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.

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**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...
Emerging studies demonstrated altered metabolite pro-
files and improve clinical diagnosis and treatment of diseases [23].
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 metabolic 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 infarction (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.

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

2. Materials and methods

2.1. Reagents and materials

Liquid chromatography-Mass Spectrometry (LC-MS) grade water (H₂O), methanol (MeOH), acetonitrile (ACN), 0.1% formic acid (FA) in water, 0.1% FA in ACN and HPLC grade chloroform (CHCl₃) and isopropanol (IPA) were purchased from Honeywell (Muskegon, MI, USA). Ammonium fluoride (NH₄F), ammonium hydroxide (NH₄OH), acetonitrile (ACN) and formic acid (FA) were 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 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 UHPLC analysis. Pulse 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 multi-variative 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 PPh3 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 solution to make a 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 (CHCl₃/MeOH/NH₃·H₂O, 60/34/5.5/0.5, by vol) to 100% B (CHCl₃/MeOH/NH₃·H₂O, 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 metabolic 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 (H₂O/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/m²) | 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.
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 (R²), an indicator of model robustness, and cross-validated predictive ability (Q²) were obtained as follows: HC versus SA, cumulative R² at 0.98 and Q² at 0.46 (Fig. 2B); SA versus MI, R² at 0.96 and Q² at 0.62 (Fig. 2C); HC versus MI, R² at 0.89 and Q² 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 |
|-------------|-----------|-------------|---------|
| 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: phosphatidylethanolamine; LysoPE: lyso-phosphatidylethanolamine.

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

| Metabolites | VIP value | Fold Change | p value |
|-------------|-----------|-------------|---------|
| 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 | 0.002 |
| SM (d16:1/26:1) | 4.21 | 0.35 | < 0.001 |
| PC (14:1/4:0) | 4.08 | 6.35 | 0.003 |
| 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 | 10.96 | 0.001 |
| PC (16:0/20:1) | 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 |
| L-Adenosine monophosphate | 1.69 | 2.87 | 0.003 |
| L-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 |
| t-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 | 2.96 | 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 | 0.011 |
| Urocanic acid | 1.06 | 1.86 | 0.011 |
| PC (22:2/18:4) | 1.02 | 1.82 | 0.011 |

PC: phosphatidylethanolamine; PG: phosphatidyglycerol; SM: sphingomyelin; MG: monoacyl-glycerol; PE: phosphatidylinositol; PS: phosphatidylserine; PA: phosphatidic acid; 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 (oxPLs and IsoPs) 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, D2/E2-IsoPs, 5-HETE, and 9/13-HODE were hydrolyzed products of D2/E2-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.
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 A2 [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, PCEPC, HODE-PC, KDdA-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]. PCEPC 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 KDdA-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/E2-IsoP-PC, precursors of a newly discovered deoxy-A2/J2-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 metabolic 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 PI, 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 also be 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 in identifying 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.

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