Review

# Investigating oxidized lipids in an omics way: Oxidative lipidomics in biological applications using mass spectrometry

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**Abstract** Oxidized lipids have been well known as potential markers of oxidative stress or other disorders in physiological/pathological processes, such as cardiovascular diseases, neurodegenerative diseases, and even some cancers. Lipidomic analysis is a modern and advanced approach to conducting informative elucidation for lipid molecules, which benefits biomarker hunting, clinical determination, and biological pathway exploration. In recent years, as mass spectrometry technology advances, lipidomics is extending the investigating range towards oxidized lipids, which is called oxidative lipidomics. This approach combines the complexity of the lipidome and focuses attention on oxidation. Because oxidized lipids are usually at low levels and artificial oxidation is undesired, oxidative lipidomics is more challenging than ordinary lipidomics, particularly in sample preparation, standards, and spectrum analysis. Nowadays, oxidative lipidomic analysis has been applied to biomedical science for various diseases in clinics and animal experiments on disease models. This review aims at the strategies and methods of oxidative lipidomics and the summary of current applications. The development of chromatographic-MS spectrometric technology is hopeful to allow a deeper and broader understanding of lipids and oxidation concerning human health.

**Key words:** oxidative stress, lipid metabolism, lipid oxidation, hydroperoxide, LC-MS/MS

## 1. Introduction

Lipids are a large class of compounds that are easily soluble in organic solvents and are heterogeneous in chemical composition and structure, mainly including fatty acids and their naturally occurring derivatives as well as their biosynthetic and functionally related compounds[1]. Lipids, known as primary structural elements, represent a pretty varied group of cellular components of biological membranes and are involved in intercellular signaling and energy storage[2,3]. Studies have shown that mammalian cells contain thousands of kinds of lipids. Moreover, with the continuous development of new technologies and methods, various new lipid molecules are still being discovered. According to the comprehensive classification system for lipids proposed by the Lipid Metabolites And Pathways Strategy (LIPID MAPS), lipids are generally divided into eight categories: fatty acyls (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), prenol lipids (PR), saccharolipids (SL), and polyketides (PK)[4], as shown in Fig. 1.

The diversity of lipid structures endows lipids with a variety of critical biological functions. Lipid metabolism participates in regulating various life activities, such as producing energy, transporting materials, recognizing information, transmitting messages, being involved in cell development, differentiation, apoptosis, and others. At the same time, abnormal changes in lipid metabolism lead to influ-

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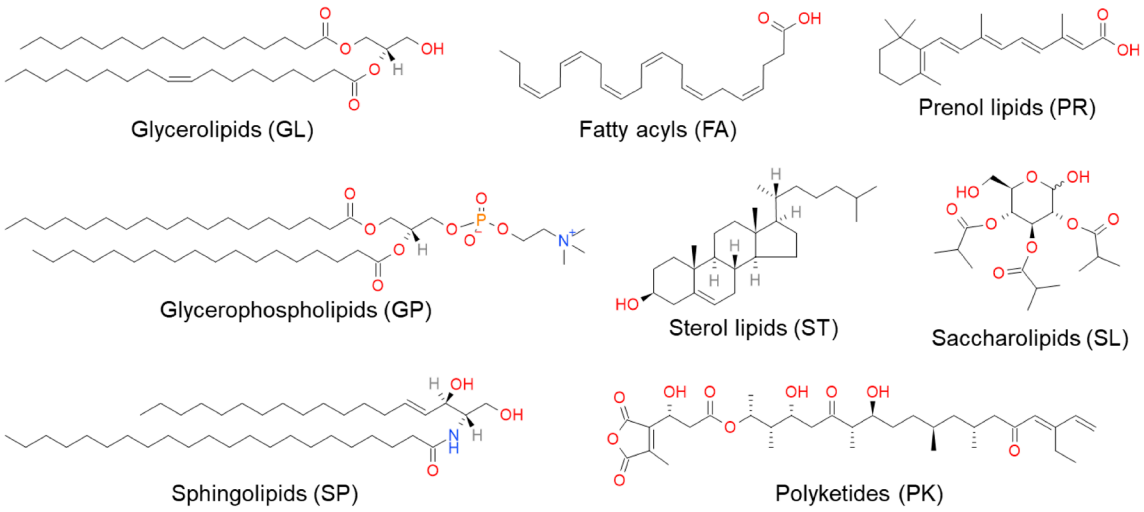



**Fig. 1. Lipid classification systems and the representative lipid molecules in each class. Fatty acyls (FA): 4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid (DHA, C22:6n-3,6,9,12,15,18); glycerolipids (GL): 1-hexadecanoyl-2-(9*Z*-octadecenoyl)-*sn*-glycerol [DG(16:0/18:1(9Z)/0:0)]; glycerophospholipids (GP): 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine [PC(18:0/18:0)]; sphingolipids (SP): N-(docosanoyl)-sphing-4-enine [Cer(d18:1/22:0)]; sterol lipids (ST): cholest-5-en-3*β*-ol (cholesterol); prenol lipids (PR): 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2*E*,4*E*,6*E*,8*E*-tetraenoic acid (retinoic acid); saccharolipids (SL): 2,3,4-*O*-triisobutyryl d-glucose (pennelliiside A); polyketides (PK): tautomycetin.**

ences on cellular homeostasis, contributing to severe illnesses covering from cardiovascular diseases (e.g., coronary heart disease, hypertension, hyperlipidemia, arteriosclerosis)[5,6] to neurodegenerative diseases (e.g., Alzheimer's disease and Parkinson's disease)[7,8], and even some cancers[9,10]. During such dysregulated processes, specific lipid mediators are generated, which can control different intracellular events.

Among such lipid mediators, one kind of molecule is defined as oxidized lipids. The unsaturated fatty chains are readily attacked by the reactive oxygen species (ROS) to form the oxygen-inserted structures, with which the lipid molecules are classified as oxidized lipids. The oxidized fatty chains can exist as free from, i.e., free fatty acids (FFA), or as esterified form, i.e., esterified fatty acids (EFA), such as glycerolipids, glycerophospholipids, or others. Lipid oxidation proceeds in vivo due to the endogenous or exogenous free radicals. A key initial step of lipid oxidation is the generation of lipid hydroperoxides, which contains an added hydroperoxyl group and the C=C double bond rearrangement, as revealed previously[11]. According to different sources of oxidation, such as radical oxidation (e.g., thermal oxidation, auto-oxidation), enzymatic oxidation (e.g., lipoxygenase), and singlet oxygen oxidation (e.g., photo-oxidation, inflammation), the formed hydroperoxides (−OOH) present varied and specific characteristics[12], as shown in **Fig. 2**. The continued chain reactions resulted in various oxidation products, such as hydroxides (−OH), aldehydes (−CHO), ketones (=O), epoxides (*–epo*), degraded small fragments, and others. These oxidation products are known to cause biological membrane disruption, DNA damage, enzyme inactivation, and many other abnormal functions, and they have even been proposed to be clinical biomarkers of some oxidative stress-related diseases[13]. Therefore, it is of great importance to pay attention to such special lipid molecules.

Lipidomics, along with genomics, transcriptomics, proteomics, and metabolomics, have been recognized as a holistic approach to simultaneously measuring multiple (as many as hundreds or thousands) targets, which construct a global view of the status of the study objects[14]. Technically, lipidomics is performed using liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS), nuclear magnetic resonance (NMR), or others[15]. From the acquired massive data covering structure identification to quantitative/semi-quantitative determination, researchers can obtain comprehensive and systematic characteristics of the lipids, understand their composition and function, and reveal the interactions between the physiological/pathological changes and lipid metabolism. As lipidomics represents the downstream products and tells the researchers "what indeed happened", it has been widely applied in the whole organisms, organs or tissues, cells, and even organelles, to contribute to molecular physiology/

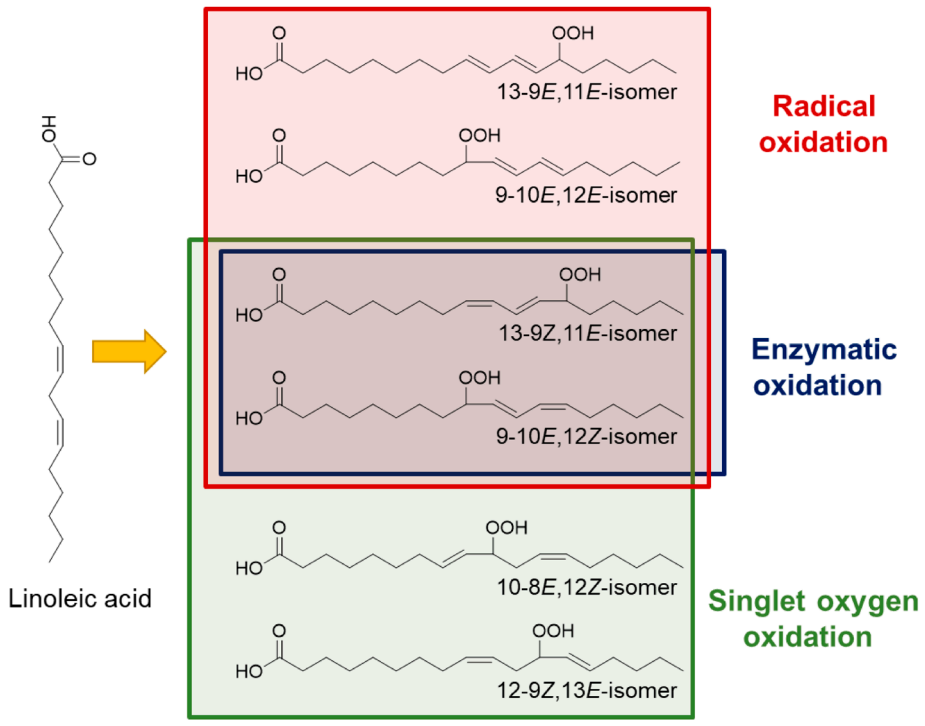



**Fig. 2. Structure variety of linoleic acid hydroperoxide isomers, namely 13−9*E*,11*E*-HPODE, 9−10*E*,12*E*-HPODE, 13−9*Z*,11*E*-HPODE, 9−10*E*,12*Z*-HPODE, 10−8*E*,12*Z*-HPODE, and 12−9*Z*,13*E*-HPODE.**

pathology discovery, nutritional and environmental management, drug development, and other fields. In particular, as one of the recently proposed strategies, oxidative lipidomics focuses on the oxidized lipids and their oxidation/degradation products, connects lipids and oxidation, and enhances the understanding of the role of oxidized lipids in cellular homeostasis and disease pathogenesis[16].

In the following sections, we will review the recent research progress in oxidative lipidomics in biological applications, mainly including these parts: (1) the methods, strategies, and workflow of conducting oxidative lipidomics; (2) the applied samples and clinical diseases (or disease models), and the uncovered findings associated with human health issues.

## 2. Strategies and Methods of Conducting Oxidative Lipidomics

### *2.1. Traditional approaches to oxidized lipid analysis*

Because the lipid oxidation reactions are consecutive and the oxidized lipids are at a much lower level than the intact lipids, in general, analysis of oxidized lipids is relatively difficult. Traditionally, analysis of oxidized lipids was based on indirect investigation of the lipid oxidation end products, and those small molecules can be measured using colorimetric assays, immunoassays, or electron-spin-resonance[16]. Those methods include thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) test[17−21], ferrous oxidation in xylenol orange (FOX) test[22,23], conjugated diene test[24], and others. But the obtained results are usually considered as the indicator of the lipid oxidation degree, but not the detailed lipid changes.

Besides, it is also needed to decide whether the targets are the total amount of certain lipid classes or individual species within single or multiple classes before analyzing. It is comparably easy to measure the total amount of certain classes, e.g., total aldehydes. While in terms of individual species, previous separation and fractionation are commonly needed, increasing labor work and increasing the risk of artificial lipid oxidation. Moreover, more fractions may lead to more complicated results.

For example, typically, after lipid extraction, the intact lipids and the oxidized lipids are yielded as a mixture. Next, the mixture is purified predominantly on column chromatography with silica gel, alumina, or other sorbents. Consequently, the unoxidized lipids with lower polarity are eluted using the nonpolar solvent, while the oxidized lipids are collected with a stronger elution (with more polar solvents). One risk is that, for some highly polar (or even polymeric) oxidation products, the final collection may be insufficient for removing them from the column, so the recovery

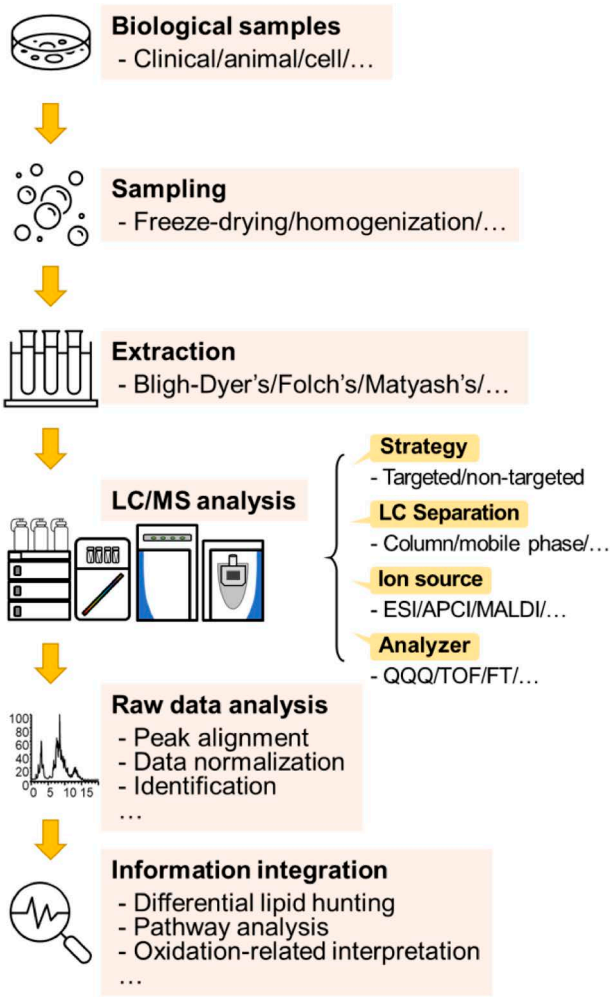
of oxidized lipids is possible to be unsatisfactory.

Therefore, it is less difficult to directly analyze the samples as the whole mixtures than to perform extra isolation and purification for the oxidized lipid fractions from the complex substances, i.e., the sample matrix. Therefore, methods with high selectivity, high sensitivity, and high specificity are desired for achieving this aim.

### *2.2. MS-based detection of the oxidized lipids*

While other analytical methods require selection and adaption for measuring lipid oxidation products, MS technology overcomes the problems of sensitivity, specificity, accuracy, and dynamic range to a great extent and enables high throughput analysis[25]. Since lipid oxidation products are always generated as a convoluted mixture with largely varying levels and structural diversities, MS-based detection of the oxidized lipids is considered to be a promising approach[26]. While the combination of chromatography and MS have been applied in biology and clinical samples as essential platforms in analyzing oxidized lipids, such as GC/MS, LC/MS, or even supercritical fluid chromatography (SFC)/MS, and nowadays, LC/MS technique (particularly LC coupled to tandem MS, i.e., LC/MS/MS) is the dominant approach for oxidative lipidomic applications. Structurally, oxidized lipids refer to the extra oxygen atoms attached to the lipid molecules, including the insertion of the peroxyl/hydroperoxyl/epoxyl group and the deletion of the C=C double bond[27]. Therefore, using MS (especially high-resolution MS), the lipid molecules with oxidized functional groups can be captured, and using MS/MS, the structure confirmation can be conducted, including the confirmation of oxygen existence, the determination of oxidizing position, and the discovery of specific fragmentation from precursor ions to product ions.

In general, there are two strategies to analyze oxidized lipids: the targeted analysis and the non-targeted analysis[28]. For the known oxidized lipids (especially when they are also interesting to investigate), the targeted strategy is usually conducted, such as the oxidized free fatty acids (oxFFA), oxidized cholesterol or other oxysterols, oxidized cholesteryl esters (oxCE), and oxidized phospholipids (oxPL)[26]. While for simultaneously viewing "every possibly detectable lipid species within multiple classes", the non-targeted strategy is preferable. Most of the studies on oxidized fatty acids and oxidized carnitines are conducted by targeted analysis, while oxidative lipidomic studies focusing on more classes belong to the non-targeted analysis.




**Scheme 1. Workflow of oxidative lipidomics in biological applications.**

### *2.3. Workflow of MS-based oxidative lipidomics*

As the same as ordinary lipidomics, oxidative lipidomics (typically using LC/MS) also follows the steps below (Scheme 1). Sampling: collection of clinical, animal, plant, cell, or other samples, as well as necessary and appropriate pretreatments; Extraction: commonly being accorded to Bligh-Dyer, Folch, Matyash, or others, with monophase, biphase, or triphase extraction[29–32]; LC/MS analysis: injecting the carefully prepared lipid samples into the instruments using optimized conditions, which can be either targeted or non-targeted according to experiment design; Raw data analysis & Information integration: interpreting the data of oxidized lipids, digging the deep meaning, and connecting to the aim(s) of the research. It should be paid extreme attention to that, compared with ordinary lipids, the oxidized lipids, usually at a very low level, are more

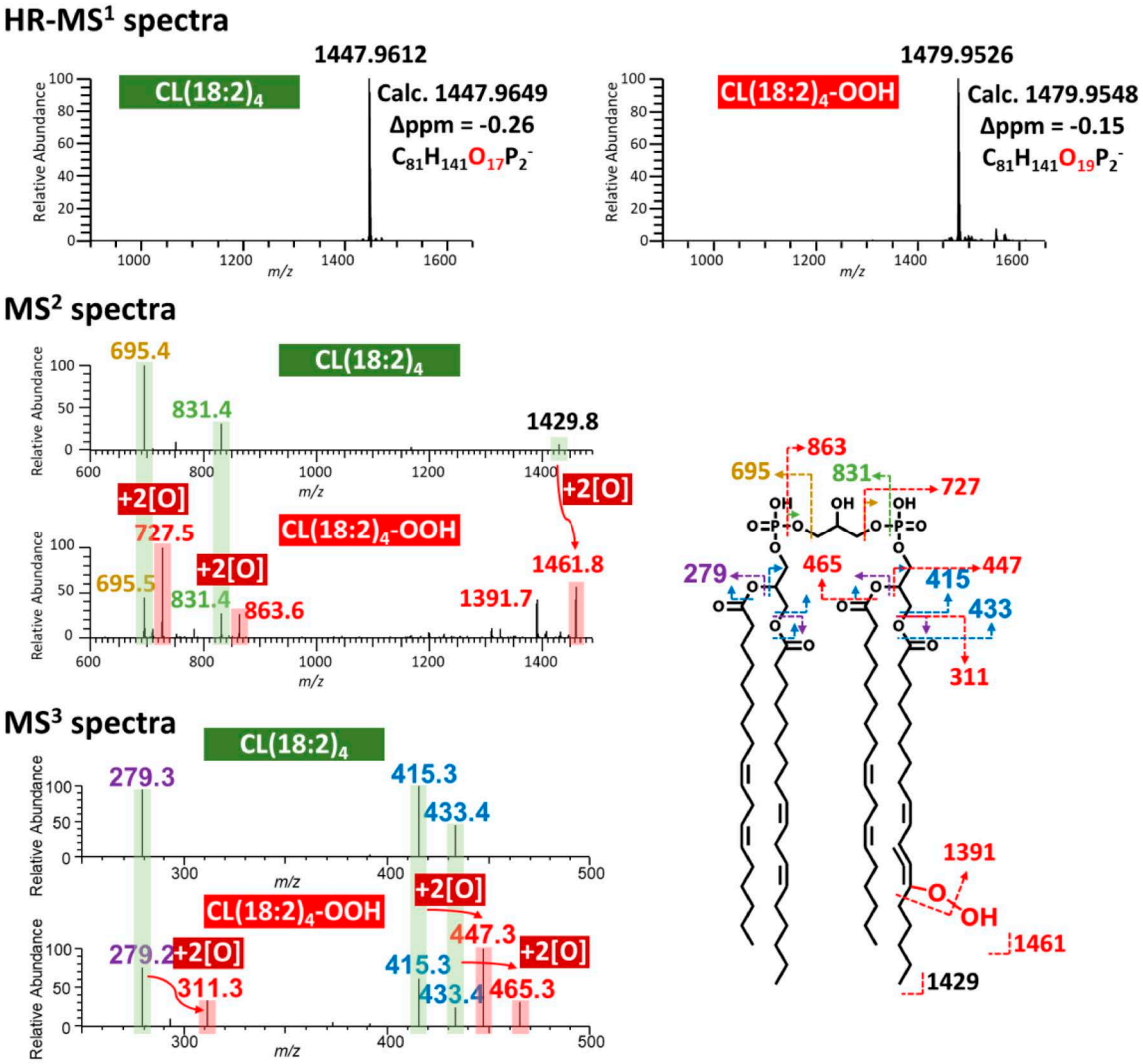



**Fig. 3. Characterized MS signals of $CL(18:2)_4$-OOH compared with $CL(18:2)_4$.**

unstable. Therefore, from sampling, all the operations should avoid auto-oxidation and degradation. After collection from the living organism, the samples should be extracted immediately or be quickly frozen using dry ice or liquid nitrogen and kept in an atmosphere of noble gas in glass containers; homogenization and vortex with ice-cold solvents at the lowest temperature whenever practicable; addition of antioxidants for protection, typically butylated hydroxytoluene (BHT). Not only air/oxygen but also proteins should be noticed: on the one hand, the hydroperoxide groups of the oxidized lipids can react with certain proteins to form covalent bonds, causing false-negative results; on the other hand, the oxygenated fatty acids can be artefactually produced by lipoxygenases[33], therefore causing false-positive results. Sometimes the purification of the samples is needed using solid-phase extraction (SPE), which should also be carefully operated. Besides, for the studies focusing on the small molecules as the oxidation/degradation products of lipids, as those compounds are not as non-volatile as lipids, long-time evaporation at high temperature and low pressure may cause the target loss.

With appropriate sampling and pretreatment, the injected samples were subjected to MS detection, in which the oxidized lipids are theoretically ionized and fragmented. The resulted MS and MS/MS signals provided structural information for identification. Taking $CL(18:2)_4$-OOH as an example (Fig. 3), in our experiment, HR-MS showed that the $[M-H]^-$ of oxidized $CL(18:2)_4$ (*m/z* 1479.9526) was 31.9914 Da more than the intact $CL(18:2)_4$ (*m/z* 1447.9612), suggesting the elemental composition

$C_{81}H_{141}O_{19}P_2^-$, which can be assigned as $CL(18:2)_4$+2[O]. The further $MS^2$ spectrum of the oxidized $CL(18:2)_4$ gives not only *m/z* 695 and *m/z* 831 (assigned as $[PA36:4-H]^-$ and $[M-PA36:4-H]^-$, respectively), but also *m/z* 727 and *m/z* 863 as the $[PA36:4-H+2O]^-$ and $[M-PA36:4-H+2O]^-$, respectively. Moreover, *m/z* 1391 suggested the position of hydroperoxidation at the C-13 position, which was a characteristic fragmentation pattern of hydroperoxide, as reported previously[34,35]. Therefore, by HR-MS and MS/MS, we can clarify the structure of the oxidized lipid molecular species, which is critical for understanding the characteristics and building the in-house library. Nevertheless, a better way to elucidate the structure of the oxidized lipids, especially distinguishing hydroperoxy, (di)hydroxy, and (hydroxy)epoxy compounds, is to compare their fragments using authentic standards. But in most cases, unfortunately, such lipid standards are not available. Therefore, it is of great importance to set up the fragmentation database and to recognize the MS/MS pattern.

Such identification and the subsequent quantitation (or semi-quantitation) can be executed manually or using automated software. Besides the workstation software provided by the manufacturers (e.g., Xcalibur by Thermofisher Scientific, Inc.), there are increasing data process platforms to conduct chromatographic peak alignment, deconvolution, peak identification from the commercial solutions (e.g., LipidSearch, Progenesis QI) to the open-source programs (MZmine, LipidFinder). Typically, the open-source software MS-DIAL developed by RIKEN enables the data-independent MS/MS deconvolution for comprehensive lipidomic analysis[36]. It should be noted that the users usually wish to build their own in-house libraries to get the optimal matches of spectrum comparison. Finally, the obtained information on oxidative lipids is required to be integrated with a biological system or disease mechanism, just as the intact lipidomics.

## 3. The Applied Samples and Clinical Diseases (or Disease Models)

Lipids have been reported to be involved in a variety of physiological and pathological procedures. As the analytical techniques develop, this field is rapidly growing, and researchers are expanding the coverage of the lipidome from the intact lipids to the oxidized lipids. Since there have been many reports on the observation of oxygenated lipid molecules, which were inherently linked to oxidative stress and appeared in a dysregulated state in different diseases, researchers express an increasing interest in these oxidized lipids and consider them "potential biomarkers" for certain diseases. Based on lipid class, oxidized structure, and formation routine, a recent review[28] divided all the oxidized lipids into three classes: (1) lipids containing hydroxy fatty acids, including 2-hydroxy-ceramides, 2-hydroxy-sphingomyelins, 2-hydroxy-cerebrosides, 2-hydroxy-gangliosides, and hydroxized acyl-L-carnitines; (2) lipids containing PUFA peroxidation products, including oxidized phosphatidylcholine (ox-PC), oxidized phosphatidylethanolamine (ox-PE), oxidized phosphatidylserine (ox-PS), oxidized cardiolipin (ox-CL), and ox-CE; (3) oxidized free cholesterol (oxysterols). In this review, we would like to summarize the applications of the oxidative lipidomic approach, focusing on the findings concerning diseases, aging, injuries, and other factors. A collection of representative works related to oxidative lipidomics in biological (clinical, animal, or cell levels) samples is listed in Table 1.

### *3.1. Oxidative stress-related metabolic diseases*

Oxidative stress is one of the main factors involved in the pathogenesis of cardiovascular disease (CVD), which is the leading cause of morbidity and mortality in the world[37]. The overproduced ROS not only breaks the balance between oxidative stress and antioxidant defense but also directly attacks the intact lipid molecules to generate oxidative lipid species.

#### 3.1.1. Alcoholic and non-alcoholic fatty liver diseases

Both alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD) mean the accumulation of fat in the liver, resulting in an enlarged and damaged liver and hepatic steatosis. Unlike AFLD, which is initialized by drinking a large amount of alcohol, NAFLD is caused by a build-up of fat. Currently, NAFLD and non-alcoholic steatohepatitis (NASH) have become common causes of chronic liver disease in the world[38], which have been reported to be associated with oxidative stress[39]. In terms of lipid oxidation, in both human and mouse models with either AFLD or NAFLD, researchers have found oxidized lipid species, which mainly include the addition of carbonyl, hydroxyl, and hydroperoxyl groups to TG, PC, and FFA[40–43].

#### 3.1.2. Diabetes mellitus

Diabetes mellitus includes type 1 diabetes (accounted for approximately 10%) and type 2 diabetes (accounted for

**Table 1. Diseases (and disease models) investigated using the lipidomic approach for oxidized lipid species**

| Entry | Disease/disorders | Biological sample | | Analytical method | | | Oxidized lipids investigated | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | Origin | Specimen | Strategy | Extraction protocol | Platform | | |
| 1 | Alcoholic fatty liver disease | Mouse | Liver | Non-targeted | Bligh-Dyer | LC/MS | TG=O | 40) |
| 2 | Alcoholic liver disease | Human | Plasma | Targeted | N/A | LC/MS | PC-OH, PC-OOH | 41) |
| 3 | Non-alcoholic steatohepatitis | Mouse | Liver, kidney | Non-targeted | Folch | LC/MS | TG-OOH, PC-OOH | 42) |
| 4 | Nonalcoholic fatty liver disease | Human | Liver | Targeted | isopropanol/hexane[43)] | LC/MS | FFA-OH, FFA-OOH | 43) |
| 5 | Type 2 diabetes mellitus | Mouse | Serum, liver, brain, ventricle, atrium, renal medulla, renal cortex, spleen | Non-targeted | Folch, Bligh−Dyer | LC/MS | TG-OOH, PC-OOH, PE-OOH, PI-OOH | 46) |
| 6 | Type 2 diabetes mellitus | Zebrafish | Plasma | Non-targeted | Bligh−Dyer | LC/MS | PC-OOH, PE-OOH, PC-CHO | 47) |
| 7 | Type 2 diabetes mellitus | Mouse | Plasma, urine | Targeted | Methanol | LC/MS | Carnitine-OH | 48) |
| 8 | Type 1 diabetes mellitus | Mouse | Plasma | Targeted | Methanol, with derivatization[83)] | LC/MS | Carnitine-OH | 49) |
| 9 | Obesity | Mouse | Liver | Targeted | Methanol | SFC/MS | PC-OH, PC-OOH, PC-epo | 50) |
| 10 | Obesity | Mouse | Liver, subcutaneous adipose tissue | Targeted | Folch | LC/MS | Cholesterol-OH, cholesterol=O, cholesterol-epo | 51) |
| 11 | Metabolic syndrome (genetically obese spontaneously hypertensive) | Rat | Plasma | Targeted | Methanol, with SPE | LC/MS | FFA-OH, FFA-OOH | 54) |
| 12 | Chronic kidney disease | Human | Plasma | Targeted | Matyash | LC/MS | PC-CHO, PC-COOH | 55) |
| 13 | Immunoglobulin A nephropathy | Human | Serum, urine | Targeted | SPE[74)] | LC/MS | FFA-OH, FFA-OOH, FFA-epo | 56) |
| 14 | Alzheimer's disease | Human | Serum | Targeted | Matyash | LC/MS | PC-CHO | 58) |
| 15 | Alzheimer's disease | Human | Brain | Non-targeted | Ethanol phosphate buffer | MS | SM-OH | 59) |
| 16 | Parkinson's disease | Rat | Substantia nigra, plasma | Non-targeted | Folch, Bligh−Dyer | LC/MS | EFA-OH, EFA-epo, oxCL | 61) |
| 17 | Parkinson's disease | Human | Plasma | Non-targeted | Methanol, with hydrolyzation and derivatization[84)] | GC/MS | FA-OH, cholesterol-OH, cholesterol=O, | 62) |
| 18 | Coronary artery disease | Human | Plasma | Non-targeted | Modified Folch[85)] | LC/MS | OxPA, oxPC, oxPE | 63) |
| 19 | Coronary artery disease | Human | Platelet | Non-targeted | Methanol | LC/MS | PC-CHO, PC-COOH | 64) |
| 20 | Acute coronary syndrome | Human | Plasma | Targeted | Folch | LC/MS | PC-CHO, PC-COOH | 65) |
| 21 | Atherosclerosis | Human | Plasma, atherosclerotic plaques | Targeted | Folch | LC/MS | OxPC | 66) |
| 22 | Peripheral atherosclerotic disease | Human | Arterial intima tissue | Targeted | Bligh-Dyer, with SPE[86)] and derivatization | GC/MS | cholesterol-OH | 67) |
| 23 | Kawasaki disease | Human | Serum | Targeted, non-targeted | N/A | LC/MS | PC-OH, PE-OH, PI-OH | 68) |
| 24 | Non-pancreatic neuroendocrine tumor | Human | Plasma | Non-targeted | Methanol/ethanol | LC/MS | FFA-OH, LPC-OH | 70) |
| | Hepatocellular carcinoma | Human | Liver | Targeted | Modified Bligh-Dyer | LC/MS | CL-OH | 71) |
| 26 | Oncocytic thyroid tumor | Human | Thyroid | Non-targeted | N/A | MS imaging | CL-OH, CL-OOH | 72) |
| 27 | Medium-chain acyl-CoA dehydrogenase deficiency | Human | Blood | Non-targeted | Methanol | LC/MS | PC-COOH | 73) |
| 28 | Septic inflammatory | Mouse | Bronchiolar alveolar lavage fluid, serum | Targeted | N/A | LC/MS | FFA-OH, FFA-epo | 74) |
| 29 | Periodontal disease | Human | Saliva, serum | Targeted | Hexane/methyl *t*-butyl ether | LC/MS | FFA-OH | 75) |
| 30 | Aging and ischemia/reperfusion | Mouse | Heart | Non-targeted | Folch | LC/MS | OxCL | 77) |
| 31 | Transient hepatic ischemia | Rat | Liver | Targeted | Folch | LC/MS | OxCL | 78) |
| 32 | Hyperoxia | Mouse | Lung | Non-targeted | Folch | LC/MS | CL-OH, PS-OH | 79) |
| 33 | Cardiac surgery | Human | Plasma | Targeted | Methanol, with hydrolyzation and SPE | LC/MS | FA-OH, FA-OOH | 80) |
| 34 | Iatrogenic plaque disruption | Human | Blood | Targeted | Folch | LC/MS | PC-CHO, PC-COOH, PC=O, CE-OH | 81) |

N/A: Not available (not mentioned in the literature).

approximately 90%)[44]. Both have been recognized to be related to oxidative stress, especially for diabetic complications[45]. Animal studies on different samples (blood, urine, and multiple organs and tissues) have proved the accumulation of TG-OOH, PC-OOH, PE-OOH, and PI-OOH species by non-targeted approach[46,47] and the elevation of carnitine-OH by targeted approach[48,49]. It is noted that the lipid hydroperoxides might be the potential biomarkers for not only diabetes itself but also diabetic complications.

3.1.3. Obesity

For obesity, mouse model experiments revealed a series of PC oxidation products[50] and various oxidized cholesterol molecules (oxysterols), such as cholesterol-OH, cholesterol=O, and cholesterol-epo[51]. Although oxysterols had been studied as the potential biomarkers of rare genetic diseases, such as Niemann-Pick disease type C[52] and cerebrotendinous xanthomatosis[53], they have also been recognized as the indicators for the excess and oxidation of cholesterol. Additionally, a targeted lipidomic analysis of rat plasma of a metabolic syndrome model suggested FFA-OH and FFA-OOH species as feasible biomarkers for diagnostic and therapeutic/pharmacological evaluation[54].

3.1.4. Renal diseases

As one of the most severe renal diseases, chronic kidney disease (CKD) involves a gradual loss of kidney function, which is also called chronic kidney failure. By using a quantitative lipidomic method, a study found that the CKD patient plasma samples showed an altered oxPC signature discrimination between the conditions with or without periodontal comorbidity[55]. Another study on immunoglobulin A nephropathy (IgAN) measured the oxFFA profile (including FFA-OH, FFA-OOH, FFA-*epo* species) in human serum and urine samples using a targeted analysis with SPE pretreatment[56]. The authors also proposed these oxFFA species as biomarkers for IgAN diagnosis and evaluation index of n-3 fatty acid supplementation.

3.1.5. Neurodegenerative diseases

In the past decade, oxidative stress has been considered as a major role in the process of neurodegenerative diseases, for example, Alzheimer's disease (AD)[57]. The accelerated oxidation of glycated proteins might contribute to the accumulation of advanced glycation endproducts in amyloid plaques. In terms of oxidative lipidome, the oxidized phospholipids have been revealed, such as PC-CHO species in human serum[58] and SM-OH species in human brain[59]. For another progressive nervous system disorder, Parkinson's disease (PD), oxidative stress also plays a key role in the degeneration of dopaminergic neurons and disruption of the physiologic maintenance[60]. Using non-targeted approaches via LC/MS or GC/MS, studies have expressed the oxidized lipid molecules covering oxFA, oxCL, and oxysterols[61,62], as well as their potential as valuable biomarkers.

3.1.6. Other metabolism disorder-related vascular diseases

Besides the reviewed conditions above, oxidized lipidomics has also been applied to other metabolic disorders. In clinical, coronary artery disease (CAD)[63,64], acute coronary syndrome (ACS)[65], and atherosclerosis[66] have been proved to be connected to oxidized phospholipids. Especially, the PC oxidation products PC-CHO and PC-COOH species have been proposed by both non-targeted and targeted methods in CAD patients. Moreover, one study measured cholesterol-OH species using GC/MS in the arterial intima tissue from peripheral atherosclerotic disease (PAD) patients, and found that these oxysterols were issue and plasma were highly related to systemic inflammatory activity[67]. In addition, a blood vessel inflammation syndrome, Kawasaki disease (KD), has also been reported to link to oxidized phospholipids[68]. The untargeted lipidomic profiling and targeted analysis indicated that oxPC species were related to coronary arteritis, suggesting that the oxidized phospholipids activated the inflammatory signals in KD.

### *3.2. Cancers*

Since researchers have realized that the unbalance between oxidation and reduction happened due to the over-production of active oxygen by tumor cells and the abnormal oxidation-reduction control[69], oxidative lipidomics has been applied to some cancer studies in the clinic, namely, non-pancreatic neuroendocrine tumor[70], hepatocellular carcinoma[71], and oncocytic thyroid tumor[72]. In addition to FFA-OH and LPC-OH, the oxCL species (e.g., CL-OH and CL-OOH) were found as elevated indexes, and such abnormality might be explained as the uncontrolled mitochondrial dysfunction in tumor cells.

### *3.3. Other diseases*

Oxidative lipidomic analysis has also been applied to medium-chain acyl-CoA dehydrogenase deficiency[73], septic inflammatory[74], periodontal disease[75], and others. A representative and well-developed method is the triple

quadrupole MS-based quantitative determination of FFA-OH, FFA-OOH, and FFA-epo as the targeted approach, which has been used for blood (either plasma or serum) and other body fluids (e.g., urine and saliva).

### *3.4. Surgery- and injury-induced disorders*

Not only diseases but also surgery- and injury-induced disorders are practicable for oxidative lipidomic analysis. Ischemia/reperfusion (I/R) is a typical injury that causes oxidation damage to organs[76]. In animal I/R organ samples, both non-targeted and targeted studies discovered oxCL species as significantly increased indicators[77,78]. The hydroxyl CL molecules were also found in the lung of the hyperoxia mouse model, as well as hydroxyl PS (PS-OH)[79]. From human plasma of cardiac surgery, targeted lipidomics determined FA-OH and FA-OOH as damage markers, but since the pretreatment included hydrolyzation, the source of these oxidized fatty acyls remained unknown[80]. Moreover, in the patient blood of iatrogenic plaque disruption, a series of oxPC, including PC-CHO, PC-COOH, and PC=O, were quantitatively investigated together with CE-OH[81].

## 4. Limitations and Prospects of Oxidative Lipidomics

Although studies on oxidative lipidomics are increasing rapidly, some limitations are worth noticing. Basically, oxidative lipidomics shares all the boundaries of ordinary lipidomics. Like metabolomics and other downstream omics, oxidative lipidomics lacks the interpretation of upstream information and underlying mechanism (metabolic pathways). In terms of operation, oxidative lipidomics always faces the challenges of sampling, pretreating, and storing[82]; it is also hard to deal with the distinguishment of artificial oxidation products. For data processing, there are usually difficulties in accomplishing structural validation and conducting multi-component absolute quantitation, which needs the preparation of oxidized lipid molecule standards. Especially, the trace amount of oxidized lipids makes them even more problematic for identification.

Nevertheless, it is believed that, as the MS technique develops, the analytical procedure will be easier-handling and more reliable; the effective distinguishment of artificial oxidation products can/will be conducted by the detailed isomer resolution[12]. Such works depend on not only the chemical preparation (i.e., synthesis) of oxidized lipid standards but also the library establishment, even including the in-silico calculation, prediction, and simulation. Furthermore, the expansion of bioinformatics will contribute to a better understanding of lipid dynamics and integration of other omics (e.g., genomics and proteomics). Another aspect is the development of imaging, which can be more intuitive and visually direct for specific studies and innovative diagnoses.

## 5. Summary

As a branch of lipidomics, oxidative lipidomics focuses on the oxidized lipid molecules of different lipid classes, including lipid-OH, lipid-OOH, lipid-epo, lipid=O, and even the degraded lipid oxidation products and other related molecules. These characterized lipid species have been recognized as useful biomarkers for both diagnosis and treatment evaluation. The application of oxidative lipidomics by chromatographic separation and MS detection covers both oxidative stress-related metabolic diseases and other disorders, from animal experiments to clinical tests. Currently, compared with "ordinary" lipidomics, i.e., intact lipidomics, oxidative lipidomics faces more challenges, especially the lack of standards and compound database. Nevertheless, it is a promising way to explore the field of biomedical studies. As MS technological advances, a deeper and broader understanding of lipids and oxidation concerning human health will be achieved.

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## Conflict of Interest

All authors declare that they have no conflict of interest.

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