7–13.
[25] T. Miyazawa, K. Yasuda, K. Fujimoto, T. Kaneda, Presence of phosphatidylcholine hydroperoxide in human plasma, J. Biochem. 103 (1988) 744–746.
[26] N. Noguchi, E. Niki, Dynamics of free radical formation from the reaction of peroxides with haemproteins as studied by stopped-flow chemiluminescence, Free Radic. Res. 23 (1995) 329–338.
[27] K. Nakagawa, J.H. Oak, O. Higuchi, T. Tsuzuki, S. Oikawa, H. Otani, M. Mune, H. Cai, T. Miyazawa, Ion-trap tandem mass spectrometric analysis of Amadori-glycated phosphatidylethanolamine in human plasma with or without diabetes, J. Lipid Res. 46 (2005) 2514–2524.
[28] V.A. Tyurin, Y.Y. Tyurina, V.B. Ritov, A. Lysytsya, A.A. Amoscato, P.M. Kochanek, R. Hamilton, S.T. Dekosky, J.S. Greenberger, H. Bayir, V.E. Kagan, Oxidative lipidomics of apoptosis: quantitative assessment of phospholipid hydroperoxides in cells and tissues, Methods Mol. Biol. 610 (2010) 353–374.
[29] Z.Y. Jiang, J.V. Hunt, S.P. Wolff, Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein, Anal. Biochem. 202 (1992) 384–389.
[30] K. Akasaka, H. Ohrui, H. Meguro, M. Tamura, Determination of triacylglycerol and cholesterol ester hydroperoxides in human plasma by high-performance liquid chromatography with fluorometric postcolumn detection, J. Chromatogr. 617 (1993) 205–211.
[31] Y. Okimoto, A. Watanabe, E. Niki, T. Yamashita, N. Noguchi, A novel fluorescent probe diphenyl-1-pyrenylphosphine to follow lipid peroxidation in cell membranes, FEBS Lett. 474 (2000) 137–140.
[32] M. Takahashi, M. Shibata, E. Niki, Estimation of lipid peroxidation of live cells using a fluorescent probe, diphenyl-1-pyrenylphosphine, Free Radic. Biol. Med. 31 (2001) 164–174.
[33] T. Ohshima, A. Hopia, J.B. German, E.N. Frankel, Determination of hydroperoxides and structures by high-performance liquid chromatography with post-column detection with diphenyl-1-pyrenylphosphine, Lipids 31 (1996) 1091–1096.
[34] Y. Kawai, M. Miyoshi, J.H. Moon, J. Terao, Detection of cholesteryl ester hydroperoxide isomers using gas chromatography–mass spectrometry combined with thin-layer chromatography blotting, Anal. Biochem. 360 (2007) 130–137.
[35] R.C. Seet, C.Y. Lee, E.C. Lim, J.J. Tan, A.M. Quek, W.L. Chong, W.F. Looi, S.H. Huang, H. Wang, Y.H. Chan, B. Halliwell, Oxidative damage in Parkinson disease: measurement using accurate biomarkers, Free Radic. Biol. Med. 48 (2010) 560–566.
[36] N. Noguchi, H. Yamashita, J. Hamahara, A. Nakamura, H. Kühn, E. Niki, The specificity of lipoxygenase-catalyzed lipid peroxidation and the effects of radical-scavenging antioxidants, Biol. Chem. 383 (2002) 619–626.
[37] W. Liu, H. Yin, Y.O. Akazawa, Y. Yoshida, E. Niki, N.A. Porter, Ex vivo oxidation in tissue and plasma assays of hydroxyoctadecadienoates: *Z,E/E,E* stereoisomer ratios, Chem. Res. Toxicol. 23 (2010) 986–995.
[38] D.A. Pratt, K.A. Tallman, N.A. Porter, Free radical oxidation of polyunsaturated lipids: new mechanistic insights and the development of peroxyl radical clocks, Acc. Chem. Res. 44 (2011) 458–467.
[39] C. Gniwotta, J.D. Morrow, L.J. Roberts II, H. Kühn, Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesions, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 3236–3241.
[40] J.D. Morrow, K.E. Hill, R.F. Burk, T.M. Nammour, K.F. Badr, L.J. Roberts II, 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.
[41] G.L. Milne, H. Yin, K.D. Hardy, S.S. Davies, L.J. Roberts II, Isoprostane generation and function, Chem. Rev. 111 (2011) 5973–5996.
[42] L.J. Roberts II, J.P. Fessel, S.S. Davies, The biochemistry of the isoprostane, neuroprostane, and isofuran pathways of lipid peroxidation, Brain Pathol. 15 (2005) 143–148.
[43] M.B. Kadiiska, B.C. Gladen, D.D. Baird, D. Germolec, L.B. Graham, C.E. Parker, A. Nyska, J.T. Wachsman, B.N. Ames, S. Basu, N. Brot, G.A. Fitzgerald, R.A. Floyd, M. George, J.W. Heinecke, G.E. Hatch, K. Hensley, J.A. Lawson, L.J. Marnett, J.D. Morrow, D.M. Murray, J. Plastaras, L.J. Roberts II, J. Rokach, M.K. Shigenaga, R.S. Sohal, J. Sun, R.R. Tice, D.H. Van Thiel, D. Wellner, P.B. Walter, K.B. Tomer, R.P. Mason, J.C. Barrett, Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl_4 poisoning? Free Radic. Biol. Med. 38 (2005) 698–710.
[44] A.E. Feldstein, R. Lopez, T.A. Tamimi, L. Yerian, Y.M. Chung, M. Berk, R. Zhang, T.M. McIntyre, S.L. Hazen, Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, J. Lipid Res. 51 (2010) 3046–3054.
[45] W. Liu, J.D. Morrow, H. Yin, Quantification of F_2-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.
[46] J.D. Morrow, L.J. Roberts, V.C. Daniel, J.A. Awad, O. Mirochnitchenko, L.L. Swift, R.F. Burk, Comparison of formation of D2/E2-isoprostanes and F2-isoprostanes in vitro and in vivo—effects of oxygen tension and glutathione, Arch. Biochem. Biophys. 353 (1998) 160–171.
[47] S.E. Farias, M. Basselin, L. Chang, K.A. Heidenreich, S.I. Rapoport, R.C. Murphy, Formation of eicosanoids, E2/D2 isoprostanes, and docosanoids following decapitation-induced ischemia, measured in high-energy-microwaved rat brain, J. Lipid Res. 49 (2008) 1990–2000.
[48] J. Lykkesfeldt, Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking, Clin. Chim. Acta 380 (2007) 50–58.
[49] H. Kosugi, T. Kato, K. Kikugawa, Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid, Anal. Biochem. 165 (1987) 456–464.
[50] H.C. Yeo, H.J. Helbock, D.W. Chyu, B.N. Ames, Assay of malondialdehyde in biological fluids by gas chromatography–mass spectrometry, Anal. Biochem. 220 (1994) 391–396.
[51] D.W. Wilson, H. Metz, L.M. Graver, P.S. Rao, Direct method for quantification of free malondialdehyde with high-performance capillary electrophoresis in biological samples, Clin. Chem. 43 (1997) 1982–1984.
[52] K. Uchida, Histidine and lysine as targets of oxidative modification, Amino Acids 25 (2003) 249–257.
[53] S.L. Hazen, F.F. Hsu, A. d'Avignon, J.W. Heinecke, Human neutrophils employ myeloperoxidase to convert alpha-amino acids to a battery of reactive aldehydes: a pathway for aldehyde generation at sites of inflammation, Biochemistry 37 (1998) 6864–6873.
[54] F. Gueraud, M. Atalay, N. Bresgen, A. Cipak, P.M. Eckl, L. Huc, K. Uchida, Chemistry and biochemistry of lipid peroxidation products, Free Radic. Res. 44 (2010) 1098–1124.
[55] G. Poli, R.J. Schaur, W.G. Siems, G. Leonarduzzi, 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest, Med. Res. Rev. 28 (2008) 569–631.
[56] Y. Riahi, G. Cohen, O. Shamni, S. Sasson, Signaling and cytotoxic functions of 4-hydroxyalkenals, Am. J. Physiol. Endocrinol. Metab. 299 (2010) E879–E886.
[57] I. Milic, R. Hoffmann, M. Fedorova, Simultaneous detection of low and high molecular weight carbonylated compounds derived from lipid peroxidation by electrospray ionization-tandem mass spectrometry, Anal. Chem. 85 (2013) 156–162.
[58] L.M. Balogh, W.M. Atkins, Interactions of glutathione transferases with 4-hydroxynonenal, Drug Metab. Rev. 43 (2011) 165–178.
[59] J. Alary, F. Bravis, J.P. Cravedi, L. Debrauwer, D. Rao, G. Bories, Mercapturic acid conjugates as urinary end metabolites of the lipid peroxidation product 4-hydroxy-2-nonenal in the rat, Chem. Res. Toxicol. 8 (1995) 34–39.
[60] M.T. Quinn, S. Parthasarathy, D. Steinberg, Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 2805–2809.
[61] G. Schmitz, G. Lizard, L. Iuliano, Ed. Special issue to LipidomicNet and ENOR Joint Workshop, "Analysis and function of oxysterols and other regulatory and lipotoxic molecular lipid species", Chem. Phys. Lipids 164 (2011) 409–624.
[62] P. Wentworth Jr., J. Nieva, C. Takeuchi, R. Galve, A.D. Wentworth, R.B. Dilley, G.A. DeLaria, A. Saven, B.M. Babior, K.D. Janda, A. Eschenmoser, R.A. Lerner, Evidence for ozone formation in human atherosclerotic arteries, Science 302 (2003) 1053–1056.
[63] S. Tomono, N. Miyoshi, H. Shiokawa, T. Iwabuchi, Y. Aratani, T. Higashi, H. Nukaya, H. Ohshima, Formation of cholesterol ozonolysis products in vitro and in vivo through a myeloperoxidase-dependent pathway, J. Lipid Res. 52 (1) (Jan 2011) 87–97.
[64] A.J. Brown, W. Jessup, Oxysterols: sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis, Mol. Aspects Med. 30 (2009) 111–122.
[65] K.J. McLean, M. Hans, A.W. Munro, Cholesterol, an essential molecule: diverse roles involving cytochrome P450 enzymes, Biochem. Soc. Trans. 40 (2012) 587–593.
[66] L.L. Smith, Cholesterol autoxidation 1981–1986, Chem. Phys. Lipids 44 (1987) 87–125.
[67] E.G. Lund, T.A. Kerr, J. Sakai, W.P. Li, D.W. Russell, cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesize a potent oxysterol regulator of lipid metabolism, J. Biol. Chem. 273 (1998) 34316–34327.
[68] S. Dzeletovic, O. Breuer, E. Lund, U. Diczfausy, Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry, Anal. Biochem. 225 (1995) 73–80.
[69] A. Babiker, U. Diczfalusy, Transport of side-chain oxidized oxysterols in the human circulation, Biochim. Biophys. Acta 1392 (1998) 333–339.
[70] T.P. Tuomainen, U. Diczfalusy, J. Kaikkonen, K. Nyyssönen, J.T. Salonen, Serum ferritin concentration is associated with plasma levels of cholesterol oxidation products in man, Free Radic. Biol. Med. 35 (2003) 922–928.
[71] H. Larsson, Y. Böttiger, L. Iuliano, U. Diczfalusy, In vivo interconversion of 7beta-hydroxycholesterol and 7-ketocholesterol, potential surrogate markers for oxidative stress, Free Radic. Biol. Med. 43 (2007) 695–701.
[72] C.Y. Lee, S.H. Huang, A.M. Jenner, B. Halliwell, Measurement of F2-isoprostanes, hydroxyeicosatetraenoic products, and oxysterols from a single plasma sample, Free Radic. Biol. Med. 44 (2008) 1314–1322.
[73] D. Steinberg, S. Parthasarathy, T.E. Carew, J.C. Khoo, J.L. Witztum, Beyond cholesterol. Modifications of lowdensity lipoprotein that increase its atherogenicity, N. Engl. J. Med. 320 (1989) 915–924.
[74] H. Esterbauer, J. Gebicki, H. Puhl, G. Jürgens, The role of lipid peroxidation and antioxidants in oxidative modification of LDL, Free Radic. Biol. Med. 13 (1992) 341–390.

[75] N. Noguchi, E. Niki, Dynamics of vitamin E action against LDL oxidation, Free Radic. Res. 28 (1998) 561–572.
[76] J.A. Berliner, N. Leitinger, S. Tsimikas, The role of oxidized phospholipids in atherosclerosis, J. Lipid Res. 50 (2009) S207–S212, (Suppl.).
[77] X. Jiang, Z. Yang, A.N. Chandrakala, D. Pressley, S. Parthasarathy, Oxidized low density lipoproteins—do we know enough about them? Cardiovasc. Drugs Ther. 25 (2011) 367–377.
[78] W. Palinski, S. Ylä-Herttuala, M.E. Rosenfeld, S.W. Butler, S.A. Socher, S. Parthasarathy, L.K. Curtiss, J.L. Witztum, Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein, Arteriosclerosis 10 (1990) 325–335.
[79] P. Holvoet, J. Donck, M. Landeloos, E. Brouwers, K. Luijtens, J. Arnout, E. Lesaffre, Y. Vanrenterghem, D. Collen, Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure, Thromb. Haemost. 76 (1996) 663–669.
[80] H. Itabe, Oxidized low-density lipoprotein as a biomarker of in vivo oxidative stress: from atherosclerosis to periodontitis, J. Clin. Biochem. Nutr. 51 (2012) 1–8.
[81] S. Ehara, M. Ueda, T. Naruko, K. Haze, A. Itoh, M. Otsuka, R. Komatsu, T. Matsuo, H. Itabe, T. Takano, Y. Tsukamoto, M. Yoshiyama, K. Takeuchi, J. Yoshikawa, A.E. Becker, Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes, Circulation 103 (2001) 1955–1960.
[82] S. Tsimikas, E.S. Brilakis, E.R. Miller, J.P. McConnell, R.J. Lennon, K.S. Kornman, J.L. Witztum, P.B. Berger, Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease, N. Engl. J. Med. 353 (2005) 46–57.
[83] T. Ishii, S. Kumazawa, T. Sakurai, T. Nakayama, K. Uchida, Mass spectroscopic characterization of protein modification by malondialdehyde, Chem. Res. Toxicol. 19 (2006) 122–129.
[84] D. Tsikas, A.A. Zoerner, J. Jordan, Oxidized and nitrated oleic acid in biological systems: analysis by GC–MS/MS and LC–MS/MS, and biological significance, Biochim. Biophys. Acta 1811 (2011) 694–705.
[85] N. Khoo, B.A. Freeman, Electrophilic nitro-fatty acids: anti-inflammatroy mediators in the vascular compartment, Curr. Opin. Pharmacol. 10 (2010) 179–184.
[86] J. Aranceta, C. Pérez-Rodrigo, Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review, Br. J. Nutr. 107 (Suppl. 2) (2012) S8–S22.
[87] R.C. Seet, C.Y. Lee, W.M. Loke, S.H. Huang, H. Huang, W.F. Looi, E.S. Chew, A.M. Quek, E.C. Lim, B. Halliwell, Biomarkers of oxidative damage in cigarette smokers: which biomarkers might reflect acute versus chronic oxidative stress? Free Radic. Biol. Med. 50 (2011) 1787–1793.
[88] S.W. Leonard, R.S. Bruno, E. Paterson, B.C. Schock, J. Atkinson, T.M. Bray, C.E. Cross, M.G. Traber, 5-nitro-gamma-tocopherol increases in human plasma exposed to cigarette smoke in vitro and in vivo, Free Radic. Biol. Med. 35 (2003) 1560–1567.
[89] M. Serafini, G. Morabito, The role of polyphenols in the modulation of plasma non-enzymatic antioxidant capacity (NEAC), Int. J. Vitam. Nutr. Res. 82 (2012) 228–232.
[90] H. Sies, Total antioxidant capacity: appraisal of a concept, J. Nutr. 137 (2007) 1493–1495.
[91] E. Niki, Assessment of antioxidant capacity in vitro and in vivo, Free Radic. Biol. Med. 49 (2010) 503–515.
[92] A.R. Collins, J. Cadet, L. Möller, H.E. Poulsen, J. Viña, Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? Arch. Biochem. Biophys. 423 (2004) 57–65.
[93] V. Leoni, D. Lütjohann, T. Masterman, Levels of 7-oxocholesterol in cerebrospinal fluid are more than one thousand times lower than reported in multiple sclerosis, J. Lipid Res. 46 (2005) 191–195.
[94] R. Wilson, K. Lyall, L. Smyth, C.E. Fernie, R.A. Riemersma, Dietary hydroxy fatty acids are absorbed in humans: implications for the measurement of 'oxidative stress' in vivo, Free Radic. Biol. Med. 32 (2002) 162–168.
[95] H. Nakanishi, Y. Iida, T. Shimizu, R. Taguchi, Analysis of oxidized phosphatidylcholines as markers for oxidative stress, using multiple reaction monitoring with theoretically expanded data sets with reversed-phase liquid chromatography/tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 1366–1374.
[96] H. Yin, T. Davis, N.A. Porter, Simultaneous analysis of multiple lipid oxidation products in vivo by liquid chromatographic-mass spectrometry (LC-MS), Methods Mol. Biol. 610 (2010) 375–386.
[97] L.J. Sparvero, A.A. Amoscato, P.M. Kochanek, B.R. Pitt, V.E. Kagan, H. Bayir, Mass-spectrometry based oxidative lipidomics and lipid imaging: applications in traumatic brain injury, J. Neurochem. 115 (2010) 1322–1336.
[98] D. Il'yasova, P. Scarbrough, I. Spasojevic, Urinary biomarkers of oxidative status, Clin. Chim. Acta 413 (2012) 1446–1453.
[99] Y. Saito, M. Waki, S. Hameed, T. Hayasaka, M. Setou, Development of imaging mass spectrometry, Biol. Pharm. Bull. 35 (2012) 1417–1424.
[100] M. Girod, Y. Shi, J.X. Cheng, R.G. Cooks, Mapping lipid alterations in traumatically injured rat spinal cord by desorption electrospray ionization imaging mass spectrometry, Anal. Chem. 83 (2011) 207–215.
[101] A. Yoritaka, N. Hattori, K. Uchida, M. Tanaka, E.R. Stadtman, Y. Mizuno, Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 2696–2701.
[102] K.C. Briley-Saebo, P.X. Shaw, W.J. Mulder, S.H. Choi, E. Vucic, J.G. Aguinaldo, J.L. Witztum, V. Fuster, S. Tsimikas, Z.A. Fayad, Targeted molecular probes for imaging atherosclerotic lesions with magnetic resonance using antibodies that recognize oxidation-specific epitopes, Circulation 117 (2008) 3206–3215.
[103] S. Tsimikas, P.X. Shaw, Non-invasive imaging of vulnerable plaques by molecular targeting of oxidized LDL with tagged oxidation-specific antibodies, J. Cell. Biochem. (Suppl. 39) (2002) 138–146.
[104] Y. Saito, T. Hamakubo, Y. Yoshida, Y. Ogawa, Y. Hara, H. Fujimura, Y. Imai, H. Iwanari, Y. Mochizuki, M. Shichiri, K. Nishio, T. Kinumi, N. Noguchi, T. Kodama, E. Niki, Preparation and application of monoclonal antibodies against oxidized DJ-1. Significant elevation of oxidized DJ-1 in erythrocytes of early-stage Parkinson disease patients, Neurosci. Lett. 465 (2009) 1–5.
[105] R.C. Seet, C.Y. Lee, B.P. Chan, V.K. Sharma, H.L. Teoh, N. Venketasubramanian, E.C. Lim, W.L. Chong, W.F. Looi, S.H. Huang, B.K. Ong, B. Halliwell, Oxidative damage in ischemic stroke revealed using multiple biomarkers, Stroke 42 (2011) 2326–2329.
[106] L. Xu, Z. Korade, D.A. Rosado Jr., W. Liu, C.R. Lamberson, N.A. Porter, An oxysterol biomarker for 7-dehydrocholesterol oxidation in cell/mouse models for Smith–Lemli–Opitz syndrome, J. Lipid Res. 52 (2011) 1222–1233.
[107] K. Nakagawa, D. Ibusuki, Y. Suzuki, S. Yamashita, O. Higuchi, S. Oikawa, T. Miyazawa, Ion-trap tandem mass spectrometric analysis of squalene monohydroperoxide isomers in sunlight-exposed human skin, J. Lipid Res. 48 (2007) 2779–2787.
[108] J. Proudfoot, A. Barden, T.A. Mori, V. Burke, K.D. Croft, L.J. Beilin, I.B. Puddey, Measurement of urinary F(2)-isoprostanes as markers of in vivo lipid peroxidation-A comparison of enzyme immunoassay with gas chromatography/mass spectrometry, Anal. Biochem. 272 (1999) 209–215.
[109] Z. Yan, E. Mas, T.A. Mori, K.D. Croft, A.E. Barden, A significant proportion of F2-isoprostanes in human urine are excreted as glucuronide conjugates, Anal. Biochem. 403 (2010) 126–128.