Review

Oxidized lipid species in lipoproteins: Significance and analytical considerations

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Abstract Lipids are ubiquitous in the human body, playing diverse biochemical and physiological functions. Unfortunately, lipids are susceptible to oxidation *in vivo* by free radicals. Lipid hydroperoxides (LOOH) are one of the primary products of lipid peroxidation which play a crucial role in the pathogenesis of oxidative stress, inflammation, and atherosclerosis. Understanding the underlying molecular mechanism of these diseases is critically important for risk stratification, prevention, and therapeutic intervention. Despite its importance in the atherosclerotic process, the qualitative and quantitative evaluations of LOOH in the biological samples have been largely limited, because of structural variability, instability and rapid clearance from circulation. Application of liquid chromatography/mass spectrometry (LC/MS) has revolutionized the study of lipidomic and eased the identification and quantification of hundreds of molecular species of oxidized lipids derived from fatty acids, cholesteryl esters, triacylglycerol and phospholipids in various biological samples. In this review, we will be discussing oxidized lipid species in human lipoproteins, its analytical consideration and significance. An overview of lipids and its oxidative products in lipoproteins, technological advances its identification and quantification using mass spectrometry, and its possible linkage to disorders of lipid metabolism will be discussed.

Key words: LC/MS, cholesteryl ester, triacylglycerol, hydroperoxide, phospholipid, oxHDL, oxLDL

Introduction

Lipids are ubiquitous in the human body, playing diverse biochemical and physiological functions. Most importantly, they serve as a reserve source of energy. Excess calories in our body are converted into fatty acids (FA), which subsequently are esterified with glycerol forming triacylglycerols (TG) and stored particularly in adipose tissue. When there is a demand for energy, TG is mobilized by cellular enzymes into FA, which are then distributed via blood to fat utilizing tissues like muscles and heart to fulfill the physiological need. Similarly, lipids particularly phospholipids (PL) are essential components of biological membranes. The membrane also contains cholesterol that modulates the fluidity of membrane. It also serves as a precursor for several steroid hormones, vitamin D and bile salts. As being hydrophobic in nature, except for free FA, which are transported binding to albumin, lipid molecules are transported in systemic circulation in the form of lipoproteins. Unfortunately, lipids are equally associated with several diseases. Any distortion or alternation in this metabolic pathway can lead to adverse conditions and some of them remain as a global health problem in today's clinical practice. In addition, unsaturated hydrocarbons of lipids are susceptible to oxidation by reactive oxygen species (ROS) generated by in our body. There is increasing evidence that such oxidized lipids play a crucial role in the pathogenesis of oxidative stress, inflammation, atherosclerosis, and coronary heart disease (CHD). Understanding the underlying molecular mechanism of these diseases is critically important for risk

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stratification, prevention, and therapeutic intervention. In this review, we will be discussing oxidized lipid species in human lipoproteins, its analytical consideration and significance. An overview of lipids and its oxidative products in lipoproteins, technological advances its identification and quantification using mass spectrometry (MS), and its possible linkage to disorders of lipid metabolism will be discussed in brief.

Lipids and Lipoproteins

Exogenous or dietary fats (mainly TG) are carried into systemic circulation by chylomicrons, whereas endogenous, that is fat synthesized *de novo* by the liver, are carried by very low-density lipoproteins (VLDL). As these TG-rich lipoproteins (TRL) passes through circulation, Apo CII activates the lipoprotein lipase, which is an extracellular enzyme anchored by heparan sulfate to the capillary wall. This enzyme rapidly hydrolyzes TG in TRL into free FA which is then taken up by the adipose tissue for storage, and by muscles for its utilization[1]. As TG get utilized, the VLDL becomes much smaller and dense. Further, TG is transferred to HDL in exchange for cholesteryl ester (CE). This process is mediated by cholesteryl ester transfer protein (CETP). Some amount of these remnant VLDL, also called as intermediate-density lipoprotein (IDL), are taken up by the liver via ApoE receptor-mediated endocytosis. In the remaining, TG are further utilized by hepatic lipase forming cholesterol-rich low-density lipoprotein (LDL), which functions to deliver cholesteryl ester (CE) to peripheral tissue. The LDL are endocytosed by extra-hepatic tissue via ApoB receptor-mediated endocytosis[2]. The synthesis of the LDL receptor must be under negative feedback regulation of intracellular cholesterol concentration in order to prevent excessive deposition of CE in peripheral tissues[3]. High-density lipoproteins (HDL), on the other hand, are responsible for reverse-cholesterol transportation. That is, it receives the cholesterol from the peripheral tissues by the process mediated by ATP-binding cassette transport protein (ABCA1). The cholesterols are then rapidly esterified to CE using FA from *Sn-2* position of phosphatidylcholine (PC) catalyzed by lecithin cholesteryl ester transport proteins (LCAT)[4]. HDL thus carries excessive cholesterol from the peripheral tissues back to the liver for its excretion and conversion to bile salt. An overview of lipoprotein metabolism is shown in Fig. 1.

In clinical practice, serum TG, total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) are routinely measured for the diagnosis and monitoring of various dyslipidemia and also to evaluate the risk of atherosclerotic cardiovascular disease (ASCVD). There is almost universal agreement that LDL plays a central role in the pathogenesis of atherosclerosis and coronary artery disease (CAD). The overall relationship between LDL-C and ASCVD is curvilinear. Therefore, LDL-C is considered as a prime target of therapy for the primary prevention of CAD[5]. HDL-C, on the other hand, protects against the development of atherosclerosis and low levels of HDL-C are associated with an increased incidence of ASCVD in epidemiological studies[6]. Due to technical limitations and controversy in the measurement of LDL-C, calculation of non-HDL-cholesterol (nonHDL-C), which includes cholesterol in LDL and other proatherogenic TRL, is gaining its popularity in clinical practice. Individuals with abdominal obesity, metabolic syndrome, or diabetic lipid disorders often have increased TG, low HDL-C, and non-increased calculated LDL-C. These patients produce highly atherogenic lipoproteins such as VLDL and IDL as well as small dense LDL (sdLDL) particles. For this reason, non-HDL-C is a better marker of cardiovascular disease (CVD) in both primary and secondary prevention studies. Therefore, the 2018 ACC/AHA guideline endorsed its routine use alternative to LDL-C[7].

We have been known for several decades that almost half of all myocardial infarction (MI) occurs among individuals who have average if not low level of plasma cholesterol. Therefore, a considerable amount of research is being done to reveal biomarkers that can provide information about future cardiovascular risk above and beyond cholesterol[8]. Measurement of oxidized lipoproteins is of great interest to improve the risk stratification and possibly as a therapeutic target.

Oxidized Lipoproteins

Oxidation of lipoproteins is another key factor that can elicit the atherogenesis[9,10]. Therefore, oxidized lipoproteins have received great attention, both to understand the pathophysiology of atherosclerosis and as a potential biomarker for risk stratification of ASCVD. The original idea of proatherogenicity of structural modification of circulating LDL was reported by Brown and Goldstein[11]. Various ROS generated during the normal cellular metabolism or induced by various environmental factors like pollutants, smoking,

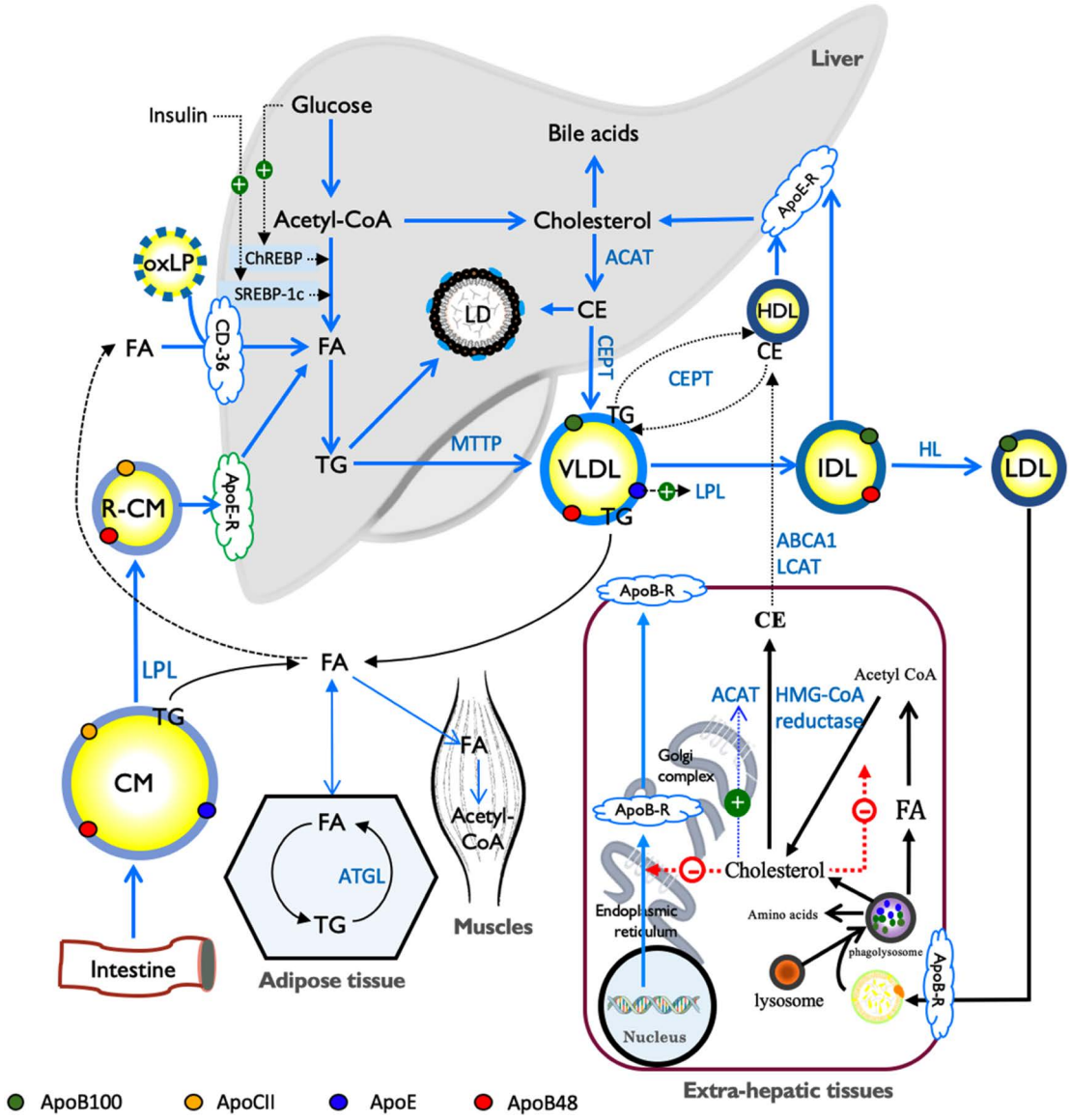

Fig. 1. Overview of lipoprotein metabolism.

ApoB-R: ApoB100-receptor, ApoE-R: apo E-receptor, ATGL: adipose tissue triglyceride lipase, CE: cholesteryl ester, CETP: cholesteryl ester transfer protein, ChREBP: carbohydrate-response element-binding protein, CM: chylomicrons, FA: fatty acids, HL: hepatic lipase, LCAT: lecithin cholesterol acyltransferase, LD: lipid droplets, LPL: lipoprotein lipase, MTTP: microsomal triglyceride transfer proteins, oxLP: oxidized lipoproteins, R-CM: Remnant chylomicrons, SREBP: sterol regulatory element-binding proteins, TG: triacylglycerol.

toxin, and drugs can oxidatively modify the lipoproteins. The oxidized LDL (OxLDL) may trigger the inflammation in the local arteries. In response to endothelium injury, the monocytes adhere to endothelial cells, and then move to the sub-endothelium layer and finally get transformed into macrophages. The macrophages contain non-specific, low-affinity scavenger receptors mainly CD36 that can bind with OxLDL. Unfortunately, these scavenger receptors are not regulated by the cellular cholesterol level, therefore, macrophages consume excess amounts of modified lipoproteins and ultimately becoming foam cells, which is a hallmark in the process of atherogenesis[12]. Finally, these foam cells accumulate, releasing various growth factors and cytokines that stimulate the proliferation of smooth muscle cells and accumulate more lipids and ultimately activate the thrombotic event resulting in atherosclerosis[13–18]. It is now well evident that LDL oxidation can occur *in vivo* and OxLDL have been identified in human atherosclerotic lesions, further supporting the involvement of OxLDL in ASCVD[19,20]. Thus, it appears that the measurement of OxLDL could serve as a better marker for atherosclerosis. Direct detection of OxLDL can be done with anion-exchange chroma-

tography, size-exclusion chromatography, agarose gel and capillary electrophoresis and has been reviewed by Yamaguchi et al[21]. Considerable numbers of enzyme immunoassay (EIA) specific for various epitopes of OxLDL are currently available. Antibodies directed against malondialdehyde (MDA)-LDL or OxPL-LDL are widely used in the research settings. Analytical importance and limitation of various assays for the direct evaluation of OxLDL have been appropriately reviewed by Itabe and Ueda[22]. Although accumulating information supports the importance of OxLDL measurement as a potential biomarker for the risk stratification of ASCVD, the clinical relevance of measurement of OxLDL is yet to be established; hence, are not in routine use. Alternatively, EIA to detect autoantibodies against OxLDL are available which correlates with the direct OxLDL measurement. However, a large prospective clinical study on the Framingham Offspring Study failed to show its association with the incidence of CHD or CVD[23]. Measurement of OxLDL and anti-oxLDL antibodies for the risk stratification of ASCVD and its future implication has been recently reviewed[24]. MS-based approaches for the analysis of oxidative modification of apoproteins will not be discussed here but has been excellently reviewed by Afonso et al[25].

Although OxLDL are primarily blamed for atherosclerosis, increasing evidence is revealing the potentiality of VLDL and IDL to initiate the process of atherosclerosis; therefore, role of the oxidized form of these TRL cannot be negated[26–29]. Both VLDL and IDL have been isolated from the human aorta and atherosclerotic plaques[30–32]. Furthermore, oxidized TRL are considered as proatherogenic and can increase cellular CE uptake by macrophages resulting in the formation of foam cells[33]. On the other hand, a study by Bowry et al. indicated that HDL is the main transport vehicle for circulating lipid hydroperoxides (LOOH) and suggested that HDL lipids are more rapidly oxidized than those in LDL[34]. Some reports show the involvement of oxidized HDL (OxHDL) in the pathogenesis of various diseases[35–38].

Oxidized Lipids

Oxidation of lipoproteins generates a complex mixture of bioactive lipids and accumulating evidence showed that they have profound implications in the atherogenesis. Oxidized lipids commonly refer to lipid derivatives in which oxygen is incorporated with the lipid molecules through enzymatic or non-enzymatic reactions. Many oxidized forms of cholesterol and its ester, TG, PL, glycolipids, and FA have been documented *in vitro* and many of them are also identified *in vivo*. Presence of oxidized lipids has been well documented in human atherosclerotic lesions[39,40]. Lipoproteins are predominantly composed of PL, CE, and TG. Apart from the structural modification apoproteins, oxidized lipoproteins can contain oxidized PL, CE and TG [Fig. 2]. Since the presence of oxidized lipid species can increase its atherogenicity, studies on these oxidized lipids may provide valuable information on the process of atherogenesis. For instance, CE hydroperoxides (CEOOH) are the major biologically active components of OxLDL, which in turn stimulate a wide variety of cellular and molecular processes involved in atherosclerosis[41]. Oxidized lipids are believed to play an important role in inflammation, oxidative stress, immune response and tumorigenesis[42–48].

The precise mechanism of the lipid peroxidation (LPO) *in vivo* is largely unknown. FA, particularly polyunsaturated fatty acids (PUFA), are vulnerable for the peroxidation[49]. It is generally believed that ROS generated in our body, either during normal metabolism or due to environmental factors, are responsible for non-enzymatic oxidative modification of biomolecules[50]. In addition, oxidative stress induced by waning antioxidant systems may play a crucial role in LPO. The oxidation is usually initiated by the abstraction of a hydrogen atom from a carbon adjacent to double bonds, resulting in the formation of conjugated dienes. Subsequently, oxygen molecule is incorporated into it, leading to the formation of LOOH. Therefore, LOOH is considered as an early product indicator of LPO[51]. LOOH can further degrade to the final products like malondialdehyde (MDA) and 4-hydroxynonenal. Furthermore, LOOH can generate other highly reactive and oxidizing radicals like lipid-peroxyl ($LOO^{\bullet}$), oxyl ($LO^{\bullet}$), and epoxy-allylic peroxyl ($OLOO^{\bullet}$). All these radicals, in turn, can lead to peroxidation of adjacent lipids; therefore, initiating a chain peroxidation reaction[52]. The mechanism of such free radical derived oxidation of lipid molecules has been well reviewed[49,51,53]. The resultant effects of LPO products are complex with diverse structural variability. Increase in the number of unsaturated carbons in the lipid generate more complex mixture of oxidized lipids[54]. Since LOOH are primary products of oxidative modification, its demonstration in the lipoproteins can be considered as an early marker of lipid oxidation[55]. Importantly, LOOH may also oxidize

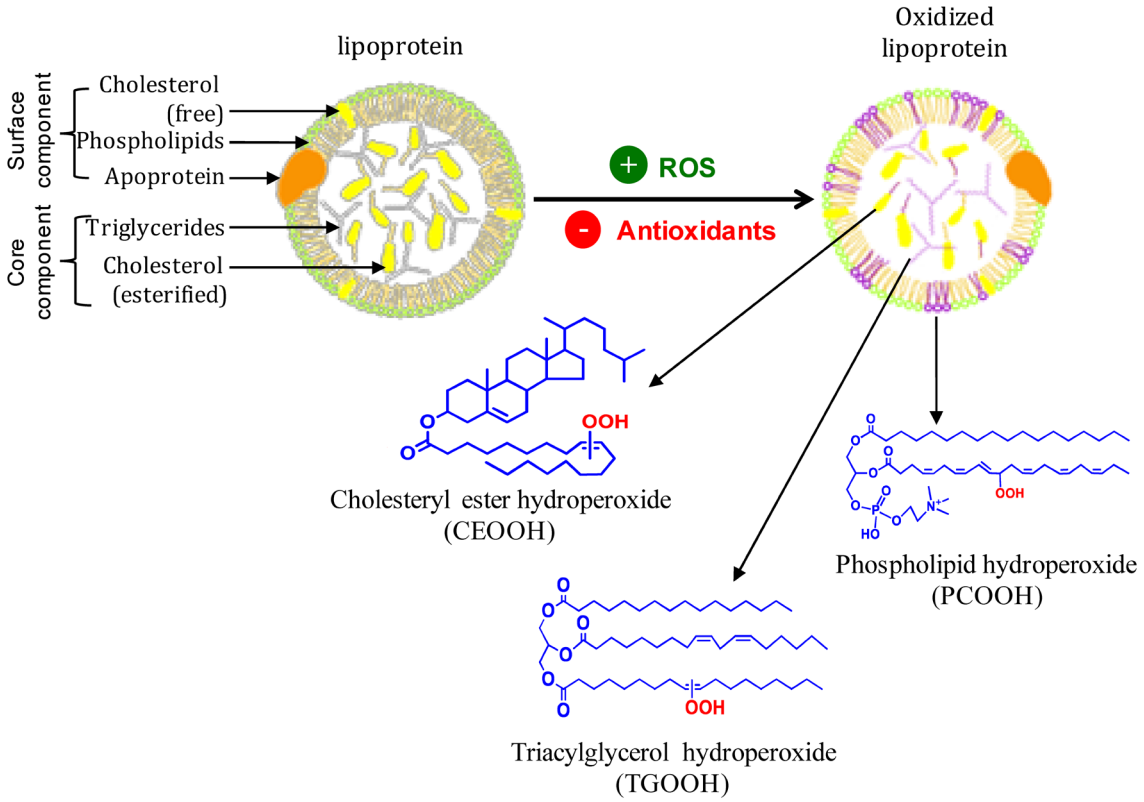

Fig. 2. Structure of lipoprotein and lipid hydroperoxides.

apolipoproteins, making it unrecognizable by its receptors and disrupting its metabolic function[56]. It is well-known that oxidative modification of lipids and inflammation are major hallmarks in atherosclerosis. These oxidized lipids play a crucial role to initiate inflammatory responses in atherosclerosis by interacting with macrophage and endothelial cells[57,58]. Therefore, oxidized lipid in the lipoprotein is one of the key promoting factors for its atherogenicity. Although oxidized lipids are of great interest in oxidative stress, inflammation, and atherosclerosis, they are also associated with number of other pathological conditions like aging, neurodegenerative diseases including Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (ALS), pre-eclampsia and foetal vascular dysfunction, diabetes and its long-term complications, chronic kidney diseases, non-alcoholic fatty liver diseases and steatohepatitis, and various cancers[59].

Analytical Consideration in Assessing Lipid Peroxidation

Since LOOH are the major reaction products of lipid oxidation, its detection in plasma or lipoprotein fraction is an indicator of the oxidative change[60]. Despite its importance in the atherosclerotic process, the qualitative and quantitative evaluation of LOOH in the biological samples have been largely limited, because of structural variability and instability, rapid clearance from circulation, too low concentration for easy detection, and the lack of appropriate internal standards for identification and quantification. Challenges and precautions in the analysis of lipid oxidation products have been beautifully reviewed[61]. Thiobarbituric acid reactive substances (TBARS) assay is perhaps the most frequently used markers as an indirect reflection of LPO[62]. Grintzalis et al. have developed a simple spectrometric method for the simultaneous measurement of plasma malondialdehyde and total LOOH based on the reaction of Fe (3^+) with xylenol orange[63]. However, it has low specificity, as it detects a wide group of aldehydes and alcohols present in the sample. Similarly, monitoring the formation of conjugated dienes by measuring optical absorbance can provide information about the oxidative change in the lipids but it is largely limited to monitor oxidative change during artificial oxidation of lipids. Likewise, the colorimetric assays by ferrous oxidation using xylenol orange[64], iodometric method[65], fluorometric method[66] have been used for the detection of total LOOH and reported measurable amount of lipid peroxides in lipoproteins. More specific methods using high-performance liquid chromatography

(HPLC) with post-column detection based on fluorometry[67,68], chemiluminometry[69–71] and electrochemistry[72,73] have been reported for the quantification of CEOOH, phosphatidylcholine hydroperoxides (PCOOH) and TG hydroperoxides (TGOOH). Using HPLC, we previously reported that normal young human plasma contains 189±87 nM of CEOOH but no TGOOH[74]. In contract, advanced liver disease is associated with significant elevation of both CEOOH and TGOOH. PCOOH has been demonstrated in human plasma ranging from 0.01 to 0.5 μM using chemiluminescence HPLC. PCOOH concentration is significantly elevated in diseased condition like diabetes, and it is mainly carried in LDL and VLDL[75]. Simpler approach for the evaluation of LPO can be the analysis of fatty acid hydroperoxides (FAOOH) in the plasma or lipoprotein fraction after saponification. Browne et al. previously reported up to 13 distinct regioisomers of FAOOH on oxidatively modified human plasma using HPLC[76]. However, total lipid was extracted from plasma, therefore the source of FAOOH, whether it is derived from PL, CE, TG or free FA cannot be determined. Also, the chemical saponification using strong alkali itself can increase the instability of LOOH.

The utilization of mass spectrometry (MS) coupled with gas chromatography (GC) or liquid chromatography (LC) has revolutionized the study of lipidomic and oxidized lipids as it can selectively identify individual or group of compounds. This analytical tool has distinct advantages of sensitivity and specificity with the ability to identify and quantify numerous analytes simultaneously based on their mass to charge (*m/z*) ratio. Recent progress in lipidomic in health and disease, with a primary focus on analytical approaches, has been recently reviewed[77]. With the development of MS-based lipidomics, hundreds of lipid oxidation products have been identified[78]. From the past few decades, liquid chromatography/mass spectrometry (LC/MS) has been widely used for the identification and quantification of oxidized lipids derived from FA, CE, and PL in various biological samples and food products. Electrospray ionization (ESI), Matrix-assisted laser desorption/ionization (MALDI) and atmospheric pressure chemical ionization (APCI) are commonly used ionization techniques in the mass spectrometric analysis of non-volatile polar lipids including oxidized lipids. Advantages and limitations of using various forms of ionization techniques in lipidomic have been reviewed[79]. After ionization, the detection of analytes in MS depends on their movement in an electric or magnetic field based on their *m/z*. Quadrupole time of flight (Q-TOF), Orbitrap MS, and Fourier-transform ion cyclotron resonance (FT-ICR) are commonly used for the identification of oxidized lipids to obtain high-resolution mass spectra with mass accuracy within a few ppm of the theoretical value[80]. MALDI-MS has several important advantages and useful for routine analysis with detailed insights into the sample on a molecular level. The use of MALDI-TOF MS in the analysis of lipid research has been excellently reviewed by Fuchs, et al[81]. For the quantification of LOOH, triple quadruple MS using selective reaction monitoring (SRM) or multi reaction monitoring (MRM) should be used by selecting targeted parent ion in Q1, collision-induced dissociations (CID) in Q2, and monitoring the characteristic fragments of the parent ion in Q3. Either stable isotope (deuterium, 13C or 15N) labeled or lipids with odd chain FA (generally C17 or C19) are used as internal standards in such quantification. High-resolution chromatographic separation of lipid and oxidized lipid molecules is another important aspect. Although GC is routinely used to separate volatile compounds in the gas phase, the use of LC for the separation of lipids has been increasing[82]. Since lipid oxidation products are more hydrophilic than original lipids, the use of LC is more suitable tool for the analysis of oxidized lipids than conventional GC-MS[80]. ESI-MS is most commonly used in the lipidomic study of lipoproteins and is very sensitive for the detection of thermally unstable LOOH[83]. Mass spectra can be obtained without prior derivatization and by direct LC/MS with a few preanalytical steps[84]. Quality of sample is another important issue in the LOOH measurement in lipoproteins. Since LOOH can also be generated from the auto-oxidation, every effort should be considered to prevent it during sample preparation. In our lab, we use EDTA for the collection of plasma as it acts as an antioxidant by chelating cationic metals. Similarly, whenever possible use of EDTA-containing buffer is desirable during isolation and processing of lipoproteins fractions. In order to ensure minimal oxidative changes, sample preparation should be done at below 4°C and storage should be at below −80°C. Repeated freeze-thaw cycles should be avoided whenever possible for the analysis of LOOH[85]. Also, we routinely use 0.005% (w/v) 2,6-di-*tert*-butyl-*p*-cresol (BHT; Butylated hydroxytoluene) in acetonitrile as an antioxidant in order to prevent auto-oxidation of lipid during lipid extraction for MS analysis.

We have reported a novel LC/MS approach for the analy-

sis of CEOOH, PCOOH, and TGOOH[86–88]. Using highly sensitive LC/LTQ Orbitrap MS, we identified several molecular species of CEOOH and TGOOH in artificially-oxidized LDL and HDL[86,88]. The development of MS-based assay for evaluation of LPO has opened a new door to researchers to gain more insights into its physiological and pathophysiological roles in various diseases. A well-known clinical study PREVENT (Prospective Randomized Evaluation of the Vascular Effects of Norvasc) Trial provided strong evidence of the predictive value of LOOH levels in adverse cardiovascular outcomes in patients with stable CHD and is independent of traditional risk factors and inflammatory markers[89]. A Scientific Statement from the American Heart Association (AHA) recommended measuring specific products of LPO such as LOOH which can be valuable in addressing specific mechanistic questions[90].

The MS-based assay is also valuable in analyzing oxidized free FA. Lui et al. have reported a negative ESI-MS method for the comprehensive study of OxFA in biological samples[91]. Since lipoproteins contain only a trace amount of free FA, free FAOOH will not be discussed in this review but have excellently covered by Massey et al[92]. Instead, hydroperoxides of other major molecular species in lipoproteins will be discussed as follow.

Oxidized Phospholipids

The surface of lipoproteins is covered primarily by phospholipid that is dominated by phosphatidylcholine (PC) and is directly exposed to various ROS during its systemic circulation. Furthermore, the *sn*-2 position of glycerol backbone of PC is often esterified with a PUFA making it susceptible to oxidation. Under oxidative stress, such PUFA in PL can be easily attached by ROS to generate various oxidized phospholipids (OxPL) which have a wide variety of biological activities. About 400 different types of OxPL have been identified applying the powerful tool of untargeted LC/MS techniques[93]. More than 30 types of OxPL have been identified in atherosclerotic lesions[94]. PCOOH are one of the oxidation products of PC, and are known to be involved in the pathogenesis of various diseases such as atherosclerosis, Alzheimer's disease, Parkinson's disease, multiple sclerosis, rheumatoid arthritis, diabetes, oxidative stress, and chronic alcoholism[95–100]. Number of regioisomeric hydroperoxides of PC formed depends on the number of unsaturated hydrocarbons in the PC molecules. For example, in PC34:2 two regioisomeric hydroperoxides is possible, one at C-9 and other at C-13 of linoleic acid. These hydroperoxides can be reduced to corresponding hydroxides or dehydrated to its corresponding ketone[57]. A well-known reaction, involving such reduction of FAOOH is the formation of truncated PC, which in turn undergo fragmentation to the 5-oxovaleric acid ester of 1-palmitoyl-sn-glycero-3-phosphocholine (POVPC) or glutaric acid ester of 1-palmitoyl-sn-glycero-3-phosphocholine (PGPC)[58]. Detail review on the chemistry of phospholipid oxidation has been published[101]. In general, the formation of OxPL compounds is largely influenced by the degree of unsaturation on acyl chains of PL[102].

A huge number of studies have been published providing a plethora of evidence on the biological significance of OxPL in lipoproteins in inflammation. OxPL are biologically potent mediators of various cellular processes mediating inflammation and responses[103–108]. These responses are ultimately linked with ASCVD. Thus it is believed that OxPL is one of the key players in the process of atherogenesis[109–112]. OxPL can interact with several cellular receptors, including scavenger receptors, platelet-activating factor receptors, peroxisome proliferator-activated receptors, and Toll-like receptors[97]. Thus, OxPL play an important role in many physiological and pathophysiological processes, such as platelet activation, atherosclerosis, apoptosis, and ferroptosis[113]. In addition, the reactive group of oxPL can covalently bind to proteins resulting in its dysfunction and promote atherogenesis[110]. OxPL can be recognized by the scavenger receptors including CD36 resulting in unregulated uptake of LDL by macrophage, thus initiating number of events in the cascade of atherogenicity including, inflammation, endothelial cells injury, smooth muscle cells proliferation and chemoattraction of monocytes[103,114,115]. Itabe et al. have reported that minimally oxidized LDL is enriched with oxPC which have profound effects to increase the pro-inflammatory function of LDL[116]. Watson et al. have identified the bioactive OxPL in minimally oxidized LDL using LC/MS. Such oxidized lipids in LDL can participate in atherosclerosis by activating endothelial cells to express monocyte specific adhesion molecules and chemokines[117]. Lu et al. performed comprehensive metabolomics in human plasma of patients with CHD and reported that OxPL were significantly elevated in the patient with MI compared to stable angina and healthy control which further supports the oxidative role in athero-

genesis[118]. In addition, a high level of OxPL is found in human atherosclerotic lesions suggesting they are actively involved in the progression of atherosclerosis[119–122]. Ravandi et al. have exhaustively analyzed all the species of OxPL in the different developmental stages of human atherosclerotic plaques[95]. Although there was great variability in the proportions of various oxidation products of PL, the level of PCOOH is almost similar to other advance oxidation products in advanced stage of the plagues. Therefore, PCOOH can be a suitable target for risk evaluation of atherosclerosis. Similarly, POVPC is another proinflammatory OxPL which is believed to play crucial role on the development of atherosclerosis. POVPC exert wide varieties of biological effects and have been identified in human atherosclerotic lesions. Cellular studies have provided abundance evidences on its association with chronic inflammation and vascular proliferation by inducing monocytes chemotactic proteins-1, IL-8, p38 mitogen-activated protein kinase, and c-jun N-terminal kinase[123–125]. It can also induce muscle cell proliferations and neutrophil activation and contribute to platelet aggregation[126,127]. Recent study by Yan et al, demonstrated that POVPC is responsible for impaired endothelial function by uncoupling and inhibiting endothelial nitric oxide synthase (eNOS) and by inducing endothelial cell apoptosis[125]. Both POVPC and PGPC in minimally oxidized LDL can induce the apoptotic signaling via activation of acid sphingomyelinase in arterial smooth muscle cells[128]. Therefore, oxPL may play an important role in the development of atherosclerosis and may be considered as a potential therapeutic target for atherosclerosis.

PL with PUFA are susceptible to oxidation and can generate an antigenic epitope. Antibodies against such PL are associated with rheumatologic diseases and antiphospholipid antibody syndrome (APS). Dominant epitopes in OxLDL are OxPL and antibodies against OxPL have also been used to detect OxPL-protein adducts in human LDL[129]. Accumulating evidence from large-scale clinical studies show OxPL/apoB has significant diagnostic value and can also be targeted for therapeutic intervention. Data from Bruneck study showed that OxPL/apoB and OxPL/Lp(a) are strongly and significantly associated with the presence, extent, and development of atherosclerosis and are significant predictors of CV events[130]. Dallas Heart Study found that elevated OxPL/apo level can predict secondary major adverse cardiovascular events (MACE) in patients with stable CHD[131]. A recently completed randomized clinical trial of small scale also showed that the elevated OxPL/apoB levels are predictive of secondary MACE in patients with stable CHD and the risk can be mitigated therapeutically[120]. Results from the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) Trial reported a remarkable increase in OxPL/apoB levels in patients with acute MI in response to atorvastatin[132]. The study suggested that the increase in OxPL/apoB levels in response to intervention may reflect plaque regression and/or stabilization. Recent study by Philippova and colleagues have reported LC/MS-based assay to quantify 8 truncated molecular species of OxPL including POVPC, PGPC, PONPC and PAzPC. ESI was used as ion source and MS analysis was performed in negative-ion mode. Interestingly, a strong correlation was reported between individual molecular species of OxPL but there were weak or no correlation between LC/MS OxPC quantification and immunoassays based on monoclonal antibody against OxPL in lipoproteins[133]. Since LC/MS method allows the direct quantification of individual species of OxPL, it is far more superior to immunoassays.

From the last decade, MS analysis is the method of choice for the measurement of oxPL. LC/MS methods for targeted OxPL species have been reported that enable high sensitivity quantitation in clinical and biological samples[134]. Soft ionization techniques—ESI and MALDI are most commonly used in the analysis of PL. Although ESI-MS is commonly used for the analysis of PCOOH, it does not provide structural information about the position of oxidation on the acyl-chain. The negative ion ESI−MS method reported by Hall and Murphy can give structural information about the position of oxidation on the side chains[135]. Yin et al. have successfully used negative ESI ion-trap MS for the identification and characterization of OxPL in LDL based on CID fragmentation[136]. MALDI-MS has been applied to a wide range of OxPL[137]. Stubiger et al. used a MALDI-TOF MS approach to profile atherogenic PL in human plasma and lipoproteins demonstrated a positive correlation between oxidized PC and ASCVD[138]. Some researchers have applied Ag^{+}coordination ion-spray (CIS)-MS to improve the ionization, particularly for the analysis of highly hydrophilic molecules like CE[139]. Milne and Porter have applied CIS-MS for the identification of PCOOH and claimed superior to conventional ESI-MS[140]. MS analysis of OxPL has been extensively reviewed[134, 141,142].

Most targeted approaches for MS analysis are based on a precursor ion acquisition of *m/z* 184 (phosphocholine head-group) for the detection of PC, and the neutral loss of water (− 18 Da) for hydroxides and hydrogen peroxide (− 34 Da) for hydroperoxides derivatives. A typical way for the identification of PCOOH using high-resolution MS is shown in Fig. 3. As shown in Fig. 3, the synthetic PC16:0/18:2-OOH (PC34:2-OOH) eluted at 8.17 min and its corresponding mass spectrum contained the $[M+H]^+$at *m/z* 818.5904 (elemental composition $C_{44}H_{85}O_{10}NP$, theoretical mass 818.5904). The EIC at *m/z* 818.5904 for VLDL showed a single peak at RT of 8.18. The base peak observed at *m/z* 818.5907 in (b′) were corresponding to the $[M+H]^+$with the same elemental composition and exact mass as the corresponding ion for the synthetic standard. Thus, the EIC peaks of (b) can be identified as PC34:2-OOH. Similarly, the MS2 and MS3 [Fig. 3 (a″) and (a‴)] spectra of the standard show a base peak at *m/z* 800.5 and *m/z* 782.6 corresponding to $[M+H-H_2O]^+$and $[M+H-2H_2O]^+$, respectively. Ion peaks corresponding to $[M+H-H_2O-O]^+$, $[M+H\text{-}FA18:0]^+$, $[M+H\text{-}FA18:2\text{-}OOH]^+$can also be detected supporting its identification. In general, the major fragment ions of PCOOH observed in MS2 spectra were a result of the loss of H_2O from the hydroperoxide group, FA from *sn*-1 carbon and FAOOH from *sn*-2 carbon. In contrast, the fragment ions of PC in MS2 spectra were formed by the natural loss of the phosphatidylcholine head-group and FA. In the targeted MS method for OxPL, the most common diagnostic scan is for *m/z* 184.1 of the phosphocholine head group. Hence, scanning for the precursor of *m/z* 184.1 enables the detection of all PC and OxPL. For instance, POVPC and PGPC can be easily identified by monitoring ion fragmentation at *m/z* 594 → 184 and 610 → 184, respectively whereas PAPC shows characteristic fragmentation at *m/z* 782 → 184 . Similarly, SOVPC and SGPC show characteristic fragmentation at *m/z* 622 → 184 and 638 → 184, respectively.

A couple of years ago, we published LC/MS-based assay for the identification and quantification of PCOOH in human plasma and lipoprotein fractions[87]. We used in house synthesized 1-palmitoyl-2-linoleoyl-PC monohydroperoxide (PC34:2-OOH) and 1-stearoyl-2-linoleoyl-PC monohydroperoxide (PC36:2-OOH) as standards for identification and 1-palmitoyl-2-heptadecenoyl-PC monohydroperoxide (PC33:1-OOH) as internal standard (IS) for quantification[143]. Lipid extracts from the lipoproteins were injected into the reversed-phase column with mobile phase composed of 10 mM aqueous ammonium acetate, acetonitrile, and 2-propanol. ESI-MS analysis was performed in both positive- and negative-ion mode in LTQ/Orbitrap MS and PCOOH were detected as $[M+H]^+$and $[M+CH_3COO]^-$, respectively. The MS1 data can be obtained in Fourier transform mode, with resolving power of 60,000 and 2 Hz scan speed. The ion spray voltage was set at 5.0 kV, with a scan range of *m/z* 250−1000. The trap fill-time was set at 500 ms. Nitrogen was used as sheath gas (set at 50 arbitrary units). The tandem MS data were acquired using the collision-induced dissociation in ion-trap mode and data-dependent acquisition, which include scans on the most intense ions in MS2 (collision energy of 35 V) and MS3 (collision energy of 45 V), respectively. We identified several molecular species of PCOOH in artificially oxidized LDL and HDL. For the quantification, selective reaction monitoring (SRM) mode was used with the targeted parent to product ions at *m/z* 778.6 → 760.6 (PC33:1-OOH, IS), *m/z* 790.6 → 772.6 (PC34:2-OOH) and *m/z* 818.6 → 800.7 (PC34:2-OOH). Our method has relatively higher sensitivity with the limit of detection and quantification of 0.01 and 0.08, respectively[87]. Recently, we reported 12 molecular species of PCOOH in human plasma and TRL based on calculated elemental composition and high-resolution *m/z* with mass accuracy within ±5 ppm, fragmentation pattern in MS2 and MS3, and RT in LC[144]. PC36:4-OOH was most often detected in VLDL, IDL, and plasma. PC34:2-OOH, PC36:2-OOH, and PC36:4-OOH were detected in all plasma samples. PC34:2-OOH, PC36:2-OOH, PC36:4-OOH, PC38:4-OOH, PC38:6-OOH, and PC40:6-OOH were major PCOOH species detected in TRL. Compared to IDL, detection of PCOOH in VLDL is more frequent with constant detection of PC36:4-OOH and PC38:6-OOH. The concentration of PCOOH in lipoprotein increases with the increase in the degree of oxidation. Therefore, the measurement of PCOOH reflects the degree of oxidation of lipoproteins. We found that PC with mono-unsaturated FA (PC34:1 and PC36:1) was found least susceptible to oxidation, while PC with PUFA particularly, PC34:3, PC36:3, PC 36:5, and PC38:5 were comparatively more susceptible to oxidation[144]. Our demonstration of the existence of PCOOH in TRL may provide new insight to understand the pathogenicity of TRL. Alterations of TRL are linked with endothelial dysfunction and considered to be atherogenic even when LDL-C are low[145,146]. Hence, the

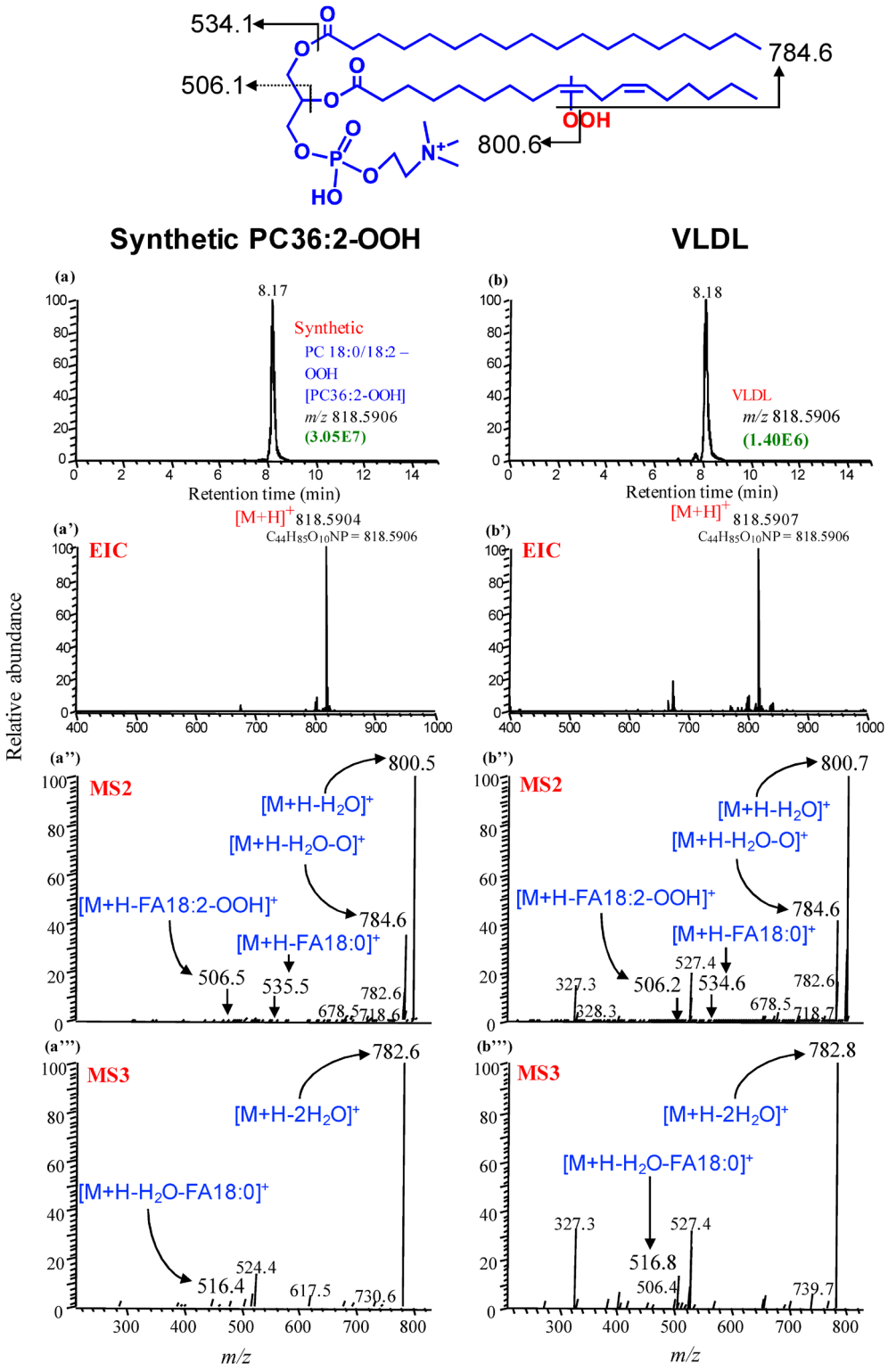

Fig. 3. Identification of PCOOH using LC/LTQ Orbitrap in positive-ion mode: (a) extracted ion (*m/z* 790.5593) chromatogram of synthetic PC36:2-OOH (40 pmol); (a′) mass spectrum of the peak at RT 8.17 min in (a); (a″) and (a‴) MS2 and MS3 spectra of PC36:2-OOH at *m/z* 818.5904, respectively (b) extracted ion (*m/z* 818.5906) chromatogram of VLDL; (b′) mass spectrum of the peak at RT 8.18 min in (b); (b″) and (b‴) MS2 and MS3 spectra of PC36:2-OOH at *m/z* 818.5904 in VLDL, respectively.

detection of lipid oxidation products in TRL might provide a basis for explaining the atherogenicity of TRL[33]. PCOOH, therefore, can be potential targets to be used as biomarkers in the risk stratification of ASCVD or may represent a potential target for therapeutic intervention. Further clinical studies are required to assess the clinical utility of PCOOH.

In summary, OxPL are potent biologically active compounds in human lipoproteins contributing to the development of atherosclerosis. Thus, are potential biomarkers for cardiac risk prediction and therapeutic intervention. Technological advances in the OxPL measurement will certainly help to better understanding the chemical and biological properties of these compounds and provide new insights into its role in the pathophysiology of human diseases.

Oxidized Cholesteryl Esters

Several oxidative products of cholesterol and its ester have been identified in lipoproteins and human atherosclerotic lesions[147–149]. There are a plethora of studies showing an involvement of oxidized sterols and cholesteryl esters in the development of ASCVD[57]. Cholesterol can be enzymatically oxidized to generate several oxidized cholesterol metabolites of oxysterols. It is widely accepted that oxysterols play a key role in the development of atherosclerosis and several studies have been published quantifying it in human plasma[150–153]. Majority of cholesterol carried in the plasma lipoproteins are esterified with FA to form corresponding CE. CE with linoleic acid (FA18:2) is the most predominant in human plasma, followed by oleic acid (FA18:1) and palmitic acid (FA16:0)[154]. Trace amounts of CE esterified with stearic acid (FA18:0), linolenic acid (FA18:3), arachidonic acid (FA20:4) and DHA (FA22:6) are also present in the lipoproteins. CE are the most abundant lipid components in LDL and HDL. PUFA in the CE are also susceptible to free radical-mediated peroxidation. Peroxidation of CE results in similar types of oxidative products as PL. However, unlike PL, which is present in the outer surface, CE is in the inner core of lipoproteins. Therefore, these two classes of lipids are presumably exposed to different oxidative environments in vivo. For example, oxidation of CE18:2 initially generates four primary hydroperoxides at 9- and 13- position of linoleic acid[57]. Reduction or dehydration of these hydroperoxides generates corresponding hydroxides or ketone, respectively.

CEOOH are the major lipid oxidation products in human lesions and hydroxylated FA are associated with plaque instability[40]. The study also demonstrated the presence of CEOOH in plasma of atherosclerotic patients at different stages. Gerry et al. reported that the oxidation of LDL is associated with a significant generation of CEOOH which increases its uptake by macrophage[155]. Therefore, CEOOH are the major component of OxLDL and human atherosclerotic lesions. CEOOH are biologically active compound that can modulate various cellular process associated with the progression of ASCVD[156]. Harkewicz et al. have shown that CEOOH are the major biologically active components of minimally oxidized LDL, which in turn stimulates wide varieties of cellular and molecular processes involved in atherosclerosis[41]. In addition, the accumulation of CEOOH has been well documented in human atherosclerotic lesions and the deposition is disease stage-dependent[39,40]. Using LC/MS method, Hutchins et al. reported that approximately 23% of CE-18:2, 16% of CE-20:4, and 12% of CE-22:6 in human atherosclerotic lesions were oxidized[157]. The same group later reported that up to 92% of the CE-PUFA pool is oxidized in atherosclerotic plaques[119]. They identified several molecular species of CEOOH in the embolized material captured by distal protection filter devices in cardiovascular patients during percutaneous interventions. The role of oxidized CE in inflammation and its importance in ASCVD has been extensively investigated and reviewed by Choi et al[158].

Initially, HPLC-based measurements of CEOOH were popular. Kenar et al. group were first for the study of CEOOH in OxLDL and OxHDL. However, because of technical limitations of HPLC single species of CEOOH were included[148]. The development of MS-based assay for the analysis of CEOOH has widened its scope and assisted many clinical and biological studies. Hutchin et al. have used ESI-MS/MS in human plasma and atheroma samples and reported that CE18:2 is most predominantly oxidized[157]. Recent studies by Guo et al. found a positive correlation of the levels of CEOOH with different types of ASCVD and CEOOH in lipoprotein can increase plasma cholesterol levels by inhibiting its uptake by hepatocytes by decreasing LDL-receptor contributing to the pathogenesis of ASCVD[159,160]. Cholesteryl oleate monohydroperoxide (CE18:1-OOH), cholesteryl arachidonate monohydroperoxide CE20:4-OOH were measured in the study using ESI-MS in positive-ion mode.

Our original method for the analysis of CEOOH in lipo-

proteins and plasma were based on high-resolution mass spectra using LC/LTQ Orbitrap[86]. We used in-house synthesized CE18:1-OOH, cholesteryl linoleate monohydroperoxide (CE18:2-OOH), and cholesteryl linolenate monohydroperoxide (CE18:3-OOH) as standards for equivocal identification. After extraction of lipids from the lipoprotein, the extracts were injected into a reversed-phase HPLC column with a mobile phase composed of 10 mmol/L aqueous ammonium acetate and 2-propanol. ESI-MS analysis was performed in both positive- and negative-ion mode. CEOOH were detected as $[M+NH4]^+$and/or $[M+Na]^+$in positive-ion mode and as $[M+CH_3COO]^-$ in negative-ion mode with mass accuracy of 5.0 ppm. We identified six molecular species of CEOOH-CE18:1-OOH, CE-18:2-OOH, CE-18:3-OOH, CE-20:4-OOH (arachidonic acid), CE-20:5-OOH (EPA), CE-22:6-OOH (DHA) in OxLDL and OxHDL. Based on the high-resolution mass spectra, characteristic retention time in LC and MS/MS spectra, we also identified 6 molecular species of CEOOH in TRL[161]. We provided the first evidence that CEOOH are present in VLDL and IDL in its native form. We confirmed that CEOOH in plasma are predominantly carried in TRL. In vitro studies have shown that both VLDL and IDL can induce the formation of foam cells and CEOOH has been identified in human atherosclerotic lesions. It can be speculated that perhaps VLDL and IDL are responsible for the delivering CEOOH in the atherosclerotic lesions[26,162]. Therefore, the presence of CEOOH in TRL might support the possible involvement of these lipoproteins in the atherogenic process and can be targeted as biomarkers beyond cholesterol.

Because of poor ionization of CEOOH by ESI or APCI, some researchers have used a silver ion (Ag^+)-based coordination ion spray (CIS) ionization technique for its MS analysis. CEOOH can be easily detected as Ag^+ adducts and a Hock cleavage of the hydroperoxides induced by Ag^+ causes region-specific fragmentation enabling the identification position of the hydroperoxides[139,149,163]. Immunoassay using antibodies directed against oxidized CE are not popular as OxPL. However, Gonen and co-workers have recently reported immunoassay that can specifically detect oxidized CE associated with apo AI and apoB-100 lipoproteins in human plasma[164].

Oxidized Triacylglycerols

Many epidemiological, clinical and experimental studies continued to support both fasting and non-fasting plasma TG as an independent risk factor for ASCVD. Plasma TG are predominantly carried by TRL which have density＜1.019 kg/L and include chylomicrons, VLDL and IDL. Accumulating evidence supports the association of TRL with atherosclerosis and CHD. Although chylomicrons and VLDL are large enough to enter the arterial endothelial layer, during its circulation lipoprotein lipase utilizes their TG forming intermediate-sized TRL[165]. Such TRL can participate in atherogenesis like LDL. TRL remnants are considered as major atherogenic lipoprotein in plasma[166]. However, whether triglycerides itself is causative or the metabolic changes that it brings, e.g., reduction of HDL or its indirect reflection of remnant lipoproteins remains to be fully elucidated. Therefore, the specific role of triglycerides in TRL towards the progression of CHD is uncertain and has long been controversial[167–169].

Oxidized lipids in the TRL may be one of the promoting factors for its atherogenicity[33]. TG contain a significant amount of unsaturated FA mainly, FA18:1 and FA18:2, and are susceptible to oxidation. Oxidized PL and CE were the major focus for many researchers, but less attention is given to oxidized TG. A number of studies have demonstrated that VLDL and IDL can contribute to the formation of foam cells, a hallmark of early atherosclerosis and oxidized forms of these lipoproteins have been identified in atherosclerotic lesions[26,30]. *In vitro* studies have shown that lipolysis of TRL leads to release several potentially toxic oxidized FA which can elicit inflammatory responses[170,171]. Therefore, the study of oxidized TG in TRL can be fruitful for a deeper understanding of the atherogenicity of TRL.

Our laboratory has identified several molecular species of TGOOH in human plasma and lipoproteins. We use high-resolution ESI-LTQ Orbitrap MS in positive-ion mode mass spectra were obtained in Fourier-transform mode. We used in-house synthesized standards of TGOOH for equivocal identification[172,173]. It includes-1-Oleoyl-2-linoleoyl-3-palmitoylglycerol monohydroperoxide (TGOOH-18:1/18:2/16:0), 1,2-dioleoyl-3-palmitoylglycerol monohydroperoxide (TGOOH-18:1/18:1/16:0), and triolein monohydroperoxide (TGOOH-18:1/18:1/18:1). TGOOH are detected as the $[M+NH_4]^+$. MS2 and MS3 spectra show of TGOOH show characteristic fragmentation pattern with peak corresponding to $[M+H-H_2O]^+$, $[M+H-H_2O-O]^+$, and small peak corresponding to natural loss of FA and FA-OOH. For example, MS2 spectra of the ion at *m/z* 906.7748 (TG52:3-OOH)

Table 1. Summary of literature reviews of analysis of oxidized lipids in biological samples

Oxidized form	Detection methods	Specimen	Targeted diseases	Reference
Phosphatidylcholine				
Hydroperoxides	HPLC-chemiluminescent, Total PCOOH	Serum	Hyperlipidemia, oxidative stress	98, 100
	HPLC, Total PCOOH	Plasma	—	72, 75, 141
	ESI, LC-MS/MS	Serum	Chronic alcoholism	99
	ESI, LC-MS/MS	Atheroma	CVD	95
	ESI LC-MS/MS	LDL, HDL, Plasma	Artificial oxidation	87
	ESI, LC-MS/MS	—	Artificial oxidation	102
	ESI, LC-MS/MS	VLDL, IDL	Artificial oxidation	144
	CIS-MS	—	—	140
Hydroxides	ESI, LC-MS/MS	—	Artificial oxidation	102
Epoxides	ESI, LC-MS/MS	Atheroma	CVD	95
Aldehydes	HPLC-fluorescent	LDL	Artificial oxidation	116
	ESI, LC-MS/MS	Atheroma	CVD	95
	ESI LC-MS/MS	Plasma	Stable angina, MI	118
	ESI LC-MS/MS, ELISA	Plasma	CVD	119
	ESI, LC-MS/MS	Plasma	ACS, CAD	114, 133
	ESI, LC-MS/MS	Atheroma	Atherosclerosis	175
	HPLC, ESI LC-MS/MS, Derivatization	LDL	Artificial oxidation	117
	ESI LC-MS/MS, MALDI-TOF MS	HDL	—	176
	MALDI-TOF-MS/MS	Plasma, VLDL, LDL, HDL	Hyperlipidemia	138
Carboxylate	ESI, LC-MS/MS	Atheroma	CVD	95
	ESI LC-MS/MS	Plasma	Stable angina, MI	118
	ESI, LC-MS/MS	Plasma	ACS, CAD	133
	ESI LC-MS/MS, ELISA	Plasma	CVD	119
	ESI, LC-MS/MS	Atheroma	Atherosclerosis	175
	MALDI-TOF-MS/MS	Plasma, VLDL, LDL, HDL	Hyperlipidemia	138
	HPLC, ESI LC-MS/MS, Derivatization	LDL	Artificial oxidation	117
OxPC	EIA, Total	Plasma	CHD	120, 131
	EIA, Total	Plasma	Atherosclerosis	130
	EIA, Total	Plasma/LDL	MI	132
	EIA, Total	Plasma	ACS, CAD	133
Cholesterylester				
Hydroperoxides	HPLC, Total CEOOH	Plasma	—	68, 72, 73
	HPLC, Total CEOOH	LDL	—	148
	HPLC, Total CEOOH	LDL	—	155
	ESI LC-MS/MS	Plasma	CVD	119
	ESI LC-MS/MS	Plasma	CHD, MI	159
	ESI LC-MS/MS	Atheroma	Atherosclerosis	157
	ESI LC-MS/MS	VLDL, IDL	—	161
	ESI LC-MS/MS	LDL, HDL, Plasma	—	86
Keto	ESI LC-MS/MS	Plasma	CVD	119
Hydroxy	ESI LC-MS/MS	Plasma	CVD	119
	ESI LC-MS/MS	Plasma	CHD, MI	159
Epoxy	ESI LC-MS/MS	Plasma	CVD	119
Oxysterol	APCI LC-MS, ESI LC-MS	Plasma	CVD	150
	HPLC	LDL	—	155
OxCE	EIA	Plasma	CVD	164
TG				
Hydroperoxides	HPLC, Total TGOOH	Plasma	—	63, 64, 65, 67, 68, 71
	ESI LC-MS/MS	VLDL, IDL	—	174
	ESI LC-MS/MS	LDL, HDL, Plasma	Artificial oxidation	88

Abs. CVD: cardiovascular diseases, MI: myocardial infarction, CHD: coronary heart disease, ACS: acute coronary syndrome, CAD: coronary artery disease.

shows peaks at *m/z* 871.6 corresponding to $[M+H-H_2O]^+$ by loss of 18 mass units, *m/z* 855.5 corresponding to $([M+H-H_2O-O]^+$by the loss of oxygen, *m/z* 577.48 corresponding to $[M+H\text{-}FAOOH18:2]^+$by loss of linoleic acid monohydroperoxide, (FAOOH 18:2), *m/z* 615.46 corresponding to $[M+H-FA16:0]^+$ by loss of palmitic acid (FA16:0), and *m/z* 589.43 corresponding to $[M+H-H_2O-FA18:1]^+$, which deduced from the precursor ion at *m/z* 871.64 by loss of FA18:1. In the negative-ion mode, TGOOH can be detected as $[M+CH_3COO]^-$. However, the absolute intensity of the same concentration of TGOOH standards is about 350 times higher in positive-ion mode[88]. Therefore, for greater sensitivity positive-ion mode is recommended for quantification of TGOOH.

Using this high-resolution MS, we were able to identify 11 molecular species of TGOOH in TRL[174]. TG50:2-OOH is the most predominant TGOOH consistently detected in VLDL, IDL, and plasma. TG52:3-OOH, TG52:4-OOH, TG54:3-OOH, TG54:4-OOH, TG54:5-OOH were also predominant in TRL. EPA and DHA containing TGOOH-TG54:6-OOH, TG56:7-OOH were also identified. Although the mean percentage content of TG in IDL is almost half of VLDL, we found that the mean concentration of TGOOH is higher in IDL compared to that of VLDL.

We confirm that TRL carries several species of TGOOH but not by LDL and HDL. When TGOOH containing TRL are hydrolyzed by lipoprotein lipase, an enzyme anchored to endothelial wall, the arterial walls are constantly exposed to the liberated FAOOH can further elicit inflammatory responses and trigger the process of atherogenesis. Therefore, it appears that significant amounts of oxidized FA are released during lipolysis of TRL and perhaps this phenomenon may result in endothelial dysfunction and inflammation[170]. Thus, the presence of TGOOH in VLDL and IDL might support a possible involvement of TG and TRL in the atherogenic process. The atherogenicity of TRL can be partly explained by the existence of TGOOH, along with CEOOH and PCOOH. Clinical utility of measuring TGOOH in these lipoproteins needs to be investigated for risk assessment of CHD and further works are needed to elucidate the association of such oxidized TG in atherosclerosis.

Summary

In summary, it is now widely accepted that LOOH generated by LPO of lipoproteins play a crucial role to induce inflammation and atherogenesis. Molecular and structural diversity, instability and trace abundance of LOOH are major challenges in the analysis of LOOH. However, the availability of MS-based assay has largely overcome such challenges by providing sensitive and specific detection, characterization, and quantitation of such complex heterogeneous class of oxidized lipids. LC/MS analysis of LOOH has opened a new door for better understanding its biological and clinical implications, particularly in inflammation and ASCVD. Wide varieties of methods have been applied in biological samples to identify and quantify lipid oxidation products in order to determine its relevance in various diseases, which has been summarized in Table 1. So far, the results of studies of LOOH were exciting and providing deeper insights into lipid metabolism. LOOH can be considered as an emerging biomarker for risk stratification of CHD. As the incidence of ASCVD continues to rise globally, LOOH can be one of the potential candidate biomarkers for the primary prevention of ASCVD and can possibly be targeted for therapeutic intervention to reduce the risk. More works are needed in both analytical aspects of LOOH and its clinical assessments to add value in the diagnostic investigations of various diseases and predictive value for CHD. The availability of LC/MS assay for LOOH in the clinical laboratory will certainly help to generate more data on its clinical utility and aid on the evidence-based evaluation LOOH in clinical practice.

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Conflict of Interest

All authors declare that they have no conflict of interest.

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