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Method

Comprehensive metabolomics identified lipid peroxidation as a prominent feature in human plasma of patients with coronary heart diseases

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ABSTRACT

Coronary heart disease (CHD) is a complex human disease associated with inflammation and oxidative stress. The underlying mechanisms and diagnostic biomarkers for the different types of CHD remain poorly defined. Metabolomics has been increasingly recognized as an enabling technique with the potential to identify key metabolomic features in an attempt to understand the pathophysiology and differentiate different stages of CHD. We performed comprehensive metabolomic analysis in human plasma from 28 human subjects with stable angina (SA), myocardial infarction (MI), and healthy control (HC). Subsequent analysis demonstrated a uniquely altered metabolic profile in these CHD: a total of 18, 37 and 36 differential metabolites were identified to distinguish SA from HC, MI from SA, and MI from HC groups respectively. Among these metabolites, glycerophospholipid (GPL) metabolism emerged as the most significantly disturbed pathway. Next, we used a targeted metabolomic approach to systematically analyze GPL, oxidized phospholipid (oxPL), and downstream metabolites derived from polyunsaturated fatty acids (PUFAs), such as arachidonic acid and linoleic acid. Surprisingly, lipids associated with lipid peroxidation (LPO) pathways including oxidized PL and isoprostanes, isomers of prostaglandins, were significantly elevated in plasma of MI patients comparing to HC and SA, consistent with the notion that oxidative stress-induced LPO is a prominent feature in CHD. Our studies using the state-of-the-art metabolomics help to understand the underlying biological mechanisms involved in the pathogenesis of CHD; LPO metabolites may serve as potential biomarkers to differentiation MI from SA and HC.

1. Introduction

Coronary heart disease (CHD), one of the leading causes of death in the world, is closely associated with atherosclerosis, characterized by the formation of plaques consisting of oxidized lipids inside the coronary arteries [1–4]. Over time, the atherosclerotic plaque hardens and narrows the arteries, reducing the blood flow to the heart; once the plaque ruptures, it produces a blood clot on the surface of arteries, blocking the blood flow and leading to angina or myocardial infarction [5].

Mounting evidence has demonstrated that atherosclerosis is the leading cause for CHD [3,4]. Atherosclerosis is a chronic process initiated from the deposition of oxidized low density lipoprotein (LDL) underneath the artery wall [6,7]. It has been well established that oxidation of phospholipids and cholesterol in LDL plays an important role in the progression of atherosclerosis and recent studies suggested that oxidized phospholipids (oxPLs) can be considered as biomarkers and therapeutic targets of CHD [6,8]. However, it remains

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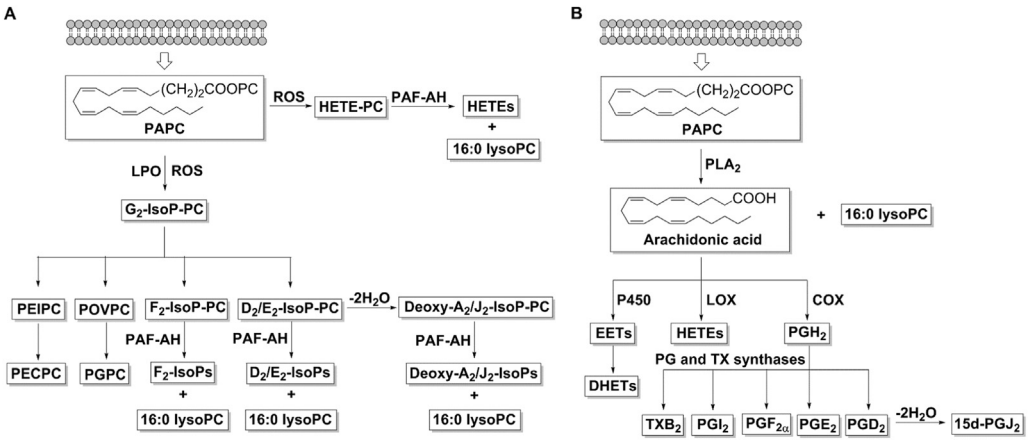

Fig. 1. Schematic outline of major pathways of PAPC metabolism: ROS induced-LPO of PAPC oxidation (A) and enzyme-catalyzed AA oxidation (B).

poorly understood how LDL is oxidized and the interplays between oxidative stress and inflammatory responses in this process [9,10]. It is generally accepted that polyunsaturated fatty acids (PUFA), such as arachidonic acid (AA) and linoleic acid (LA), esterified to phospholipids and cholesterol in LDL are primary targets for free radical-induced lipid peroxidation (LPO)[11,12] and oxidation of LDL exacerbates inflammatory responses in macrophages and endothelial cells in the artery, causing lipid deposition and endothelial dysfunctions during atherosclerosis [13]. During this process, reactive nitrogen species generated by myeloperoxidase (MPO) may convert LDL into an atherogenic form through LPO and nitration of apoB 100 [14,15]. Furthermore, hypochlorous acid (HOCl)-induced LPO also contributes to the modification of LDL [16,17]. More than 30 different oxPLs have been found in atherosclerotic lesions [9]. PAPC (1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine), a major phospholipid in LDL and cell membrane, is susceptible to oxidation by free radicals, generating multiple oxidative products including D_2/E_2-IsoP-PC, PECPC, POVPC as shown in Fig. 1A [11]. These esterified oxidation products can be hydrolyzed primarily by platelet activating factor (PAF) acetyl hydrolase (AH) to generate isoprostanes (IsoPs) and lysoPC [18]. Isoprostanes (IsoP) are isomers of prostaglandins, which are derived from ROS-induced LPO under oxidative stress [19]. On the other hand, AA released from esterified phospholipids under inflammatory stimuli can be subsequently converted into prostaglandins (PGs), hydroxyeicosatretraenoic acids (HETEs), and epoxyeicosatetraenoic acids (EETs) by cyclooxygenases (COXs), lipoxygenases (LOXs), and cytochrome P450s respectively (Fig. 1B) [20,21]. Thus, metabolism of the PUFA-containing GPL, especially PAPC, bridges the two major contributing factors – oxidative stress and inflammation – to the onset and progression of atherosclerosis [22].

CHD is a complex metabolic disorder resulting from genetic and environmental interactions. Metabolomics , a powerful technique to systematically study the entire metabolic pathways in a given biological system, has been increasingly employed to identify metabolic biomarkers and improve clinical diagnosis and treatment of diseases [23]. Emerging studies demonstrated altered metabolite profiles that reflected the onset and progression of CHD [24,25]. Some of metabolic biomarkers identified through metabolomics have a potential to provide diagnostic and predictive values of CHDs [26–30]. However, substantial amounts of validation remain to be carried out before these putative “biomarkers” can be applied for CHD diagnosis due to the inherent caveats of untargeted metabolomics [31]. Furthermore, it is a tremendous clinical challenge to differentiate myocardial infraction (MI) from stable angina (SA).

In the present study, we performed an untargeted metabolomics evaluation in plasma of SA (stable angina), MI (myocardial infarction) and HC (health controls) and identified unique features of metabolites that can be used to differentiate these three groups. In the subsequent pathway analysis, glycerophospholipid (GPL) pathway emerged at the top of these significantly altered metabolic pathways. We then applied a targeted lipidomic analysis of all the metabolites associated with GPL pathway including oxidized PL and the downstream lipid mediators. Surprisingly, the oxidized lipids, presumably derived from LPO, represent a prominent feature to differentiate MI from SA and HC, suggesting a potential to be biomarkers for CHD once validated in the future studies.

2. Materials and methods

2.1. Reagents and materials

Liquid chromatography-Mass Spectrometry (LC-MS) grade water (H_2O), methanol (MeOH), acetonitrile (ACN), 0.1% formic acid (FA) in water, 0.1% FA in ACN and HPLC grade chloroform ($CHCl_3$) and isopropanol (IPA) were purchased from Honeywell (Muskegon, MI, USA). Ammonium fluoride (NH_4F), ammonium hydroxide ($NH_3{\cdot}H_2O$) was purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Plasma samples collection

A total of 28 human subjects (10 HC, 10 SA and 8 MI) were enrolled at Beijing Tian Tan Hospital (Beijing, China). All the participants recruited in this study were diagnosed and classified based on symptoms and coronary angiography [22]. Plasma samples collected were frozen immediately for analysis. The Institutional Review Board at Tian Tan Hospital approved the study and all participants provided written informed consent.

2.3. Sample preparation for metabolomics

Plasma samples were thawed at 4 °C on the ice. An aliquot of 50 µl plasma sample was precipitated by adding 150 µl precooled methanol, vortexing for 30 s, sonicating for 10 min at 4 °C, and then incubating for 1 h at −20 °C. Precipitated protein was removed by centrifugation (13,000 rpm, 20 min) at 4 °C. Subsequently, the supernatants were transferred to LC-MS vials and stored at −80 °C until the Ultrahigh Performance Liquid Chromatography – Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF/MS) analysis. To ensure data quality for metabolic profiling, pooled quality control samples were prepared by mixing 5 µl supernatant from each sample.

2.4. UHPLC-QTOF/MS analysis

Metabolomics performed were described in a previous study [32].

In brief, plasma samples were randomly injected into a Waters ACQUITY UHPLC HSS T3 column (100 × 2.1 mm, 1.8 μm) maintained at 25 °C using an Agilent 1290 series UHPLC system coupled to an Agilent 6550 iFunnel QTOF mass spectrometer. During acquisition, one quality control sample was run after every eight injections. QC samples were also used for MS/MS data acquisition by using a Triple TOF 6600 mass spectrometer (AB SCIEX, USA).

2.5. Data preprocessing

Data preprocessing procedures were described in previous publications including nonlinear retention time alignment, peak discrimination, filtering, alignment, matching, and identification. The acquired mass spectrometry data (.d) were converted to the mzXML format using ProteoWizard and preprocessed by R package XCMS and R package CAMERA (version 3.2) generating a data matrix consisted of the retention time (RT), mass to charge ratio (*m/z*) values, and peak intensity [33]. A LOESS regression model was used to normalize each metabolite peak in all samples based on QC samples [34].

Subsequently, metabolites annotated were subjected to further statistical analysis by using Metaboanalyst 3.0 (http://www.metaboanalyst.ca/MetaboAnalyst/faces/home.xhtml). Firstly, Principal component analysis (PCA) was used to view the clustering trend for the multidimensional data. Partial least-squares discriminant analysis (PLS-DA) was then performed to further investigate the metabolic changes and screen for potential marker metabolites. In addition to the multivariate statistical method, the nonparametric test (Wilcoxon rank-sum test) was applied to determine the significance of each metabolite. Potential metabolic biomarkers were selected with VIP value more than 1 and p value less than 0.05. The differential metabolites were used to conduct hierarchical cluster analyses (HCA) and mapped into their biochemical pathways.

2.6. Lipid extraction of human plasma

Lipids were extracted using the Folch method following the previously published protocols [35]. To avoid oxidation during extraction, butylated hydroxytoluene (BHT) and PPh_3 were added into Folch solution to make a final concentration of 0.005% (wt/vol) and 0.0025 mg/ml respectively. PC internal standard (14:1–14:1 PC) of 100 ng and 5 ng of each prostaglandin internal standard (shown in Table S2) were added prior to extraction.

2.7. Detection of oxPL profile by a targeted metabolomics using normal phase LC/ESI-MS/MS

As described in our previously study, samples were injected into an Agilent Zorbax RX-silica column (150 × 2.1 mm, 5 μm) at a flow rate of 500 μl/min by using an Agilent 1260 Quad pump coupled to a Thermo Fisher TSQ Vantage triple quadrupole mass spectrometer [22]. The linear gradient started from 100% A ($CHCl_3$/MeOH/NH_3·H_2O, 80/19.5/0.5, by vol) to 100% B ($CHCl_3$/MeOH/NH_3·H_2O, 60/34/5.5/0.5, by vol) in 14 min, held at 100% B for 6 min and returned to 100% A in 2 min.

Each oxidized phospholipid was determined by three characteristic transitions as shown in Table S1. In positive ion mode, PC species is readily ionized to give a characteristic fragment of PC headgroup with *m/z* 184. In the negative ion mode, a palmitate in *sn-1* position and the side chain of oxidized fatty acids at *sn-2* position were used as important structural information to identify and quantify a specific oxidation product. All the transitions used in multiple reaction monitoring (MRM) experiments were summarized in Table S1.

2.8. Detection of fatty acid metabolites using a targeted metabolomics based on reverse phase LC/ESI-MS/MS

Systematic analysis of fatty acid metabolomic approach was performed according to our published procedure [36,37]. In brief, samples were injected onto a Phenomenex C18 column (100 × 2.1 mm, 2.6 μm) at a flow rate of 400 μl/min by using an AB Sciex 5500 QTrap hybrid quadrupole linear ion trap mass spectrometer in negative ion mode. The gradient started with 100 to 92% A (H_2O/ACN/FA, 63/37/0.02, by vol) in 6 min, held at 45% A from 6.5 to 10 min, increased to 100% B (ACN/IPA, 50/50, by vol) at 13 min, and returned to 100% A from 14 to 14.5 min. The characteristic transitions used in the MRM experiments were shown in Table S2.

2.9. Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA. A probability value of < 0.05 was considered statistically significant.

3. Results

To investigate the metabolic changes associated with CHD, we performed the untargeted metabolomic profiles of 28 plasma samples from the three groups (10 HC, 10 SA, and 8 MI patients) using UHPLC-QTOF/MS. Table 1 represented a brief clinical background of the human subjects enrolled in this study. We attempted to match the major risk factors associated with CHD including age, sex, BMI, hypertension, history of cigarette smoking, diabetes mellitus, and blood lipid profiles. Notably, patients with SA were older than HC, but there was no statistically significant difference in age between patients with SA and MI. A majority of the participants across the three groups were normal weight. Even though the healthy controls had an average BMI of 26.18 ± 3.09, there were no statistical difference of BMI among three groups. The percentage of smoker was significantly higher in MI patients compared with HC and SA patients. The lipid profiles were all well matched across three groups.

3.1. Metabolic profiles of plasma samples from healthy controls, SA and MI patients

A total of 582 molecular features (240 metabolite features in negative ion mode, and 342 metabolite features in positive ion mode in the MS analysis) were selected for subsequent analyses. The principle component analysis (PCA) was performed to view the metabolomics data set and identify characteristics for each group. As shown in Fig. S1, quality control (QC) samples, shown as blue dots, were center-clustered, indicating the good instrumental reproducibility and stability throughout the period of this metabolomics study.

Table 1
Clinical characteristics of patients enrolled in this study.

	HC (n = 10)	SA (n = 10)	MI (n = 8)
Age (years)	54.9 ± 10.92	68.5 ± 10.01	52.89 ± 26.42
Male gender (%)	70	70	87.5
BMI (kg/m^2)	26.18 ± 3.09	24.92 ± 3.53	23.11 ± 9.75
Hypertension (%)	70	90	37.5
Diabetes mellitus (%)	30	20	12.5
Current smoker (%)	30	30	87.5
Drinking history (%)	20	20	25
Triglycerides(mg/dl)	1.7 ± 1.15	1.62 ± 0.97	3.03 ± 2.06
CHO (mmol/l)	3.82 ± 0.73	4.06 ± 0.91	4.01 ± 1.6
LDL-C (mmol/l)	2.23 ± 0.66	2.41 ± 0.78	2.35 ± 0.94
HDL-C (mmol/l)	1.08 ± 0.32	0.99 ± 0.25	0.88 ± 0.35
apo-A1 (g/L)	1.23 ± 0.24	1.18 ± 0.22	1.17 ± 0.48
apo-B (g/L)	0.79 ± 0.19	0.87 ± 0.24	1.00 ± 0.62

Healthy controls: HC, stable angina: SA, myocardial infarction: MI. Values are mean ± SD or %; BMI, body mass index; CHO, total cholesterol; LDL-C, low density lipoprotein (LDL) cholesterol; HDL-C, high density lipoprotein (HDL) cholesterol.

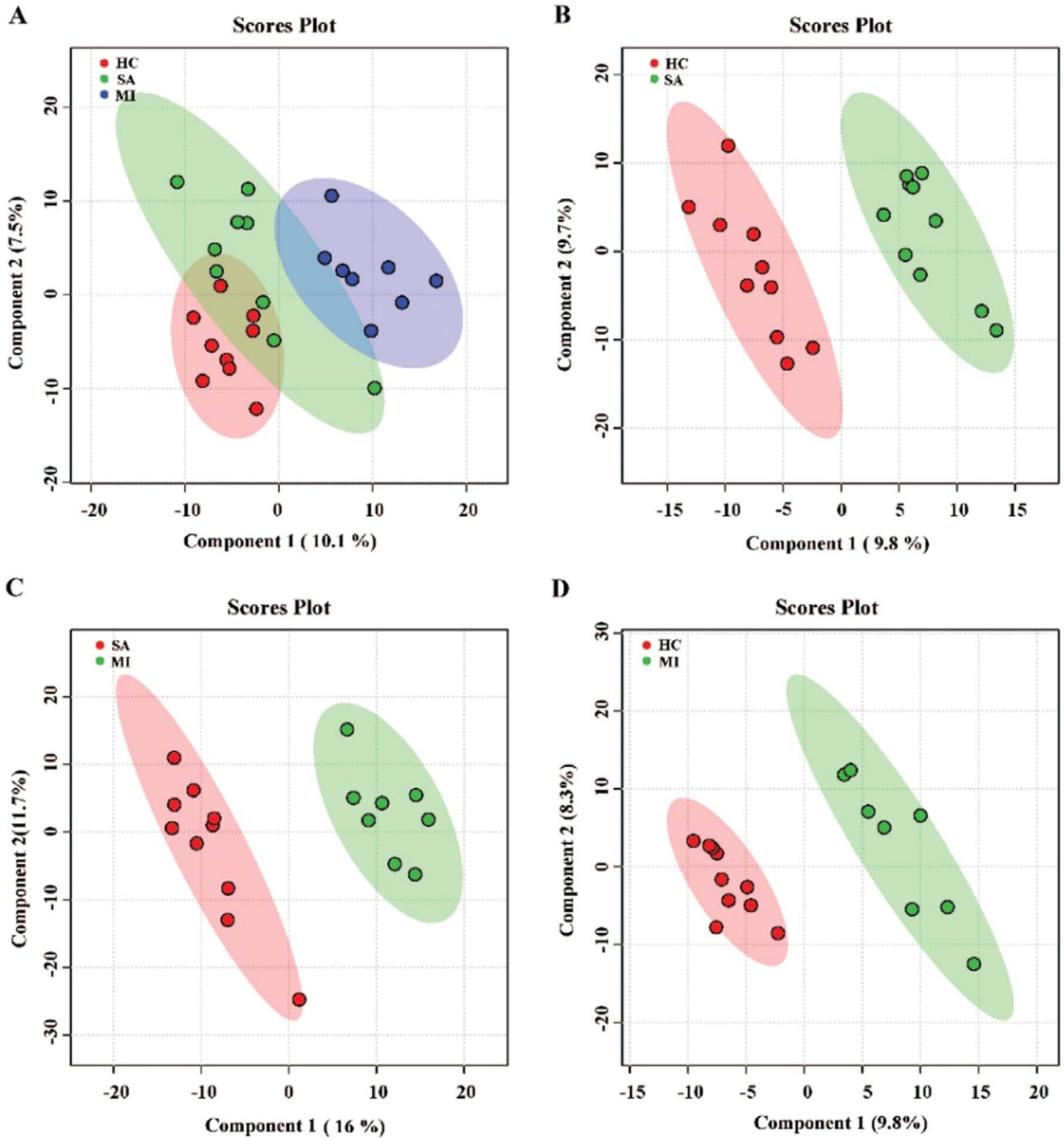

Fig. 2. PLS-DA score plot of (A) HC, SA patients and MI patients, (B) HC and SA patients, (C) SA patients and MI patients, and (D) HC and MI patients. Each dot represents the plasma metabolomic profile of a single sample.

Partial least-squares discriminant analysis (PLS-DA) model was employed to further investigate the metabolic changes and differential metabolites. In Fig. 2A, HC, SA and MI groups were separated from each other, implying distinct metabolic profiles associated with different disease stages. Then we compared SA to HC, MI to SA, as well as MI to HC; PLS-DA score plots revealed that all these three groups could be clearly discriminated (Fig. 2B–D). Parameters for explained variation (R^2), an indicator of model robustness, and cross-validated predictive ability (Q^2) were obtained as follows: HC versus SA, cumulative R^2 at 0.98 and Q^2 at 0.46 (Fig. 2B); SA versus MI, R^2 at 0.96 and Q^2 at 0.62 (Fig. 2C); HC versus MI, R^2 at 0.89 and Q^2 at 0.47 (Fig. 2D).

3.2. Hierarchical clustering analysis (HCA) of differential metabolites and pathways

The metabolite features with variable importance in the projection (VIP) values > 1.0 and p value < 0.05 were considered as the potential differential metabolites. As summarized in Tables 2–4, there were 18 specific metabolites that can distinguish SA from HC, 37 for MI from SA, and 36 for MI from HC. Interestingly, a majority of these differential metabolites were lipids. For example, lipids represent 13 out 18 (Table 2), 23 out 37 (Table 3), and 25 out 36 (Table 4). All these differential metabolites were used to conduct the HCA (Fig. 3A–C). The heatmaps showed the clear differential metabolic profiles when comparing SA to HC, MI to SA, and MI to HC. Among these three paired comparisons, the metabolic profiles of MI were better distinguished from HC group.

Next, we mapped these differential metabolites into their biochemical pathways. As shown in Fig. 3D–F, the significantly altered pathways were mainly involved in glycerophospholipid (GPL) metabolism, porphyrin and chlorophyll metabolism, and amino acids metabolism. Moreover, disturbance of GPL metabolism was ranked at the top of the

Table 2
Statistical analysis of differential metabolites to distinguish SA from HC group.

Metabolites	VIP value	Fold Change	p value
HC vs SA			
PC (18:3/2:0)	7.95	0.15	< 0.001
PC (14:1/4:0)	5.95	0.12	< 0.001
PC (17:2/2:0)	4.62	6.42	0.005
Bilirubin	3.64	3.02	0.005
LysoPC (20:0)	2.80	0.34	0.01
Biliverdin	2.31	2.31	0.009
2-Oxoadipic acid	2.22	1.73	0.03
LysoPC (22:5)	2.14	0.43	0.007
L-gamma-glutamyl-L-valine	1.79	0.49	0.015
LysoPE (18:3)	1.79	0.48	< 0.001
LysoPC (O-15:0)	1.60	1.96	0.01
Urocanic acid	1.49	0.56	0.02
LysoPC (20:2)	1.46	1.84	0.003
PC (24:1/14:1)	1.31	0.54	< 0.001
PC (12:0/24:4)	1.31	0.62	0.04
Inosine	1.27	1.67	0.01
Glycerophosphocholine	1.26	0.57	0.009
PC (10:0/26:2)	1.05	1.57	0.009

PC: phosphatidylcholine; LysoPE: lyso-phosphatidylethanolamine.

Table 3
Statistical analysis of differential metabolites to distinguish MI from SA group.

Metabolites	VIP value	Fold Change	p value
SA vs MI			
Bilirubin	5.88	0.05	< 0.001
PG (16:0/18:2)	5.08	18.63	< 0.001
PC (18:3/2:0)	4.98	4.32	< 0.001
SM (d16:1/26:1)	4.21	0.35	< 0.001
PC (14:1/4:0)	4.08	6.35	< 0.001
LysoPC (20:0)	3.82	7.45	< 0.001
5-Hydroxyhexanoic acid	3.82	6.17	< 0.001
Biliverdin	3.52	0.14	< 0.001
Adenosine monophosphate	3.29	3.35	< 0.001
Glycocholic acid	2.59	0.18	0.001
PC (10:0/20:1)	2.41	2.87	0.002
N1-Methyl-2-pyridone-5-carboxamide	2.21	0.41	0.002
Pyridoxamine 5′-phosphate	2.11	6.05	0.002
Pregnenolone sulfate	2.00	3.10	0.003
Traumatic acid	1.97	3.75	0.003
PE (P-16:0/22:5)	1.96	0.65	0.003
Hypoxanthine	1.78	3.69	0.003
16-Hydroxy hexadecanoic acid	1.69	2.87	0.003
Chlorogenic acid	1.66	3.60	0.004
PI (16:0/16:0)	1.48	2.53	0.004
LysoPA (18:2)	1.46	2.24	0.004
MG(18:0/0:0/0:0)	1.45	2.33	0.004
L-gamma-glutamyl-L-valine	1.42	1.96	0.004
2-Oxoadipic acid	1.39	1.79	0.004
PI (16:1/22:6)	1.39	1.91	0.006
L-Arginine	1.39	0.47	0.006
MG (20:3/0:0/0:0)	1.37	1.66	0.006
Palmitic acid	1.37	1.89	0.006
LysoPS (20:4)	1.31	1.89	0.006
Oxypurinol	1.31	2.50	0.006
Hydrocortisone	1.22	2.06	0.006
LysoPC (O-15:0)	1.20	0.48	0.006
SM(d14:0/12:0)	1.19	2.18	0.006
PG (18:1/0:0)	1.14	0.48	0.008
PC (10:0/26:2)	1.11	0.69	0.008
Urocanic acid	1.06	1.86	0.011
PC (22:2/18:4)	1.02	1.82	0.011

PC: phosphatidylcholine; PG: phosphatidylglycerol; SM: sphingomyelin; MG: monoacylglycerol; PI: phosphatidylinositol; PS: phosphatidylserine; PA: phosphatidic acid; PE: phosphatidylethanolamine.

Table 4
Statistical analysis of differential metabolites to distinguish MI from HC group.

Metabolites	VIP value	Fold Change	p value
HC vs MI			
PE (P-16:0/22:5)	5.35	0.54	0.04
Bilirubin	4.40	0.14	0.01
Adenosine monophosphate	4.00	2.89	0.01
1-Stearoylglycerophosphoglycerol	3.39	0.36	0.04
PC (10:0/20:1)	2.87	0.25	0.02
5′-Methylthioadenosine	2.80	5.82	0.009
LysoPS (20:4/0:0)	2.62	2.49	0.001
LysoPE (15:0/0:0)	2.57	0.19	0.02
LysoPC (20:0)	2.49	2.60	0.03
Biliverdin	2.49	0.32	0.01
Palmitic acid	2.43	2.47	0.01
16-Hydroxypalmitic acid	2.41	3.17	< 0.001
L-Tryptophan	2.38	0.43	0.04
LysoPC (20:1)	2.37	0.42	0.03
Chlorogenic acid	2.24	3.64	0.01
Pregnenolone sulfate	2.10	2.44	0.02
Traumatic acid	2.08	2.99	0.004
Gentisic acid	2.01	1.49	0.003
PI (16:1/22:6)	1.99	2.25	< 0.001
Oxypurinol	1.89	2.54	0.004
PI (16:0/16:0)	1.83	2.29	0.004
Hydrocortisone	1.66	1.86	0.008
Oleic acid	1.61	2.04	0.01
MG (18:0/0:0/0:0)	1.61	2.02	0.001
Carnitine (20:1)	1.43	1.82	< 0.001
PC (22:4/14:1)	1.41	1.64	0.006
LysoPC (20:2)	1.40	1.82	0.01
LysoPC (20:5)	1.37	0.58	0.01
PC (22:2/18:4)	1.35	1.80	0.006
PC (10:0/14:1)	1.22	1.61	0.01
PI (16:0/18:2(9Z,12Z))	1.15	1.64	0.002
PC (22:5/20:5)	1.12	1.58	0.006
MG (20:3(5Z,8Z,11Z)/0:0/0:0)	1.11	1.39	0.02
PC (18:3/2:0)	1.09	0.66	0.03
Carnitine (24:0)	1.01	1.53	0.02
Stearoylcarnitine	1.01	1.49	0.003

PC: phosphatidylcholine; PG: phosphatidylglycerol; SM: sphingomyelin; MG: monoacylglycerol; PI: phosphatidylinositol; PS: phosphatidylserine; PE: phosphatidylethanolamine.

most significantly affected pathways in all paired comparisons, consistent with the notion that dyslipidemia is a prominent feature in all stages of atherosclerosis [8].

3.3. Targeted metabolomic analysis of GPL pathways: focusing on LPO products of oxPLs and IsoPs

As shown in Fig. 1, GPL metabolism has been closely associated with inflammatory responses and oxidative stress-induced LPO, two major contributing factors for the initiation and progression of atherosclerosis [8]. We next employed a targeted metabolomic approach to systematically analyze all the major downstream metabolites associated with GPL metabolic pathways: eicosanoids derived from three major enzymatic pathways of arachidonic acid and linoleic acid, oxidized PLs (PAPC and PLPC) on the surface of LDL particles, and the hydrolyzed oxidation products from oxPL. Surprisingly, we observed overwhelmed production of LPO metabolites in the circulation comparing to the enzymatic products derived from arachidonic acids as evidenced by the multiple isomeric peaks in the MRM transitions in the MS analysis (Fig. S2) [22]. Furthermore, we found that both oxPLs and hydrolyzed fatty acid oxidation products were significantly elevated in plasma of MI patients comparing to those from HC and SA patients (Fig. 4). Among them, D_2/E_2-IsoPs, 5-HETE, and 9/13-HODE were hydrolyzed products of D_2/E_2-IsoP-PC, HETE-PC and HODE-PC respectively. Taken together, our data suggest that oxidative stress and LPO play an important role in atherosclerosis and the LPO metabolites can be potentially used to differentiate MI from SA and HC.

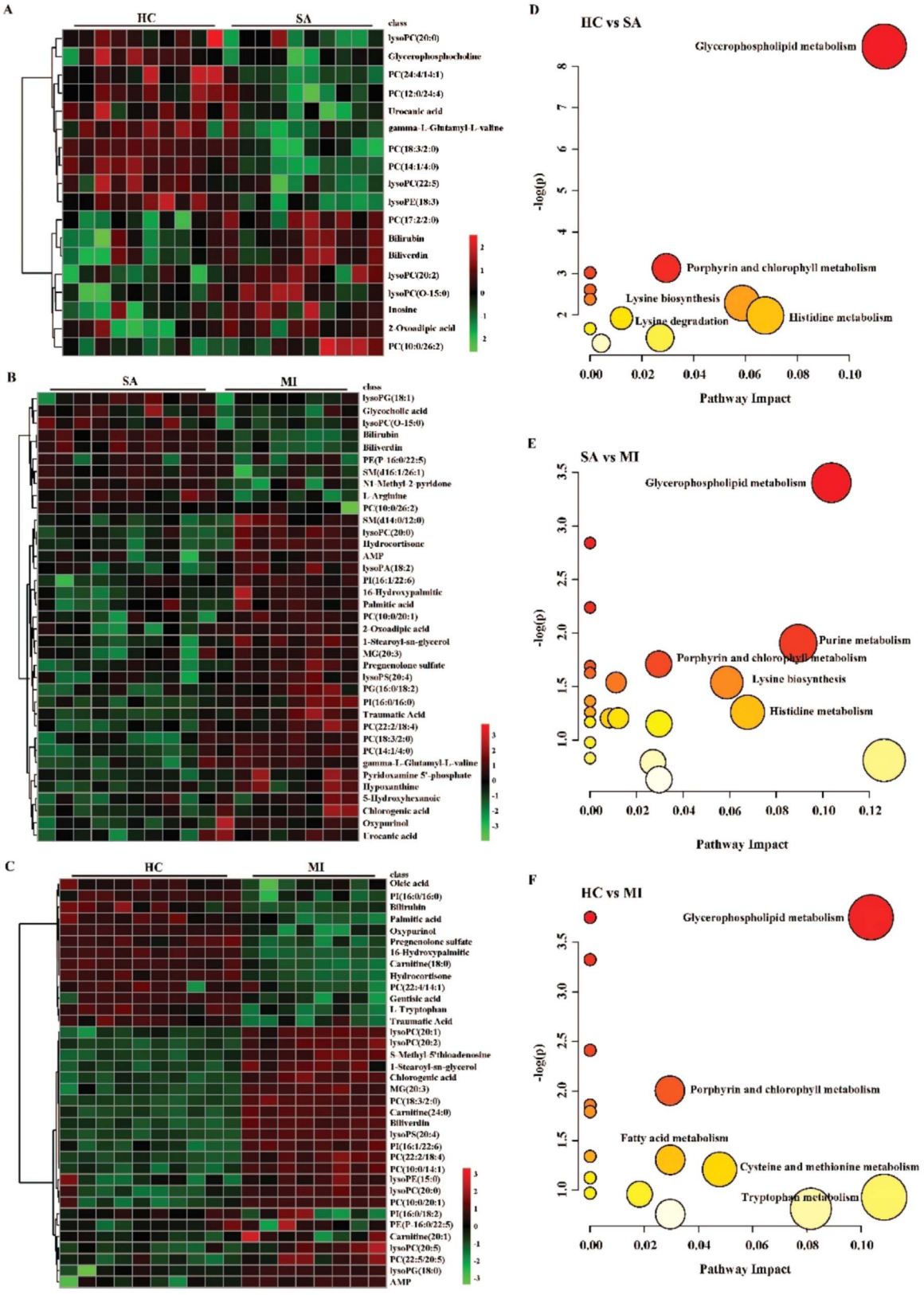

Fig. 3. Heatmap visualization of differential metabolites and related pathways. (A) HC to SA, (B) SA to MI, and (C) HC to MI. The color scale (right) illustrates the relative expression level of metabolites across all samples: red color represents an expression level above mean, green color represents expression lower than the mean. Pathway analysis of potential differential metabolites: (D) HC to SA, (E) SA to MI, and (F) HC to MI. All matched pathways are plotted according to p value from pathway enrichment analysis and pathway impact values from pathway topology analysis. Color gradient and circle size indicate the significance of the pathway ranked by p value (yellow: higher p values and red: lower p values) and pathway impact values (the larger the circle the higher the impact score). Only the significantly affected pathways with low p value and high pathway impact score are showed.

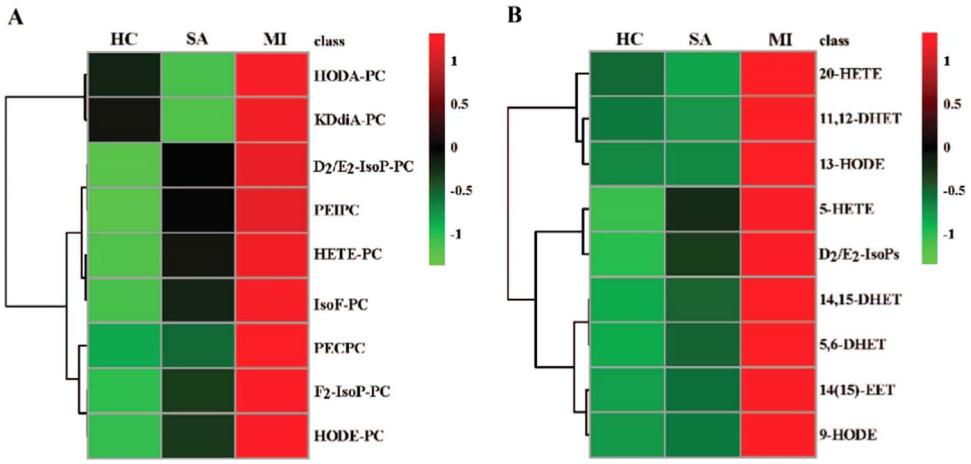

Fig. 4. Heatmap visualization of significantly changed metabolites associated with CHD: oxPLs (A) and oxidation products of hydrolyzed free fatty acids (B).

4. Discussion

In this study, we performed a comprehensive metabolomic analysis in human plasma to investigate disturbed metabolic features from healthy controls and CHD patients. Subsequent analysis demonstrated a good discrimination of HC with SA or MI patients, as well as SA with MI patients, implying a uniquely altered metabolite profile in different stages of CHD. A total of 18, 37 and 36 metabolites have been identified as differential metabolites to distinguish SA from HC, MI from SA, and MI from HC respectively. Metabolic pathway analysis of these potential metabolites suggested that disturbance of GPL metabolism was among the most significantly altered metabolic pathways in all paired comparisons. We then employed a targeted lipidomic analysis to further evaluate the downstream metabolite of major phospholipids and found that oxidized PL and their hydrolyzed fatty acids were closely associated with CHD. These oxidative stress-induced LPO products overwhelmed the enzymatic products of arachidonic acid and linoleic acid, especially in plasma from MI patients. All these data demonstrated that oxidative stress and LPO are a predominant feature and contributing factors to CHD. Even though there are some limitations associated with our current study, such as the limited size of the cohorts, significant number of cigarette smokers in MI groups comparing to SA and HC, the LPO metabolites have the potential to differentiate different stages of CHD one validated in larger cohorts.

GPL were the major differential metabolites identified in this work and these phospholipids belonged to multiple subclasses including phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC), phosphatidylethanolamine (PE), lysophosphatidylethanolamine (lysoPE), phosphatidylserine (PS), lysophosphatidylserine (lysoPS), phosphatidylinositol (PI), and lysophosphatidic acids (lysoPA). Among them, lysoPC (20:0), lysoPC (20:1), lysoPC (20:2), lysoPC (20:5), lysoPC (22:5), lysoPE (18:3), and glycerophosphocholine have been previously reported to be differential metabolites in CHDs [30]. Besides, we found lysoPA (18:2) (one of the main lysoPA species) and lysoPS (20:4) were increased in MI patients compared to healthy controls or SA patients [38]. Furthermore, we observed PC and lysoPC were the primarily disturbed subclasses especially in the comparison of SA with HC group. As shown in Fig. 1, PC can be hydrolyzed to generate lysoPC and free fatty acid catalyzed by phospholipase A_2 [39]; the free PUFAs, such as arachidonic acid and linoleic acid, can be further oxidized to prostaglandins, thromboxane, prostacyclin, and hydroxylated fatty acids by COX, LOX and cytochrome P450 [20]. All these enzymatic lipid mediators have been shown to play a critical role in inflammatory responses, immune response, blood pressure control among others in the context of CHD and atherosclerosis [40]. Furthermore, PUFA-containing PL can be oxidized by LPO in the context of CHD and generates a plethora of oxidation products [11]. The oxidized PL can be hydrolyzed by PAF-AH to form oxidized fatty acids [41,42]. Among these oxidation products, isoprostanes (IsoPs), isomers of prostaglandins, have been well characterized and studied in CHD [19,35,43]. These two processes are interrelated in the initiation and progression of CHD due to the presence of oxidative stress and chronic inflammation in atherosclerosis [44–46]. Our results showed that oxPLs were strikingly increased in MI patients. Among them, HETE-PC, PECPC, HODE-PC, KDdiA-PC and HODA-PC were the top five oxidized products. The roles of oxidized PL in atherosclerosis have been a research focus for the past decades and both anti-atherosclerotic and pro-atherosclerotic effects of these oxPLs have been reported [46,47]. PECPC is a potent anti-inflammatory lipid mediator involved in Nrf2 pathway [48] whereas POVPC exhibits pro-inflammatory bioactivity mediated by Toll-like receptor 2 [49]. Truncated products KODA-PC, HODA-PC and KDdiA-PC are ligands of CD36, which directly contribute to the development of macrophage foam cell formation [50]. We also found elevated levels of IsoPs-PC in plasma of MI patients [19,51], specifically, D_2/E_2-IsoP-PC, precursors of a newly discovered deoxy-A_2/J_2-IsoP-PC, which have potent anti-inflammatory and antioxidant properties [22]. Moreover, LA esters of cholesterol reside in the core of LDL, and HODEs are major oxidation products derived from LA [52,53]. Previous studies demonstrated that oxidation products from cholesterol esters have been associated with atherosclerosis [54,55]. Distinct from enzymatically generated eicosanoids, LPO products of IsoPs consisted of multiple peaks in MS analysis due to the presence of multiple stereoisomers [56,57] (Fig. S2). Interestingly, we observed that LPO metabolites were significantly elevated only in MI patients whereas SA patients had modest elevations comparing to control. It warrants further investigation and validation in large cohorts for this interesting and significant observation.

In addition, several amino acids or intermediates of amino acid metabolisms have been identified as differential metabolites associated with CHD. For example, MI patients had obviously decreased tryptophan and arginine levels, corroborated with previous publications [58–61].

Metabolomics, systematic analysis of all metabolites and metabolic pathways in a given biological system, has been increasingly recognized as an enabling technique in biomarker discovery and understanding disease mechanisms [23]. This technique attracts increasing attentions in CHD research [30]. Lipidomic is a branch of metabolomics, which has been considered as a powerful technique to systematically investigate all the lipids and pathways associated with lipid metabolism. Due to the importance of lipid metabolism in atherosclerosis and CHD, a number of studies have been carried out using lipidomics to identify feature of lipids associated with different stages of CHD [54,62,63]. Metabolomics and lipidomics can be carried out in a targeted and untargeted fashion and the limitations of these techniques have to be

taken into consideration in a study [31]. One of the biggest challenges in the metabolomic analysis is the quantification of a wide range of abundances of metabolites. In this study, we combined untargeted and targeted approach to identify metabolic features of different CHD. The oxidized PL and their downstream metabolites were quantified in a targeted manner using internal standards and MRM technique, which enabled us to differentiate the LPO products from enzymatic products [36,37,64]. Thus, the identification of LPO products as a predominant feature in human plasma has significant implications in understanding the pathophysiology of atherosclerosis and CHD as well as identifying potential biomarkers. It is noteworthy that oxidized cholesterol ester may be also targeted for future analysis due to the important roles of these oxidation products in atherosclerosis [54].

In summary, we observed a significantly disturbed metabolic profile and LPO in the different stages of CHD through metabolomics and identified a variety of differential metabolites to distinguish HC, SA and MI patients. Among them the metabolites associated with LPO appeared to be a predominant feature in CHD and may be used as potential biomarkers to differentiate MI from SA and HC. Our study has highlighted the power of using comprehensive metabolomic (lipidomic) approach to identify biomarkers and underlying mechanisms in CHD.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.redox.2017.04.032.

References

[1] D. Steinberg, J.L. Witztum, Oxidized low-density lipoprotein and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 2311–2316.
[2] U.P. Steinbrecker, S. Parthasarathy, D.S. Leake, J.L. Witztum, D. Steinberg, Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids, Proc. Natl. Acad. Sci. USA 81 (1984) 3883–3887.
[3] D. Mozaffarian, E.J. Benjamin, A.S. Go, D.K. Arnett, M.J. Blaha, M. Cushman, S.R. Das, S. de Ferranti, J.-P. Després, H.J. Fullerton, V.J. Howard, M.D. Huffman, C.R. Isasi, M.C. Jiménez, S.E. Judd, B.M. Kissela, J.H. Lichtman, L.D. Lisabeth, S. Liu, R.H. Mackey, D.J. Magid, D.K. McGuire, E.R. Mohler, C.S. Moy, P. Muntner, M.E. Mussolino, K. Nasir, R.W. Neumar, G. Nichol, L. Palaniappan, D.K. Pandey, M.J. Reeves, C.J. Rodriguez, W. Rosamond, P.D. Sorlie, J. Stein, A. Towfighi, T.N. Turan, S.S. Virani, D. Woo, R.W. Yeh, M.B. Turner, Executive summary: heart disease and stroke statistics—2016 update: a report from the American Heart Association, Circulation 133 (2016) 447–454.
[4] D. Mozaffarian, E.J. Benjamin, A.S. Go, D.K. Arnett, M.J. Blaha, M. Cushman, S.R. Das, S. de Ferranti, J.-P. Després, H.J. Fullerton, V.J. Howard, M.D. Huffman, C.R. Isasi, M.C. Jiménez, S.E. Judd, B.M. Kissela, J.H. Lichtman, L.D. Lisabeth, S. Liu, R.H. Mackey, D.J. Magid, D.K. McGuire, E.R. Mohler, C.S. Moy, P. Muntner, M.E. Mussolino, K. Nasir, R.W. Neumar, G. Nichol, L. Palaniappan, D.K. Pandey, M.J. Reeves, C.J. Rodriguez, W. Rosamond, P.D. Sorlie, J. Stein, A. Towfighi, T.N. Turan, S.S. Virani, D. Woo, R.W. Yeh, M.B. Turner, heart disease and stroke statistics—2016 update: a report from the American Heart Association, Circulation 133 (2016) e38–e360.
[5] C.K. Glass, J.L. Witztu, Atherosclerosis: the road ahead, Cell 104 (2001) 503–516.
[6] S. Tsimikas, Lipoprotein(a): novel target and emergence of novel therapies to lower cardiovascular disease risk, Curr. Opin. Endocrinol. Diabetes Obes. (2016).
[7] J.A. Berliner, M. Navab, A.M. Folgelman, J.S. Frank, L.L. Demer, P.A. Edwards, A.D. Watson, A.J. Lusis, Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics, Circulation 91 (1995) 2488–2496.
[8] J.A. Berliner, A.D. Watson, A role for oxidized phospholipids in atherosclerosis, N. Engl. J. Med. 353 (2005) 9–11.
[9] S. Lee, K.G. Birukov, C.E. Romanoski, J.R. Springstead, A.J. Lusis, J.A. Berliner, Role of phospholipid oxidation products in atherosclerosis, Circ. Res. 111 (2012) 778–799.
[10] S. Tsimikas, Oxidative biomarkers in the diagnosis and prognosis of cardiovascular disease, Am. J. Cardiol. 98 (2006) 9P–17P.
[11] H. Yin, L. Xu, N.A. Porter, Free radical lipid peroxidation: mechanisms and analysis, Chem. Rev. 111 (2011) 5944–5972.
[12] H. Yin, N.A. Porter, New insights regarding the autoxidation of polyunsaturated fatty acids, Antioxid. Redox Signal. 7 (2005) 170–184.
[13] Moore, Kathryn J., and Tabas, I, Macrophages in the pathogenesis of atherosclerosis, Cell 145 (2011) 341–355.
[14] E.A. Podrez, H.M. Abu-Soud, S.L. Hazen, Myeloperoxidase-generated oxidants and atherosclerosis, Free Radic. Biol. Med. 28 (2000) 1717–1725.
[15] E.A. Podrez, D. Schmitt, H.F. Hoff, S.L. Hazen, Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro, J. Clin. Investig. 103 (1999) 1547–1560.
[16] L.J. Hazell, M.J. Davies, R. Stocker, Secondary radicals derived from chloramines of apolipoprotein B-100 contribute to HOCl-induced lipid peroxidation of low-density lipoproteins, Biochem. J. 339 (1999) 489–495.
[17] E. Malle, G. Marsche, J. Arnhold, M.J. Davies, Modification of low-density lipoprotein by myeloperoxidase-derived oxidants and reagent hypochlorous acid, Biochim. Et. Biophys. Acta (BBA) - Mol. Cell Biol. Lipids 1761 (2006) 392–415.
[18] T.M. McIntyre, S.M. Prescott, D.M. Stafforini, The emerging roles of PAF acetylhydrolase, J. Lipid Res. 50 (2009) S255–S259.
[19] G.L. Milne, H. Yin, K.D. Hardy, S.S. Davies, L.J. Roberts, Isoprostane generation and function, Chem. Rev. 111 (2011) 5973–5996.
[20] H. Yin, Y. Zhou, M. Zhu, S. Hou, Z. Li, H. Zhong, J. Lu, T. Meng, J. Wang, L. Xia, Y. Xu, Y. Wu, Role of mitochondria in programmed cell death mediated by arachidonic acid-derived eicosanoids, Mitochondrion 13 (2013) 209–224.
[21] C.D. Funk, Prostaglandins and Leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[22] J. Lu, S. Guo, X. Xue, Q. Chen, J. Ge, Y. Zhuo, H. Zhong, B. Chen, M. Zhao, W. Han, T. Suzuki, M. Zhu, L. Xia, C. Schneider, T.S. Blackwell, N.A. Porter, L. Zheng, S. Tsimikas, H. Yin, Identification of a novel series of anti-inflammatory and anti-oxidative phospholipid oxidation products containing cyclopentenone moiety in vitro and in vivo: implication in atherosclerosis, J. Biol. Chem. (2017).
[23] E.P. Rhee, R.E. Gerszten, Metabolomics and cardiovascular biomarker discovery, Clin. Chem. 58 (2012) 139–147.
[24] M.A. Martinez-Gonzalez, M. Ruiz-Canela, A. Hruby, L. Liang, A. Trichopoulou, F.B. Hu, Intervention trials with the mediterranean diet in cardiovascular prevention: understanding potential mechanisms through metabolomic profiling, J. Nutr. (2016).
[25] S.H. Shah, J.R. Bain, M.J. Muehlbauer, R.D. Stevens, D.R. Crosslin, C. Haynes, J. Dungan, L.K. Newby, E.R. Hauser, G.S. Ginsburg, C.B. Newgard, W.E. Kraus, Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events, Circ.-Cardiovasc. Genet. 3 (2010) 207–U233.
[26] X. Liu, J. Gao, J. Chen, Z. Wang, Q. Shi, H. Man, S. Guo, Y. Wang, Z. Li, W. Wang, Identification of metabolic biomarkers in patients with type 2 diabetic coronary heart diseases based on metabolomic approach, Sci. Rep. 6 (2016) 30785.
[27] W. Guo, C. Jiang, L. Yang, T. Li, X. Liu, M. Jin, K. Qu, H. Chen, X. Jin, H. Liu, H. Zhu, Y. Wang, Quantitative metabolomic profiling of plasma, urine, and liver extracts by 1H NMR spectroscopy characterizes different stages of atherosclerosis in hamsters, J. Proteome Res. 15 (2016) 3500–3510.
[28] B. Garcia-Fontana, S. Morales-Santana, C. Diaz Navarro, P. Rozas-Moreno, O. Genilloud, F. Vicente Perez, J. Perez del Palacio, M. Munoz-Torres, Metabolomic profile related to cardiovascular disease in patients with type 2 diabetes mellitus: a pilot study, Talanta 148 (2016) 135–143.
[29] J. Barallobre-Barreiro, Y.L. Chung, M. Mayr, Proteomics and metabolomics for mechanistic insights and biomarker discovery in cardiovascular disease, Rev. Esp. De. Cardiol. 66 (2013) 657–661.
[30] Y. Fan, Y. Li, Y. Chen, Y.J. Zhao, L.W. Liu, J. Li, S.L. Wang, R.N. Alolga, Y. Yin, X.M. Wang, D.S. Zhao, J.H. Shen, F.Q. Meng, X. Zhou, H. Xu, G.P. He, M.D. Lai, P. Li, W. Zhu, L.W. Qi, Comprehensive metabolomic characterization of coronary artery diseases, J. Am. Coll. Cardiol. 68 (2016) 1281–1293.
[31] F. Baig, R. Pechlaner, M. Mayr, Caveats of untargeted metabolomics for biomarker discovery∗, J. Am. Coll. Cardiol. 68 (2016) 1294–1296.
[32] J.L. Wang, T. Zhang, X.T. Shen, J. Liu, D.L. Zhao, Y.W. Sun, L. Wang, Y.J. Liu, X.Y. Gong, Y.X. Liu, Z.J. Zhu, F.Z. Xue, Serum metabolomics for early diagnosis of esophageal squamous cell carcinoma by UHPLC-QTOF/MS, Metabolomics 12 (2016).
[33] C. Kuhl, R. Tautenhahn, C. Bottcher, T.R. Larson, S. Neumann, CAMERA: an integrated strategy for compound spectra extraction and annotation of liquid chromatography/mass spectrometry data sets, Anal. Chem. 84 (2012) 283–289.
[34] W.B. Dunn, D. Broadhurst, P. Begley, E. Zelena, S. Francis-McIntyre, N. Anderson, M. Brown, J.D. Knowles, A. Halsall, J.N. Haselden, A.W. Nicholls, I.D. Wilson, D.B. Kell, R. Goodacre, C. H. S. M. H, Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry, Nat. Protoc. 6 (2011) 1060–1083.
[35] H. Yin, B.E. Cox, W. Liu, N.A. Porter, J.D. Morrow, G.L. Milne, Identification of intact oxidation products of glycerophospholipids in vitro and in vivo using negative ion electrospray iontrap mass spectrometry, J. Mass Spectrom. 44 (2009) 672–680.
[36] Y. Huang, M. Zhu, Z. Li, R. Sa, Q. Chu, Q. Zhang, H. Zhang, W. Tang, M. Zhang, H. Yin, Mass spectrometry-based metabolomic profiling identifies alterations in salivary redox status and fatty acid metabolism in response to inflammation and

oxidative stress in periodontal disease, Free Radic. Biol. Med. 70 (2014) 223–232.
[37] X. Yao, R. Sa, C. Ye, D. Zhang, S. Zhang, H. Xia, Y.-c. Wang, J. Jiang, H. Yin, H. Ying, Effects of thyroid hormone status on metabolic pathways of arachidonic acid in mice and humans: a targeted metabolomic approach, Prostaglandins Other Lipid Mediat. 118–119 (2015) 11–18.
[38] M. Kurano, K. Kano, T. Dohi, H. Matsumoto, K. Igarashi, M. Nishikawa, R. Ohkawa, H. Ikeda, K. Miyauchi, H. Daida, J. Aoki, Y. Yatomi, Different origins of lysophospholipid mediators between coronary and peripheral arteries in acute coronary syndrome, J. Lipid Res. 58 (2017) 433–442.
[39] P.C. Norris, D. Gosselin, D. Reichart, C.K. Glass, E.A. Dennis, Phospholipase A2 regulates eicosanoid class switching during inflammasome activation, Proc. Natl. Acad. Sci. USA (2014).
[40] E.A. Dennis, P.C. Norris, Eicosanoid storm in infection and inflammation, Nat. Rev. Immunol. 15 (2015) 511–523.
[41] P. Kougias, H. Chai, P.H. Lin, A.B. Lumsden, Q. Yao, C. Chen, Lysophosphatidylcholine and secretory phospholipase A2 in vascular disease: mediators of endothelial dysfunction and atherosclerosis, Med. Sci. Monit.: Int. Med. J. Exp. Clin. Res. 12 (2006) RA5–RA16.
[42] G. Schmitz, K. Ruebsaamen, Metabolism and atherogenic disease association of lysophosphatidylcholine, Atherosclerosis 208 (2010) 10–18.
[43] J.D. Morrow, Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 279–286.
[44] J.L. Witztum, A.H. Lichtman, The influence of innate and adaptive immune responses on atherosclerosis, Annu. Rev. Pathol. 9 (2014) 73–102.
[45] A.E. Fraley, G.G. Schwartz, A.G. Olsson, S. Kinlay, M. Szarek, N. Rifai, P. Libby, P. Ganz, J.L. Witztum, S. Tsimikas, Relationship of oxidized phospholipids and biomarkers of oxidized low-density lipoprotein with cardiovascular risk factors, inflammatory biomarkers, and effect of statin therapy in patients with acute coronary syndromes: results from the MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) trial, J. Am. Coll. Cardiol. 53 (2009) 2186–2196.
[46] S. Tsimikas, E.S. Brilakis, E.R. Miller, J.P. McConnell, R.J. Lennon, K.S. Kornman, J.L. Witztum, P.B. Berger, Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease, N. Engl. J. Med. 353 (2005) 46–57.
[47] S. Tsimikas, J.L. Witztum, The role of oxidized phospholipids in mediating lipoprotein(a) atherogenicity, Curr. Opin. Lipidol. 19 (2008) 369–377.
[48] P. Bretscher, J. Egger, A. Shamshiev, M. Trotzmuller, H. Kofeler, E.M. Carreira, M. Kopf, S. Freigang, Phospholipid oxidation generates potent anti-inflammatory lipid mediators that mimic structurally related pro-resolving eicosanoids by activating Nrf2, EMBO Mol. Med. 7 (2015) 593–607.
[49] A. Kadl, P.R. Sharma, W.S. Chen, R. Agrawal, A.K. Meher, S. Rudraiah, N. Grubbs, R. Sharma, N. Leitinger, Oxidized phospholipid-induced inflammation is mediated by Toll-like receptor 2, Free Radic. Biol. Med. 51 (2011) 1903–1909.
[50] E.A. Podrez, E. Poliakov, Z. Shen, R. Zhang, Y. Deng, M. Sun, P.J. Finton, L. Shan, M. Febbraio, D.P. Hajjar, R.L. Silverstein, H.F. Hoff, R.G. Salomon, S.L. Hazen, A novel family of atherogenic oxidized phospholipids promotes macrophage foam cell formation via the scavenger receptor CD36 and is enriched in atherosclerotic lesions, J. Biol. Chem. 277 (2002) 38517–38523.
[51] W. Liu, J.D. Morrow, H. Yin, Quantification of F2-isoprostanes as a reliable index of oxidative stress in vivo using gas chromatography-mass spectrometry (GC-MS) method, Free Radic. Biol. Med. 47 (2009) 1101–1107.
[52] G. Spiteller, Linoleic acid peroxidation - the dominant lipid peroxidation process in low density lipoprotein - and its relationship to chronic diseases, Chem. Phys. Lipids 95 (1998) 105–162.
[53] W. Liu, H. Yin, Y.O. Akazawa, Y. Yoshida, E. Niki, N.A. Porter, Ex vivo oxidation in tissue and plasma assays of Hydroxyoctadecadienoates: Z,E/E,E stereoisomer ratios, Chem. Res. Toxicol. 23 (2010) 986–995.
[54] A. Ravandi, G. Leibundgut, M.Y. Hung, M. Patel, P.M. Hutchins, R.C. Murphy, A. Prasad, E. Mahmud, Y.I. Miller, E.A. Dennis, J.L. Witztum, S. Tsimikas, Release and capture of bioactive oxidized phospholipids and oxidized cholesteryl esters during percutaneous coronary and peripheral arterial interventions in humans, J. Am. Coll. Cardiol. 63 (2014) 1961–1971.
[55] S.H. Choi, H. Yin, A. Ravandi, A. Armando, D. Dumlao, J. Kim, F. Almazan, A.M. Taylor, C.A. McNamara, S. Tsimikas, E.A. Dennis, J.L. Witztum, Y.I. Miller, Polyoxygenated cholesterol ester hydroperoxide activates TLR4 and SYK dependent signaling in macrophages, PLoS One 8 (2013) e83145.
[56] H. Yin, T. Davis, N.A. Porter, Simultaneous analysis of multiple lipid oxidation products in vivo by liquid chromatographic-mass spectrometry (LC-MS), Methods Mol. Biol. 610 (2010) 375–386.
[57] H. Yin, L. Gao, H.-H. Tai, L.J. Murphey, N.A. Porter, J.D. Morrow, Urinary prostaglandin F_2 is generated from the isoprostane pathway and not the cyclooxygenase in humans, J. Biol. Chem. 282 (2007) 329–336.
[58] S. Zhang, S. Zhang, H. Wang, W. Wu, Y. Ye, Arginine methylation dysfunction increased risk of acute coronary syndrome in coronary artery disease population: a case-control study, Medicine 96 (2017) e6074.
[59] K.A. Polyzos, D.F.J. Ketelhuth, The role of the kynurenine pathway of tryptophan metabolism in cardiovascular disease. An emerging field, Hamostaseologie 35 (2015) 128–136.
[60] C. Murr, T.B. Grammer, M.E. Kleber, A. Meinitzer, W. Marz, D. Fuchs, Low serum tryptophan predicts higher mortality in cardiovascular disease, Eur. J. Clin. Investig. 45 (2015) 247–254.
[61] E. Yu, M. Ruiz-Canela, M. Guasch-Ferre, Y. Zheng, E. Toledo, C.B. Clish, J. Salas-Salvado, L. Liang, D.D. Wang, D. Corella, M. Fito, E. Gomez-Gracia, J. Lapetra, R. Estruch, E. Ros, M. Cofan, F. Aros, D. Romaguera, L. Serra-Majem, J.V. Sorli, F.B. Hu, M.A. Martinez-Gonzalez, Increases in plasma tryptophan are inversely associated with incident cardiovascular disease in the Prevencion con Dieta Mediterranea (PREDIMED) Study, J. Nutr. (2017).
[62] FitzGerald, Garret A, Human platelet lipidomics: variance, visualization, flux, and fuel, Cell Metab. 23 (2016) 757–759.
[63] R. Capoulade, K.L. Chan, C. Yeang, P. Mathieu, Y. Bossé, J.G. Dumesnil, J.W. Tam, K.K. Teo, A. Mahmut, X. Yang, J.L. Witztum, B.J. Arsenault, J.-P. Després, P. Pibarot, S. Tsimikas, Oxidized phospholipids, lipoprotein(a), and progression of calcific aortic valve stenosis, J. Am. Coll. Cardiol. 66 (2015) 1236–1246.
[64] S. Li, Q. Chu, J. Ma, Y. Sun, T. Tao, R. Huang, Y. Liao, J. Yue, J. Zheng, L. Wang, X. Xue, M. Zhu, X. Kang, H. Yin, W. Liu, Discovery of novel lipid profiles in PCOS: Do insulin and androgen oppositely regulate bioactive lipid production? J. Clin. Endocrinol. Metab. 2016. jc20162692.