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# Lipid peroxidation products as oxidative stress biomarkers

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**Abstract.** Oxidative stress induced by reactive oxygen and nitrogen species has been implicated in the pathogenesis of various disorders and diseases. Biomarkers are needed for assessment of oxidative stress status *in vivo* and also for health examination, diagnosis at early stage, prognosis, safe and efficient drug development, and evaluation of efficacy of drugs, foods, beverages, and supplements. Lipids are susceptible to oxidation and lipid peroxidation products are potential biomarkers for oxidative stress status *in vivo* and its related diseases. Recently, isoprostane, isoprostaglandin homologues from arachidonic acid, neuroprostanes from docosahexaenoic acid, hydroxyoctadecadienoic acid from linoleic acid, and oxysterols from cholesterol have received much attention as potential biomarkers for oxidative stress status *in vivo*. The physiological levels of these lipid peroxidation products and potential application as biomarkers will be reviewed.

Keywords: Biomarker, lipid peroxidation, oxidative stress, HODE, isoprostane, oxysterol

*Abbreviations:* CEOOH, cholesteryl ester hydroperoxide; COX, cyclooxygenase; GPx, glutathione peroxidase, H(p)ETE, hydro(pero)xyeicosatetraenoic acid; H(p)ODE, hydro(pero)xyoctadecadienoic acid; KCh, ketocholesterol, LOX, lipoxygenase; MDA, malondialdehyde; OHCh, hydroxycholesterol; PCOOH, phosphatidylcholine hydropxeroxide; RNS, reactive nitrogen species; ROS, reactive oxygen species

## 1. Introduction

Biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathologic processes, or pharmacologic responses to a therapeutic intervention [21]. The appropriate biomarkers may be used for health examination, diagnosis of pathologic processes at early stage, assessment of treatment response and prognosis, safe and efficient drug development, and evaluation of efficacy of drugs, foods, beverages, and supplements. Biomarkers are also used for precise measurement of oxidative stress status *in vivo* and non-invasive measurements of circulating levels of specific biomarker may improve risk assessment.

It is widely accepted that oxidative stress is involved in the pathogenesis of various disorders and diseases including age-related chronic diseases such as atherosclerosis, rheumatoid arthritis, ophthalmologic and neurodegenerative diseases. Oxidative stress induces cancer as well. Among the biological molecules, lipids are most susceptible to the attack of reactive oxygen and nitrogen species

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Table 1
Mechanisms of lipid peroxidation and products from linoleates

| Type | Characteristics | Isomers of HPODE | | |
|---|---|---|---|---|
| | | regio | stereo | enatio |
| 1. Enzymatic (15-LOX) | specific catalytic | 13 | *cis, trans* | $S$ |
| 2. Non-enzymatic | | | | |
| a) Free radical chain oxidation ($LO_2\cdot$) | random chain reaction | 9,13 | *cis, trans* *trans, trans* 9–*ct* = 13–*ct* 9–*tt* = 13–*tt* | $R = S$ (*racemic*) |
| b) Non-radical ($^1O_2$) | random stoichiometric | 9,10,12,13 | *cis,trans* | |

(ROS/RNS) [40]. It has been shown that lipid peroxidation induces alterations in the properties of the biological membranes such as disturbance of fine structure, functional loss and permeability of biomembranes. It also generates potentially toxic products which are chemically reactive and covalently modify critical macromolecules such as proteins [57], DNA bases [30,38], and low density lipoprotein (LDL) to proatherogenic forms [54]. Lipid peroxidation has been implicated also in the neurodegeneration [33]. The levels of lipid peroxidation products may be used as a biomarker for the measurement of oxidative stress status *in vivo*.

Proteins are also the direct target for ROS/RNS because of their high concentrations. Their oxidation may result in deamination, decarboxylation, aromatic ring modification, peptide backbone cleavage, cross-linking, and modification of side chain, leading eventually to inactivation of enzyme activity and accumulation within cells and extracellular environment [9]. These protein metabolites are also potential biomarkers. DNA bases are also oxidized to lead strand breaks, crosslinking, sugar damage and to give many kinds of base modification products [10], of which 8-oxoguanine is one of the most well known biomarkers of oxidative stress [19]. Furthermore, antioxidant levels, the ratio of their reduced forms to oxidized forms, oxidized antioxidants, and antioxidant enzymes may also be used as biomarker for oxidative stress status [23].

## 2. Lipid peroxidation

Phospholipids, cholesterol, cholesterol esters and triglycerides are major lipids *in vivo*, and both fatty acid moieties and cholesterol ring and side chain are oxidized by three different mechanisms: free radical-mediated chain oxidation, non-free radical, non-enzymatic oxidation, and enzymatic oxidation [40]. Each type of oxidation gives specific products and requires specific antioxidant to be inhibited. The characteristics of each type of oxidation and products are summarized in Table 1, taking linoleate as an example of substrate.

Lipoxygenases (LOX), cyclooxygenases (COX), and cytochrome c are important enzymes which oxidize fatty acids *in vivo*. Both free fatty acids and their esters in the membranes and lipoproteins are substrates of these enzymes. Lipoxygenase oxidizes arachidonic acid to give 5-, 8-, 12-, and 15-hydroperoxyeicosatetraenoic acid (HpETE), while cyclooxygenase metabolizes arachidonic acid to prostaglandins, prostacyclin, thromboxane, and leukotrienes. Further, arachidonic acid is metabolized via cytochrome P-450 enzymes to many positional isomers of hydroxyeicosatetraenoic acid (HETE), epoxyeicosatrienoic acids and dihydroxyeicostetraenoic acids. The oxidation by lipoxygenase proceeds

Table 2
Enzymes involved in cholesterol oxidation

| Enzymes | Oxysterol |
|---|---|
| CYP3A4 | 4*beta*-OHCh |
| 11*beta*-hydroxysteroid dehydrogenase type 1 | 7-KCh, 7-*beta*-OHCh |
| 7 *alpha*-hydroxylase (CYP7A1) | 7*alpha*-OHCh |
| 24-hydroxylase (CYP46A1) | 24S-OHCh |
| 25-hydroxylase | 25-OHCh |
| 27-hydroxylase | (CYP27) 27-OHCh |

by regio-, stereo-, and enantio-specific mechanism [52]. For example, the oxidation of linoleic acid by 15-lipoxygenase (15-LOX) gives 13(S)-9-*cis*,11-*trans*-hydroperoxylinoleic acid (HPODE) specifically, while free radical mediated-oxidation of linoleic acid gives both 9- and 13-(R,S)-cis,trans- and trans,trans-HPODE, the ratio S/R being 1. Therefore, the ratio of S/R higher than 1 of 13-H(P)ODE suggests the involvement of 15-LOX [14]. It has been found that phosphatidylserine is oxidized selectively via peroxidase activity of mitochondrial cytochrome c, which leads to its externalization and apoptosis [12].

The enzymes involved in the oxidation of cholesterol are summarized in Table 2 [59]. There are several cytochrome P-450 enzymes which oxidize cholesterol specifically. CYP46A1, 24-hydroxylase, is predominantly localized in the brain and 24(S)-hydroxycholesterol, 24(S)-OHCh, may be a biomarker for the oxidation of cholesterol in the brain [3]. 7alpha-OHCh is formed by 7alpha-hydroxylase, CYP7A1, as well as free radical mediated oxidation.

As discussed above, lipids are oxidized by three distinctive mechanisms and the responsible active species may be defined from the specific products formed. The trans,trans-forms of hydroperoxides and hydroxides of polyunsaturated fatty acids are products of free radical-mediated peroixdation. 10- and 12-HODE are specific products from linoleates by singlet oxygen [45]. Nitro-fatty acids have been found in human plasma, erythrocyte, and urine [13,50]. They are formed by the reactions of unsaturated fatty acids with nitrogen dioxide radical, nitronium ion, and/or peroxynitrite. It was reported that nitro-oleic acid were formed in about equal amount with nitro-linoleic acid and that 10- and 12-nitrolinoleic acid, rather than 9- and 13-derivatives, were formed [2]. These patterns are not in agreement with those expected from the relative reactivities of lipids based on thermochemistry. It was reported recently that 9-nitrolinoleic acid was much less stable than 10-nitrolinoleic acid [29], which may explain the above unexpected results.

Myeloperoxidase (MPO) and hypochlorous and hypobromous acids derived therefrom are also important oxidant *in vivo* and their action has been well documented [16,28]. MPO itself may be a biomarker of oxidative stress [35]. The kinetic studies imply that hypochlorite and hypobromide react primarily with proteins, while the direct reactions with lipids and antioxidants are less significant *in vivo* [28]. Nonetheless, MPO is capable of generating free radicals by the reactions with amines, tyrosine, and hydroperoxides, and inducing free radical-mediated chain oxidation of lipids [42,46,53]. Chlorohydrin and bromohydrin are specific products for the oxidation of double bonds, including cholesterol, by hypochlorite and hypobromide, respectively.

## 3. Physiological levels of lipid peroxidation products

The levels of lipid peroxidation products in biological fluids and tissues of human subjects have been measured extensively [11]. Previously, thiobarbituric acid reactive substances (TBARS), ethane and pentane in breath gas, lipid hydroperoxides, and aldehydes such as malonaldehyde (MDA) have been

Table 3
Substrate specificity of glutathione peroxidase GPx for hydroperoxides

| Type | | Substrate specificity | | | | |
|---|---|---|---|---|---|---|
| | | $H_2O_2$ | Fatty acid hpo | phospholipid hpo | CE hpo | cholesterol hpo |
| I | cGPx | +++ | ++ | − | − | − |
| II | GPx-GI | ++ | ++ | + | ? | ? |
| III | pGPx | ++ | ++ | + | − | + |
| IV | PHGPx | + | ++ | +++ | +++ | + |

often measured as a marker of lipid peroxidation *in vivo* [15]. TBARS has been used quite often but it should be bear in mind that it is neither specific nor quantitative, although it may be used to assess relative, semi-quantitative extent of lipid peroxidation under similar conditions. Lipid hydroperoxides are major primary product of lipid peroxidation and they can be measured with high sensitivity by HPLC-chemiluminescence methods [34,61] and HPLC-MS/MS [62]. Miyazawa and his colleagues observed 73.7 nM phosphatidylcholine hydroperoxide (PCOOH) in the plasma of healthy subjects ($n = 18$) [39], while Yamamoto found little PCOOH but low level of cholesteryl ester hydroperoxide (CEOOH) in human plasma. Since cholesteryl ester is oxidized more readily than phosphatidylcholine in lipoproteins and CEOOH is metabolized slower than PCOOH, CEOOH level in plasma is assumed to be higher than that of PCOOH. Care should be taken in the quantification of lipid hydroperoxides by HPLC-chemiluminescence method, since the sensitivity depends very much on the type of hydroperoxides and analytical conditions including apparatus set up [41]. Furthermore, they are substrate of enzymes such as glutathione peroxidases (GPx) and selenoprotein P and may undergo secondary reactions. There are four types of GPx which reduce hydroperoxides to the corresponding hydroxides with different substrate specificity as shown in Table 3 [56,58]. Therefore, the level of lipid hydroperoxides measured does not show the level of lipid peroxidation. Aldehydes, especially alpha, beta-unsaturated aldehydes, are not stable end products: they may be oxidized further or react readily with proteins and DNA bases [30,38, 57]. Aldehyde-modified proteins have been used frequently in the immunohistochemical analyses.

Recent advances of mass spectrometry enabled precise and accurate identification and quantification of lipid peroxidation products. Isoprostanes were found to be formed non-enzymatically by free radical-mediated oxidation of arachidonic acid, labile prostaglandin H2-like bicyclic endoperoxides being the intermediates [32]. Since its discovery in 1990 by Morrow and Roberts [37], numerous studies have been carried out and it is now accepted that isoprostanes are the most reliable biomarker of lipid peroxidation *in vivo*. More recently, neuroprostanes produced by the oxidation of DHA are measured also as a biomarker of oxidative stress *in vivo* [49].

Furthermore, hydroxyoctadecadienoic acid (HODE) produced from linoleic acid has been used also as a biomarker for lipid peroxidation *in vivo* [64]. Linoleic acid is the most abundant polyunsaturated fatty acids *in vivo* and its oxidation proceeds by much simpler mechanisms to give less complicated products than arachidonates.

The free radical-mediated oxidation of cholesterol gives 7-alpha- and 7-beta-hydro(pero)xycholesterol (7-O(O)HCh), 7-keto-cholesterol (7-KCh), 5-alpha, 6-alpha- and 5-beta-, 6-beta-epoxycholesterol (5,6-epoxyCh), and trihydroxycholesterol as major products [4,5]. 7-OOHCh is reduced by GPx to 7-OHCh [25]. Furthermore, these products are often interconverted to other oxysterols [26]. 7-Alpha-OHCh is formed by free radical-mediated oxidation, by 7-alpha-hydroxylase (CYP7A1), and by the isomarization of 5-alpha-OHCh formed in the oxidation by singlet oxygen.

These oxidation products are formed in both free forms and ester forms. The saponification will convert ester forms to free forms. The reduction of biological samples with sodium borohydride, followed by

Table 4
Total linoleate (t18:2) and cholesterol (tCh) and their oxidation products hydroxyoctadecadienoic acid (tHODE) and 7-hydroxycholesterol (7-OHCh) measured in human plasma and erythrocytes ($n = 44$)

| | tHODE | t18:2 | tHODE t18:2 | t7-OHCh | tCh | t7-OHCh tCh |
|---|---|---|---|---|---|---|
| | nM | mM | $\mu$mol mol | nM | mM | $\mu$mol mol |
| plasma | 203 | 1.28 | 194 | 243 | 6.87 | 40.9 |
| erythrocytes | 1917 | 0.66 | 3519 | 5226 | 11.0 | 686 |

saponification by potassium hydroxide will convert hydroperoixdes and ketones as well as hydroxides of free and ester forms of lipids to free fatty acid hydroxides and free hydroxycholesterol [64].

The results of analyses of 44 healthy subjects are summarized in Table 4 [66]. Both plasma and erythrocyte were treated with sodium borohydride and potassium hydroxide. The ratio of oxidation products to parent lipids is included in Table 4. The data show that the lipids in erythrocytes are relatively more oxidized than plasma lipids. The relative extent of oxidation of linoleate to cholesterol was 4.7 and 5.1 in plasma and erythrocyte respectively. It should be noted that the levels observed are determined by the rates of metabolism and excretion as well as that of formation. The levels of hydroxyeicosatetraenoic acid (HETE), isoprostane, and oxysterols in human plasma have been also reported recently [27].

The oxidative modification of LDL is accepted as an important initial event in the progression of atherosclerosis [55]. Oxidatively modified LDL is not recognized by LDL receptors but instead it is taken up by many types of scavenger receptors expressed on macrophage in artery wall, resulting in accumulation of cholesterol esters and generation of foam cells. Oxidized LDL is pro-inflammatory and immunogenic. Exposure of oxidized LDL to endothelial cells leads to induction of adhesion molecules and reduction of nitric oxide production, a key mediator of blood vessel relaxation. Many reported data show the correlations between the circulating levels of oxidized LDL and endothelial dysfunction, plaque progression, and clinical cardiovascular disease [47]. Thus, it is important to measure the levels of oxidized LDL in humans, and antibodies against oxidized LDL have been developed [7,17]. The electronegativity of LDL particle surface increases with increasing oxidation. Kitano el al. separated human LDL with anion exchange HPLC into three fractions, each fraction of which was analyzed for lipid peroxidation products [24]. The results included in Table 5 show that with increasing electronegativity of LDL particle, the relative oxidation level increased as well, the extent of LDL fraction 3 being about 1 order higher than fraction 1 and whole plasma. It should be noted that $F_2$-isoprostane shown in Table 5 includes $D_2$ and $E_2$-isoprostanes, since the samples were treated with sodium borohydride which reduces $D_2$- and $E_2$-isoprosatnes to $F_2$-isoprostane. It was reported previously that the ratios of isoprostane/arachidonic acid and HODE/linoleic acid in atherosclerotic lesions of human artery wall were 13.6 umol/mol and 4.7 mmol/mol, while those in non-atherosclerotic veins were 3.4 umol/mol and below detection limit, respectively [14].

In contrast to polyunsaturated fatty acids, cholesterol does not contain bis-allylic hydrogen. When plasma was oxidized under the constant flux of free radicals, polyunsaturated fatty acids were oxidized preferentially, and the oxidation of cholesterol proceeded after polyunsaturated fatty acids were depleted [43]. However, as shown in Table 4, the level of 7-OHCh measured in plasma and erythrocytes were quite high, the relative extent of oxidation of cholesterol being only 1/5 of linoleates. It was found that the amounts of free and ester forms of hydroxycholesterol were similar in plasma. As shown in Table 6, it was found that human atherosclerotic plaque contained high oxysterols [18]. It is not clear at present

Table 5
Lipid peroxidation levels in human LDL fractions 1, 2, and 3

| products/parent | LDL fraction | | |
|---|---|---|---|
| lipid (umol/mol) | 1 | 2 | 3 |
| tHODE/t18:2 | 227 | 782 | 3670 |
| t8$F_2$-isoP/t20:4 | 8.7 | 18.3 | 66.6 |
| t7-OHCh/tCh | 116 | 181 | 344 |

Table 6
Oxysterols in human normal artery, fatty streak, and advanced athersoclerotic lesion

| | normal artery | fatty streak | advanced lesion |
|---|---|---|---|
| Cholesterol (mg/g wet wt) | 3.71 | 25.74 | 38.57 |
| Oxysterols (mg/g Ch) | | | |
| 7-alpha-OH | 0.36 | 2.8 | 3.7 |
| 7-beta-OH | 0.16 | 1.2 | 1.6 |
| alpha-epoxy | 0.28 | 1.6 | 1.7 |
| beta-epoxy | 0.16 | 0.97 | 1.0 |
| 7-K | 0.11 | 2.2 | 2.8 |
| 27-OH | 0.32 | 4.3 | 7.5 |

why the level of oxysterols was higher than expected from the results of *in vitro* experiments. It may be because the enzymatic oxidation of cholesterol is significant and/or the metabolism and excretion of oxysterols are slower than those of fatty acid oxidation products.

Obviously, it is difficult to know where the lipid peroxidaton products measured in blood are formed *in vivo*. It is known that the composition of fatty acids varies depending on the tissues. For example, brain and eye contain more highly unsaturated fatty acids such as arachidonic acid, EPA and DHA than other tissues. Thus, the ratio of isoprostanes/HODE may imply the tissues where the lipid peroxidaiton takes place. Further, it is difficult to know the local concentrations of lipid peroxidation products at the microenvironment of cells and tissues.

## 4. Isoprostanes, HODE and OHCh as biomarker

Various lipid peroxidation products have been applied for assessment of lipid peroxidation and oxidative stress status *in vivo* as summarized in Table 7. In addition to these products, proteins and DNA bases modified by the lipid peroxidatoin products have also been used as biomarkers. Above all, the adducts of lysine residues and unsaturated aldehydes such as HNE and acrolein have been used most frequently [57]. The adduct of lysine with lipid hydroperoxide has also been identified [20] and found in human atherosclerotic lesions [22]. It was reported that the urinary metabolite of 4-HNE with GSH, 1,4-dihydroxynonene mercapturic acid, might be used as a biomarker of lipid peroxidation [1,48]. As mentioned above, lipid peroxidation products also react with and modify DNA bases [30,38].

Many studies have explored the correlation between the level of lipid peroxidaiton products and disease state and their application as biomarkers for early detection and prognosis of diseases [8,31]. Above all, isoprostanes have been most extensively studied by many groups. In some cases, but not always, a strong correlation was observed. Lipid peroxidation products have advantages and disadvantages as biomarker. Compared with proteins, a high specificity may not be expected for lipid peroxidation products. In other words, the increase in lipid peroxidation products levels suggests the elevation of oxidative stress status,

Table 7
Biomarkers of lipid peroxidation

| |
|---|
| I. Oxidized free and ester forms of fatty acids |
| Hydroperoxides |
| Hydroxides, HODE |
| Isoprostanes, Isofurans, Neuroprostanes |
| Cyclopentenone: 15-deoxy-prostaglandin J2 (15d-PGJ2) |
| Epoxide |
| Chlorohydrin |
| Conjugated diene |
| Breath hydrocarobons, ethane, pentane |
| II. Aldehydes |
| alpha,beta-unsaturated aldehydes |
| Acrolein, croton aldehyde, 2-hexenal, 2-nonenal, 4-hydroxy-2-hexenal, 2-hydroxy-2-nonenal (HNE) |
| Ketoaldehydes and dialdehydes |
| Glyoxal, methylglyoxal, malondialdehyde (MDA) |
| TBARS (thiobarbituric acid reactive substances) |
| III. Oxysterols |
| 5-OOHCh, 7-OOHCh, 7-OHCh, 7-KCh, 5,6-epoxyCh, triol, |
| Chlorohydrin |
| IV. Others |
| lysoPC |
| oxidized LDL |

but it is difficult to specify disease or tissue. On the contrary, lipid peroxidation products may be useful as an indicator of overall oxidative stress status and healthy state.

Arachidonic acid is more readily oxidized than linoleic acid and its oxidation mechanisms are well understood. Isoprostanes are unique product from the free radical-mediated oxidation of arachidonic acid. One disadvantage for isoprostanes may be that the oxidation of arachidonic acid proceeds by many competing reactions to give numerous products and that F2-isoprostanes are composed of 64 isomers, which make the selectivity of formation and concentration of isoprostane isomers very low. The amount of 8-isoprostane $F_2$ alpha is one or two orders less than HODE. On the other hand, linoleic acid is the most abundant polyunsaturated fatty acid *in vivo* and it is oxidized by a straightforward mechanism to give only four isomeric HPODE as primary product almost quantitatively. Thus, the level of HODE after reduction and saponification is higher than any other lipid peroxidation products. Another advantage for HODE is that the antioxidant capacity of the environment can be assessed from the ratio of cis,trans-HODE/trans,trans-HODE. The higher the antioxidant capacity, the larger this ratio. Therefore, the antioxidant capacity of pure compounds and also complex mixtures in foods, beverages, and supplements *in vivo* may be assessed from this ratio [65].

In general, higher levels of lipid peroxidation products are observed in the patients of various diseases than healthy subjects. Similarly, high lipid peroxidation products levels have been observed in neurological disease patients [33,36,51]. These results do not indicate that lipid peroxidation is a cause of diseases, but a positive correlation implies that these levels may be used as a biomarker, at least as surrogate biomarker for the effect of medical treatment and the efficacy of diets and drugs.

Ideally, the biomarkers and their assay should be simple, specific, accurate, sensitive, reproducible, non-invasive, high-throughput, and inexpensive. There are no such biomarkers that satisfy all of these factors yet. Various specimens have been used including plasma, serum, erythrocyte, lymphocyte, lipoprotein, tissues, cells, cerebrospinal fluid, urine, saliva, sperm, tear, breath gas, and various lavages. Non-invasive samples are recommended, but it is difficult to show where the phenomenon occurs. The standardization of specimens, such as collection, storage, processing, and assay conditions is also important.

It may also be noted that lipid peroxidation products can be absorbed from the diet. Foods contain more or less oxidized lipids, and the measurements of lipid peroxidation products in the biological fluids may be confounded by the diet. It has been reported that dietary hydroxy fatty acids labeled with $^{13}C$ are absorbed and found in plasma [36]. It is not clear how other lipid peroxidatoin products such as isoprostanes and oxysterols are absorbed, circulated, and excreted in urine. It is important to collect samples from the fasted subjects. The potential for artifacts produced during sample collection, processing, storage, and instrumental analyses should be always considered.

Overall, many data show the positive association of biomarkers of lipid peroxidation with oxidative stress status and related various diseases, although inconsistencies between various studies exist. The association of isoprostanes with various diseases has been summarized by Yin [63] in this issue. At the same time, it may be noteworthy that recently it has been found that low levels of oxidative stress and lipid peroxidation products may act as *"eustress"* by inducing adaptive response and elevating defense capacity through up-regulation of glutathione and phase II antioxidant enzymes [6]. It was found also that gamma-tocopheryl quinone, an antioxidant metabolite of vitamin E, may exert either cytotoxic or cytoprotective effect depending on the conditions [44]. The biological effects of lipid peroxidation are quite complicated. Recent studies reveal that lipid peroxidation products may be cytotoxic and cytoprotective, and pro-infllammatory and anti-inflammatory. Such an opposite effects have been observed for ROS, RNS, LOX, antioxidants as well as lipid peroxidation products. Recently, the physiological role of lipid peroxidation products has received much attention. However, it should be pointed out also that, although some lipid peroxidation may be controlled, it is difficult to control and program the time, site, and amount of many lipid peroxidation reactions, especially free radical-mediated peroxidation. This may suggest the inherent limitation of lipid peroxidation products as physiologically important signaling messenger. It is hoped that future studies will elucidate the biological effects of lipid peroxidation and how lipid peroxidation products may be used as biomarkers for diagnosis, prognosis, and assessment of efficacy of drugs, foods, and supplements.

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