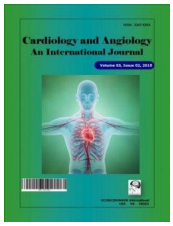

Cardiology and Angiology: An International Journal

7(4): 1-11, 2018; Article no.CA.44602
ISSN: 2347-520X, NLM ID: 101658392

Plasma and Erythrocyte Membrane Plasmalogens in Patients with Coronary Heart Diseases Undergoing Percutaneous Intervention

Takeshi Arita[1], Taku Yokoyama[1], Shohei Moriyama[1], Kei Irie[1], Mitsuhiro Fukata[1], Keita Odashiro[1], Toru Maruyama[1*], Seira Hazeyama[2], Shiro Mawatari[2], Takehiko Fujino[3] and Koichi Akashi[1]

[1]Department of Medicine, Kyushu University, Fukuoka 812-8582, Japan.
[2]Institute of Rheological Function of Foods Co., Ltd., Hisayama 811-2501, Japan.
[3]BOOCS Clinic, Fukuoka 812-0025, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Author TA performed main part of this research project and manuscript preparation. Authors TY and S. Moriyama were the main operators of PCI in the enrolled subjects and hence contributed blood sampling. Authors KI and MF performed practical data extraction from medical records. Author KO contributed to the sampling, input and screening of extracted data. Author TM contributed to the statistical analyses and manuscript preparation as a corresponding author. Authors SH and S. Mawatari performed plasma and erythrocyte membrane phospholipid assay. Author TF had the initial research concept, and author KA is a team leader and supervised the team collaboration. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CA/2018/44602
Editor(s):
(1) Dr. Gen-Min Lin, Division of Cardiology, Hualien-Armed Forces General Hospital, National Defense Medical Center, Taiwan.
Reviewers:
(1) Mra Aye, Melaka Manipal Medical College, Malaysia.
(2) Wang Jie, Guanganmen Hospital, China Academy of Chinese Medical Sciences, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/26742

Original Research Article

Received 05 August 2018
Accepted 14 October 2018
Published 22 October 2018

ABSTRACT

Aims: Plasmalogens are unique phospholipid of biological membrane and is considered to play a potent role of intrinsic antioxidant. Atherosclerosis is associated with oxidative stress, but the correlation between plasmalogens and atherosclerosis is debatable. Therefore, this study aimed to assess the plasma and erythrocyte membrane phospholipids profile in patients with coronary heart

**Corresponding author: E-mail: maruyama@artsci.kyushu-u.ac.jp;*

diseases (CHD) undergoing percutaneous coronary intervention (PCI).
Place and Duration of the Study: Vascular laboratory of Heart Center, Kyushu University Hospital, Fukuoka, Japan, from February to August 2016.
Methodology: The plasma concentrations and erythrocyte membrane contents of phospholipids were quantified in patients with CHD (n = 30, group A) and age-matched controls (n = 38, group B) using high-performance liquid chromatography with evaporative light scattering detection method.
Results: Plasma concentrations of plasmalogens in group A were significantly lower than those in group B. Similar findings were obtained from relative contents of plasmalogens in the erythrocyte membrane. Multiple regression models for plasmalogens yielded phospholipids other than plasmalogen as determinants of plasmalogens.
Conclusions: This cross-sectional study indicated that plasma and erythrocyte membrane plasmalogens are reduced in patients with CHD undergoing PCI. Further longitudinal studies are required to elucidate the clinical role of intrinsic plasmalogens as a laboratory marker of oxidative stress and extrinsic plasmalogens as a novel therapy for atherosclerosis.

Keywords: Coronary heart disease; percutaneous coronary intervention; erythrocyte membrane; plasmalogens.

1. INTRODUCTION

Atherosclerosis is a worldwide health burden and underlies coronary atherosclerosis leading to angina pectoris, acute myocardial infarction and sudden cardiac death. Vascular damage induced by oxidative stress is thought to trigger inflammatory process and play a major role in coronary atherosclerosis [1,2]. Oxidative stress alters the content and distribution of circulating erythrocyte membrane phospholipids and fatty acids [3] and impairs the rheological function of erythrocytes [4,5]. Therefore, erythrocyte membrane phospholipids contents and profiles reflect the vascular and systemic redox status [6].

Phospholipid class of plasmalogen is ubiquitously found in biological membrane and involved in cellular signalling pathway and differentiation [7]. Emerging evidence suggests that plasmalogen is a sensitive marker of oxidative stress [8,9], because this unique glycerophospholipid contains fatty alcohol with a vinyl-ether bond at the sn-1 position and is rich in polyunsaturated fatty acids at the sn-2 position of the glycerol backbone (Fig. 1). Erythrocyte membrane is a target sample of redox biology, since erythrocytes carry oxygen, have no intracellular organelle, and hence phospholipids including plasmalogen are present only in surface membrane. Plasmalogens in atherosclerotic patients are still debatable in current studies, i.e., a few studies reported that serum or erythrocyte membrane plasmalogens are diminished [8,10-12] except for one [13]. This discrepancy stems from differences of enrolled subjects' baseline characteristics and age-dependent changes of plasmalogen contents, which show a dramatic increase from neonates to adults and the following gradual decrease. This complicated alteration is related to brain development in youth and redox imbalance, peroxisome dysfunction in the senescence [11,12].

Therefore, the present study was designed to investigate the plasmalogens in plasma and erythrocyte membrane obtained from patients with coronary heart diseases (CHD) undergoing percutaneous coronary intervention (PCI).

2. SUBJECTS AND METHODS

2.1 Subjects

This study was designed as a cross-sectional case-control study using two subjects groups derived from two different facilities by means of the same plasmalogens assay techniques and performed from February to August 2016 according to the Declaration of Helsinki (2008). In this study, we enrolled 30 Japanese patients with CHD (group A: 72.3 ± 7.9 years, 6 women and 24 men) and 38 age-matched control subjects (group B: 71.6 ± 5.1 years, 25 women and 13 men). Exclusion criteria included hemodialysis [8], dementia [14] and cancer [15]. Blood examination was carried out after overnight fasting, and other routine laboratory examinations were performed in the morning. Blood pressure was measured at sitting position by sphygmomanometer after taking a few minutes rest. Body mass index (BMI) was calculated by body weight (kg) divided by square of height (m^2). Hypertension was diagnosed by

the use of antihypertensive drugs and/or systolic blood pressure (BP) ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg [16]. Diabetes was defined as a fasting blood glucose level of ≥ 126 mg/dl, a casual blood glucose level of ≥ 200 mg/dl, an HbA1c level of ≥ 6.5% and/or current antidiabetic medication. Dyslipidemia was defined as an LDL cholesterol level of ≥ 140 mg/dl, an HDL cholesterol level of < 40 mg/dl and/or the prescription of lipid-lowering agents [17,18].

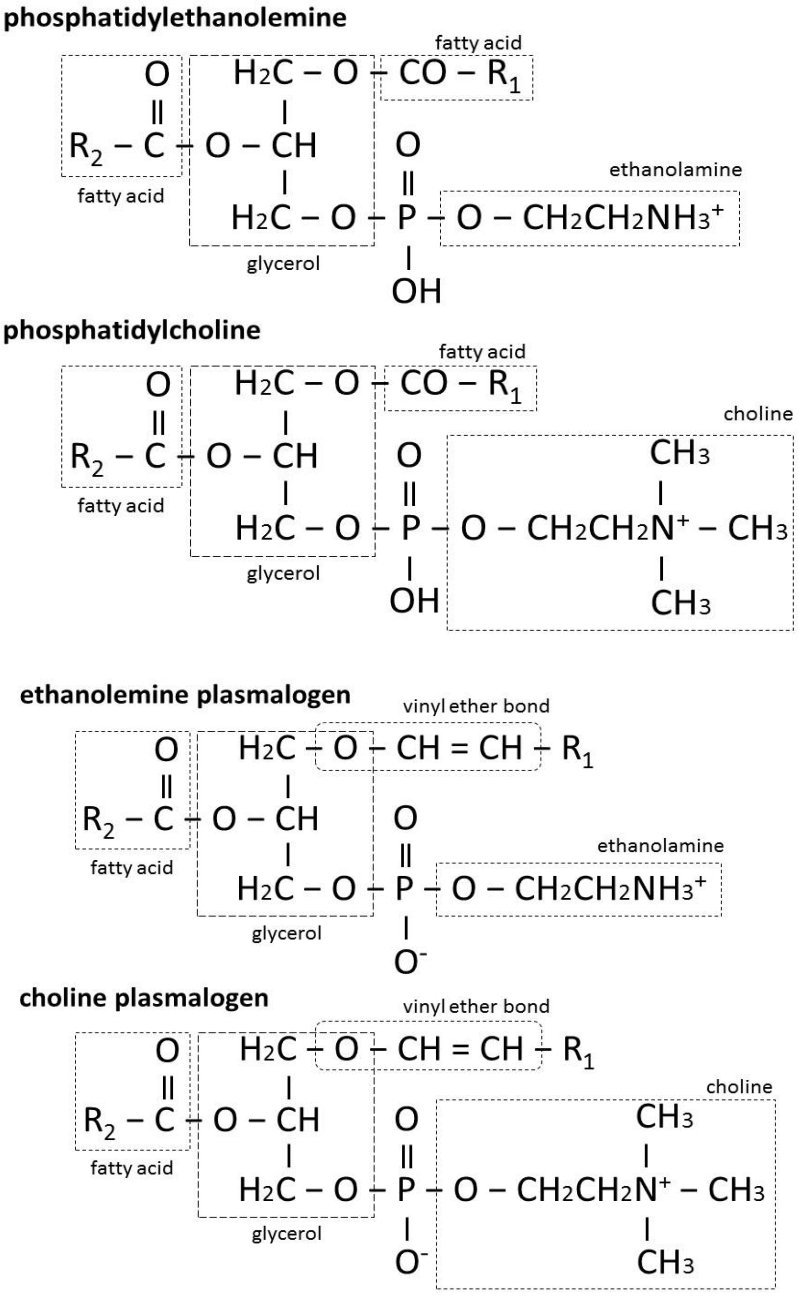

Fig. 1. Structures of phosphatidylethanolamine (PE), phosphatidylcholine (PC), ethanolamine plasmalogen (PlsEtn) and choline plasmalogen (PlsCho). R1 denotes the carbon chain at the sn-1 position, and R2 at the sn-2 position.

Patients with CHD in group A were referred to the Heart Center of the Kyushu University Hospital (Fukuoka, Japan) for PCI. These patients had coronary artery diseases such as angina pectoris (n = 13), recent myocardial infarction (n = 7) and old myocardial infarction (n = 3). PCI included plane old balloon angioplasty, cutting balloon angioplasty and coronary stent implantation. There were no current smokers, i.e., smoking cessation had been strongly recommended prior to the enrollment into this study. Most of them (90%) had at least one of the coronary risk factors such as hypertension (n = 22, 73.3%), dyslipidemia (n = 16, 53.3%) and diabetes (n = 13, 43.3%). They were treated with antihypertensive drugs including β-blocking agents (n = 15, 50%), angiotensin receptor blocking agents (n = 12, 40%) and long-acting Ca antagonists (n = 11, 36.7%), although combined antihypertensive medication was common (n = 12, 40%). Anti-platelet agents (aspirin, clopidogrel, prasugrel, and the combination of these) were prescribed in most of the patients (n = 23, 76.7%). Hydroxymethylglutaryl-CoA reductase inhibitors (so-called statin) were prescribed to lower the serum LDL cholesterol (n = 17, 56.7%). Highly purified agents of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and/or docosahexanoeic acid (DHA) were prescribed to improve the serum lipid profile, the endothelial function and the blood rheology (n = 9, 30%). These prescriptions were under the discretion of the treating physicians.

Group B contained control Japanese subjects visiting the outpatient clinic of BOOCS Clinic (Fukuoka, Japan) for the purpose of annual health screening or conservative medication of hypertension or dyslipidemia. Drug prescription in group B was less common than that in group A, i.e., they were prescribed with long-acting Ca antagonists (13.2%), statin (7.9%) and angiotensin receptor blocking agents (5.3%). There were no smokers in this group. All procedures performed in this study were in accordance with the current ethical standards of each institutional and/or national research committee and with the updated Declaration of Helsinki (2008), i.e., signed informed consent was obtained from each subject after the admission to the hospital and before the PCI in group A or at the enrollment into the study in group B.

2.2 Measurements of Plasma Concentration of Phospholipids

Venous blood was sampled by disposable syringe containing heparin, packed and cooled immediately in ice bath, then kept in refrigerator, and processed within 48 hours. Plasma was separated by centrifugation at 1,000 x *g* for 5 minutes at 4°C and kept at -80°C until measurement. Plasma concentrations of plasmalogens are too low to be detected by the conventional single run of high performance liquid chromatography (HPLC). Therefore, these concentrations were quantified after the treatment of plasma with phospholipase A_1 (PLA_1), which hydrolyses ester (acyl) bond but not ether bond, i.e., all diacyl phospholipids are completely hydrolysed, whereas sphingomyelin (SM) and ether phospholipids remain intact [18]. There are two types of ether bonds in ether phospholipids, i.e., alkyl bonds and alkenyl bond. Phospholipids with an alkenyl bond are plasmalogens (Fig. 1). PLA_1 purchased from Sigma-Aldrich Co. (Tokyo, Japan) and Mitsubishi Kagaku Foods Co. (Tokyo, Japan) was diluted with an equal volume of 0.1 M citrate buffer (pH 4.5), and 20 µl of the diluted PLA_1 was added to 80 µl of plasma and incubated at 45°C for 60 minutes.

Lipid extraction after the treatment with PLA_1 was performed by adding 800 µl of *n*-hexane/isopropanol (3:2, v/v) to the PLA_1-treated plasma. After vigorous mixing, it was placed in an ultrasound bath for 5 minutes. Then, 400 µl of Na_2SO_4 solution was added and left for 5 minutes, and 400 µl of hexane layer was transferred to a new conical Eppendorf tube. Thereafter, 400 µl of hexane/isopropanol (7:2, v/v) was added to the lower phase and vigorously mixed, and the hexane layer (300 µl) was recovered. The combined hexane layer was dried under N_2 gas and stored at -30°C until use. Separation of phospholipid classes including ether phospholipids was performed by using HPLC-ELSD system [19], which was composed of an Agilent 1100 equipped with a four-solvent delivery system, a degasser, an automatic injector, and evaporative light scattering detector (ELSD). The column was a LiChrosphere 100 Diol (250 x 2 mm), 5 µm (Merck, Germany). Mobile phase A was *n*-hexane/2-propanol/acetic acid (82:17:1, v/v/v) with 0.08% triethylamine (TEA), and mobile phase B was 2-propanol/water/acetic acid (85:14:1, v/v/v) with 0.08% TEA. The lipid extract after PLA_1 treatment of plasma was reconstituted with 200

μl of hexane/isopropanol (3:2, v/v), and 20 μl was applied to the HPLC system. The column temperature was 50°C and flow rate was 0.4 ml/min. The phospholipid classes were detected by ELSD (Agilent 1900 Infinity) with the following settings: evaporation temperature, 60°C; sensitivity gain, 6; flow rate of N_2 gas, 1 l/min. Nebuliser temperature was 30°C. HPLC-ELSD system was validated and linearity for calibration was updated periodically. To date, linear regression curves for calculation of phospholipid concentration from chromatographic peak area showed $R^2 > 0.97$ [19].

2.3 Measurements of Erythrocyte Membrane Phospholipids

Extraction of phospholipid from erythrocyte membrane was essentially performed according to the method reported previously [20]. Plasma and buffy coat were removed carefully from obtained venous blood after centrifugation at 1,000 x *g* for 5 minutes at 4°C, and the packed erythrocytes were washed three times in cold isotonic saline at 1,000 x *g* for 5 minutes at 4°C. A small portion of the supernatant was carefully aspirated at each washing. The washed erythrocytes were lysed with hypotonic buffer (10 mM Tris-HCl, pH 7.4) and were centrifuged at 25,000×g for 20 min at 4°C. This procedure was repeated four times for removing hemoglobin, and isolated erythrocyte membrane was obtained. The membranes were kept at -80°C until use.

Lipids from erythrocyte membrane were extracted with chloroform/methanol (1:2, v/v) method and total lipids were dried under N_2 gas. After the lipids were re-suspended in hexane/isopropanol (3:2, v/v), separation of erythrocyte membrane phospholipid classes was performed according to the HPLC-ELSD method [20], which can detect all the phospholipids usually found in erythrocyte membrane such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM), choline plasmalogen (PlsCho), ethanolamine plasmalogen (PlsEtn) together with ether phospholipids (ePE and ePC) by a single run of HPLC.

2.4 Statistical Analyses

Sample size was calculated to provide 90% power with an α error of 0.05. A total of ≥ 68 cases were required based on our preliminary study investigating erythrocyte membrane phospholipids distribution including plasmalogen [21]. For discrete data, comparison of data between the two groups was performed by chi-square (χ^2) test or the Fisher's exact test. Yates' correction was added if necessary. For continuous data, data are expressed as means ± SD, and the Kolmogorov-Smirnov test was first applied to investigate the normality of the data distribution. Normally distributed data were compared with unpaired Student's *t* test. Comparison of data which were not distributed normally was performed by Mann-Whitney *U* test. None of the variables with missing data qualified. Multiple regression analyses were performed preserving constant term to assess the association of plasmalogens and other data. Criteria for entering into the regression models were a significant intergroup difference or otherwise the clinical meaning of the variables. Partial regression coefficients (β), standardised β and 95% confidence of interval (CI) of β were expressed. Adjusted coefficient of determination (R^2) more than 0.200 was accepted. These analyses were performed using Bell Curve for Excels version 2.12 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Differences with two-sided $p < 0.05$ were considered significant.

3. RESULTS

3.1 Baseline Characteristics of Subjects

The baseline characteristics of the enrolled subjects were detailed in Table 1. PCI was finished electively and successfully in all the patients with CHD in group A. Although gender ratio in group A differed from the ratio in group B, there were no significant differences in age and BMI between the two groups. Hypertension, dyslipidemia and diabetes in group A were prevalent than those in group B ($p < 0.001$). However, diastolic BP in group A was lower than that in group B ($p = 0.003$) indicating that BP control in group A is strict. HbA1c in group A was significantly greater than that in group B ($p < 0.001$), but average HbA1c in group A (6.2 ± 1.6%) was within the normal range of the National Glycohemoglobin Standardization Program (NGSP), indicating that diabetic control in group A is intensive. Total, LDL and HDL cholesterol levels in group A were lower than the respective levels in group B ($p < 0.001$), indicating that lipid lowering therapy is also intensive. Group A showed the tendency of mild anemia ($p = 0.013$), hypoalbuminemia ($p < 0.001$) and renal impairment ($p = 0.020$) relative

to group B. These findings indicate that intensive medication for coronary risk factors continued in patients with CHD prior to PCI.

3.2 Plasma Concentration of Phospholipids

Plasma concentrations of phospholipids in the two groups were summarised in Table 2. The concentrations of PlsEtn (p = 0.003) and PlsCho (p < 0.001) in group A were reduced commonly relative to the respective concentrations in group B. The same was true in the case of plasma concentration of SM (p < 0.001).

3.3 Erythrocyte Membrane Phospholipids

Erythrocyte membrane phospholipid fractions were simultaneously detected by a single run of HPLC, and relative composition of phospholipid classes was calculated on the basis of each chromatographic peak area [3]. The area of PlsEtn in the erythrocyte membrane of group A was significantly reduced relative to the area in group B (p = 0.001). PC in group A was significantly lesser than that in group B (p < 0.001), whereas SM in group A was greater than that in group B (p < 0.001).

3.4 Association of Plasmalogen with Other Data

As plasma concentrations of PlsEtn and PlsCho in group A were lesser than the corresponding concentrations in group B (Table 2), association of plasmalogens with other data including baseline characteristics or laboratory data was assessed by multiple regression analyses. Plasma concentration of SM alone was the contributor to the plasma concentrations of both PlsEtn (Table 4) and PlsCho (Table 5). Multiple regression models in both cases were acceptable considering sufficient R^2 (0.215 (p = 0.002) and 0.481 (p < 0.001), respectively). Likewise, relative content of PlsEtn in the erythrocyte membrane of group A was lesser than that in group B (Table 3), and multiple regression demonstrated that both erythrocyte membrane PC and SM contents contributed negatively to the erythrocyte PlsEtn (p < 0.001 in both cases) with highly significance (Table 6) under R^2 = 0.484 (p < 0.001). Among coronary risk factors, dyslipidemia alone has negative impact on the erythrocyte PlsEtn content with modest significance (p = 0.035).

Table 1. Baseline characteristics and laboratory data in the two groups

	Group A (n = 30)	Group B (n = 38)	p value
Age (years)	72.3 ± 7.9	71.6 ± 5.1	0.456
Gender (female/male)	6 / 24	25 / 13	< 0.001
BMI (kg/m^2)	23.2 ± 3.2	22.2 ± 2.4	0.165*
Hypertension	22 (73.3)	6 (15.8)	< 0.001
Dyslipidemia	16 (53.3)	14 (36.8)	< 0.001
Diabetes mellitus	13 (43.3)	0 (0)	< 0.001
Systolic BP (mmHg)	128.4 ± 17.1	133.6 ± 14.9	0.189*
Diastolic BP (mmHg)	69.6 ± 10.0	78.2 ± 12.0	0.003*
Erythrocytes (x10^4 /μl)	395 ± 92	440 ± 31	0.013
Leukocytes (/μl)	5728 ± 1283	5106 ± 1388	0.068
Platelets (x10^4 /μl)	19.9 ± 4.7	22.6 ± 4.3	0.016*
Serum albumin (g/dl)	4.0 ± 0.5	4.5 ± 0.2	< 0.001
AST (IU/l)	23.8 ± 9.0	22.9 ± 4.5	0.846
ALT (IU/l)	23.7 ± 16.2	17.7 ± 5.4	0.241
BUN (mg/dl)	21.1 ± 9.7	16.5 ± 2.9	0.062
Uric acid (mg/dl)	5.7 ± 1.8	5.4 ± 1.2	0.351*
Creatinine (mg/dl)	1.12 ± 0.91	0.76 ± 0.17	0.020
HbA1c (%)	6.2 ± 1.6	5.6 ± 0.3	< 0.001
Total cholesterol (mg/dL)	168.6 ± 33.9	210.5 ± 33.2	< 0.001*
HDL cholesterol (mg/dL)	51.1 ± 15.4	68.0 ± 14.3	< 0.001*
LDL cholesterol (mg/dL)	93.8 ± 29.7	123.2 ± 25.6	< 0.001*
Triglyceride (mg/dL)	131.0 ± 81.7	108.1 ± 52.3	0.162
C-reactive protein (mg/dL)	0.36 ± 0.61	0.13 ± 0.15	0.264

**Data indicate that distribution shows normality. Parentheses indicate units. BMI, body mass index calculated by body weight (kg) divided by the square of height (m^2); BP, blood pressure; HbA1c, hemoglobin A1c estimated according to the National Glycohemoglobin Standardization Program (NGSP).*

Table 2. Plasma concentrations of phospholipids in the two groups

	Group A (*n* = 30)	Group B (*n* = 38)	*p* value
PlsEtn (mg/dL)	3.58 ± 0.95	4.34 ± 1.04	0.003*
PlsCho (mg/dL)	3.59 ± 0.81	4.38 ± 0.74	< 0.001*
SM (mg/dL)	23.13 ± 4.55	28.09 ± 3.60	< 0.001

**Data indicate that distribution shows normality. Parentheses indicate units. Cho, choline; Etn, ethanolamine; Pls, plasmalogen; SM, sphingomyelin.*

Table 3. Erythrocyte membrane distribution of phospholipids in the two groups

	Group A (*n* = 30)	Group B (*n* = 38)	*p* value
PlsEtn (%)	7.76 ± 0.81	8.49 ± 0.92	0.001
PE (%)	9.98 ± 1.18	10.04 ± 1.12	0.827
PC (%)	22.06 ± 2.92	24.97 ± 2.72	< 0.001
PS (%)	8.87 ± 1.02	9.27 ± 1.31	0.186
SM (%)	51.33 ± 3.73	47.22 ± 4.75	< 0.001

All data distributions show normality. Amounts of phospholipids indicate relative values based on the chromatographic peak area (%). PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine. Other abbreviations are as in Table 2.

Table 4. Multiple regression analysis for association of plasma PlsEtn with other data

	β	Standardized β	95% CI of β	*p* value
Gender	0.087	0.040	- 0.472 — 0.645	0.758
Plasma SM (mg/dL)	0.094	0.407	0.032 — 0.157	0.004
Serum albumin (g/dl)	0.141	0.055	- 0.518 — 0.801	0.670
Hypertension	- 0.411	- 0.188	- 0.967 — 0.145	0.144
Dyslipidemia	0.125	0.050	- 0.542 — 0.793	0.709
Diabetes mellitus	- 0.090	- 0.033	- 0.766 — 0.587	0.792

Adjusted coefficient of determination (R^2) = 0.215 (p = 0.002). β, partial regression coefficient; CI, confidence of interval. Other abbreviations are as in Table 2.

Table 5. Multiple regression analysis for association of plasma PlsCho with other data

	β	Standardized β	95% CI of β	*p* value
Gender	- 0.146	- 0.086	- 0.503 — 0.211	0.417
Plasma SM (mg/dL)	0.127	0.696	0.087 — 0.167	< 0.001
Serum albumin (g/dl)	0.001	0.000	- 0.421 — 0.422	0.997
Hypertension	- 0.043	- 0.025	- 0.399 — 0.312	0.808
Dyslipidemia	- 0.236	- 0.120	- 0.662 — 0.191	0.274
Diabetes mellitus	- 0.079	- 0.037	- 0.512 — 0.353	0.715

Adjusted coefficient of determination (R^2) = 0.481 (p < 0.001). Abbreviations are as in Tables 2 and 4.

Table 6. Multiple regression analysis for association of erythrocyte PlsEtn with other data

	β	Standardized β	95% CI of β	*p* value
Gender	0.188	0.100	- 0.182 — 0.558	0.314
Erythrocyte PC	- 0.234	- 0.793	- 0.353 — - 0.115	< 0.001
Erythrocyte SM	- 0.229	- 1.161	- 0.308 — - 0.150	< 0.001
Serum albumin (g/dl)	0.283	0.126	- 0.185 — 0.752	0.231
Hypertension	- 0.058	- 0.030	- 0.446 — 0.331	0.768
Dyslipidemia	- 0.514	- 0.238	- 0.990 — - 0.038	0.035
Diabetes mellitus	0.024	0.010	- 0.463 — 0.511	0.922

Adjusted coefficient of determination (R^2) = 0.484 (p < 0.001). Abbreviations are as in Tables 2, 3 and 4.

4. DISCUSSION

The main findings of this study are that both plasma and erythrocyte membrane plasmalogens were commonly reduced in patients with CHD undergoing PCI, and that this reduction is related not to the baseline characteristics including conventional coronary risk factors but to the intrinsic phospholipids profiles.

PCI for CHD has developed rapidly in recent years. Medical management of coronary risk factors is essential for indication of PCI. The patients enrolled in this study are under the intensive control of conventional risk factors such as diabetes, dyslipidemia, hypertension and of residual risk factor by the administration of polyunsaturated fatty acid agents. However, altered phospholipid profiles in patients with CHD were evident in plasma (Table 2) and erythrocyte membrane (Table 3). Statin was mainly administered to the patients in group A, and this may have influenced our outcome. The effects of statin on the plasma concentrations of plasmalogens are conflicting. In literature, pitavastatin (4 mg/day) resulted in reduction of plasma LDL cholesterol and preferential increase in PlsCho and PlsEtn [22], whereas rosvastatin (10 or 40 mg/day) reduced plasma LDL cholesterol, triglyceride, SM, PC, PlsCho and PlsEtn [23]. Such discrepancy may stem from species and dose of statin. Although possible effects of statin on our data remain, reduced plasmalogens in patients with CHD are compatible to the current studies [10-12].

Plasmalogens are novel subclass of glycerophospholipid containing fatty alcohol with a vinyl-ether bond at the sn-1 position and polyunsaturated fatty acids at the sn-2 position of the glycerol backbone (Fig. 1). This structure indicates that plasmalogens are considered to be a sensitive marker of oxidative stress [8,9]. This is supported by this study demonstrating that plasma and erythrocyte plasmalogens in patients with CHD are commonly reduced (Tables 2, 3). It is important but controversial whether reduced plasmalogens are cause or result of coronary atherosclerosis, and whether this is due to reduced synthesis or increased degradation of plasmalogens. Biosynthesis of plasmalogens is regulated by peroxisome activity, whereas acute peroxidation of human erythrocytes exposed to *tertial* butyl-hydroperoxide (tBHP) causes membrane phospholipid peroxidation and selective reduction of PlsEtn (from 14.5 ± 0.5 to 11.3 ± 0.2%) in our previous study [20]. Furthermore, administration of plasmalogen precursor attenuated the reactive oxygen species accumulation and exerted protective actions against hypoxia and oxidative insults in cultured human endothelial cells [24]. These results imply that substantial plasmalogen is consumed as an endogenous antioxidant, although hydrophilic lyso-ethanolamine plasmalogen, a product of acid hydrolysis of PlsEtn, was not detected by our erythrocyte lipid extraction method using hexane/isopropanol mixture.

The present findings of the relative increase of SM and a decrease of PC in erythrocyte membrane obtained from patients with CHD (Table 3) are compatible to the results of recent studies showing unanticipated impact of phospholipid metabolism in atherosclerosis [25]. Sphingolipids including SM are accumulated in coronary plaque and are acting as inducers of atheromatous plaque instability [26], since SM synthase activity links to peroxisome proliferator-activated receptor δ (PPAR-δ) signalling pathway causing inflammation and atherosclerosis [27]. PC and PE are most abundant in cellular membrane, and the altered individual phospholipid biosynthesis is involved deeply in lipoprotein metabolism leading to dyslipidemia and atherosclerosis [25]. It is noteworthy that the reduction of both plasma and erythrocyte membrane plasmalogens was not so related to the conventional coronary risk factors which were under the intensive medication (Tables 4 to 6). Rather, this reduction of plasmalogens reflects the intrinsic phospholipid profiles *per se*, indicating that this unique glycerophospholipid is involved in coronary atherosclerosis independently from conventional or residual risk factors in CHD patients.

5. STUDY LIMITATIONS

The findings of this study should be interpreted carefully due to inherent limitations. First, this is a case-control study containing potential bias concerning enrollment and treatment of patients. Consecutive enrollment of subjects resulted in male predominance in CHD group and female predominance in control group. However, no sex-dependent differences in human plasmalogens contents were reported to our knowledge. Treatment was under the discretion of treating physicians. However, treatment strategy was optimised by domestic guidelines [28], which was reflected by intensive control of coronary risk factors (Table 1). Second one relates to

methodology. Plasmalogens were quantified persistently by HPLC-ELSD system in our laboratory, and the authentic outcome was obtained in dementia [29]. At present, plasma ether phospholipids are quantified by tandem mass spectrometry (LC/MS/MS) [11,12]. However, a system for LC/MS/MS is too expensive and not realistic for routine clinical laboratory. Moreover, good correlation between our enzymatic assay using PLA_1 and LC/ESI-MS (R^2 > 0.94) was confirmed especially for ethanolamine ether phospholipids (ePE) [30]. Third limitation is the absence of longitudinal study, i.e., successful endovascular therapy is reported to ameliorate oxidative stress [31]. Dietary plasmalogens increase relative erythrocyte membrane plasmalogen contents in rats [32], suppress neuroinflammation and neuronal cell death induced by lipopolysaccharide in mice [33], and provide a clinical improvement of mild cognitive impairment in human [34]. Such interventional study design should be applied to CHD and may clarify the role of this unique glycerophospholipid as an intrinsic lipophilic antioxidant.

6. CONCLUSIONS

The present study demonstrated that plasma and erythrocyte membrane plasmalogens in patients with CHD undergoing PCI are lower than the corresponding plasmalogens of controls at least in subjects aged 70 years or older. Although longitudinal studies may elucidate the clinical role of plasmalogens as a laboratory marker of CHD, this study supports the hypothesis that coronary atherosclerosis is accelerated by oxidative stress [1,2] and plasmalogens are an unappreciated biomarker of redox condition and chronic inflammation underlying coronary atherosclerosis in the elderly [8,9].

CONSENT

Medical information extraction was informed to and signed informed consent was obtained from all the subjects at the enrollment.

ETHICAL APPROVAL

All the procedure performed in this study were in accordance with the ethical standards of our institutional and/or national research committee and with an updated Declaration of Helsinki (2008).

DATA SHARING STATEMENT

This study shared data with our current study in which control subjects were apparently healthy volunteers (n = 20), and multivariate analyses were not performed [21].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease. Vascul Pharmacol. 2015;71(8): 40-56.
2. Förstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res. 2017;120(4):713-735.
3. Mawatari S, Murakami K. Analysis of membrane phospholipid peroxidation by isocratic high-performance liquid chromatography with ultraviolet detection. Anal Biochem. 1998;264(1):118-123.
4. Okamoto K, Maruyama T, Kaji Y, Harada M, Mawatari S, Fujino T, Uyesaka N. Verapamil prevents impairment in filterability of human erythrocytes exposed to oxidative stress. Jpn J Physiol. 2004;54(1):39-46.
5. Lang F, Abed M, Lang E, Föller M. Oxidative stress and suicidal erythrocyte death. Antioxid Redox Signal. 2014;21(1): 138-153.
6. Siener R, Alteheld B, Terjung B, Junghans B, Bitterlich N, Stehle P, Metzner C. Change in the fatty acid pattern of erythrocyte membrane phospholipids after oral supplementation of specific fatty acids in patients with gastrointestinal diseases. Eur J Clin Nutr. 2010;64(4): 410-418.
7. Braverman NE, Moser AB. Functions of plasmalogen lipids in health and disease. Biochim Biophys Acta. 2012;1822(9): 1442-1452.
8. Stenvinkel P, Diczfalusy U, Lindholm B, Heimbürger O. Phospholipid plasmalogen, a surrogate marker of oxidative stress, is associated with increased cardiovascular mortality in patients on renal replacement therapy. Nephrol Dial Transplant. 2004;19(4):972-976.

9. Lessig J, Fuchs B. Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis. Curr Med Chem. 2009;16(16):2021-2041.
10. Nishimukai M, Maeba R, Yamazaki Y, Nezu T, Sakurai T, Takahashi Y, Hui SP, Chiba H, Okazaki T, Hara H. Serum choline plasmalogens – those with oleic acid in sn-2 – are biomarkers for coronary artery disease. Clin Chim Acta. 2014; 437(11):147-154.
11. Sutter I, Klingenberg R, Othman A, Rohrer L, Landmesser U, Heg D, Rodondi N, Mach F, Windecker S, Matter CM, Lüscher TF, von Eckardstein A, Hornemann T. Decreased phosphatidylcholine plasmalogens – a putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction. Atherosclerosis. 2016;246(5):130-140.
12. Moxon JV, Jones RE, Wong G, Weir JM, Mellett NA, Kingwell BA, Meikle PJ, Golledge J. Baseline serum phosphatidylcholine plasmalogen concentrations are inversely associated with incident myocardial infarction in patients with mixed peripheral artery disease presentations. Atherosclerosis. 2017;263(8):301-308.
13. Osipenko AN. Change of plasmalogen content of red blood cells in myocardial hypoxia and acidosis. Acta Cardiol. 2018;73(1):61-68.
14. Maeba R, Araki A, Ishii K, Ogawa K, Tamura Y, Yasunaga M, Minami U, Kamori A, Okazaki T, Nishimukai M, Hara H, Fujiwara Y. Serum ethanolamine plasmalogens improve detection of cognitive impairment among elderly with high excretion levels of urinary myo-inositol: a cross-sectional study. Clin Chim Acta. 2016;453(1):134-140.
15. Wallner S, Schmitz G. Plasmalogens the neglected regulatory and scavenging lipid species. Chem Phys Lipids. 2011;164(6): 573-589.
16. Weisser B, Mengden T, Düsing R, Vetter H, Vetter W. Normal values of blood pressure self-measurement in view of the 1999 World Health Organization-International Society of Hypertension guidelines. Am J Hypertens. 2000;13(8): 940-943.
17. Ohta Y, Tsuchihashi T, Onaka U, Hasegawa E. Clustering of cardiovascular risk factors and blood pressure control status in hypertensive patients. Intern Med. 2010;49(15):1483-1487.
18. Matsuzawa Y. Metabolic syndrome: definition and diagnostic criteria in Japan. J Atheroscler Thromb. 2005;12(6):301.
19. Mawatari S, Hazeyama S, Fujino T. Measurement of ether phospholipids in human plasma with HPLC-ELSD and LC/ESI-MS after hydrolysis of plasma with phospholipase A_1. Lipids. 2016; 51(8):997-1006.
20. Mawatari S, Okuma Y, Fujino T. Separation of intact plasmalogens and all other phospholipids by a single run of high-performance liquid chromatography. Anal Biochem. 2007;370(1):54-59.
21. Noda H, Moriyama S, Irie K, Fujita K, Yokoyama T, Fukata M, Arita T, Odashiro K, Maruyama T, Mawatari S, Fujino T, Akashi K: Erythrocyte membrane plasmalogen contents diminished in severe atherosclerotic patients undergoing invasive endovascular therapy. Membrane. 2017;42(6):242-249.
22. Orsoni A, Thérond P, Tan R, Giral P, Robillard P, Kontush A, Meikle PJ, Chapman MJ. Statin action enriches HDL3 in polyunsaturated phospholipids and plasmalogens and reduces LDL-derived phospholipid hydroperoxides in atherogenic mixed dyslipidemia. J Lipid Res. 2016;57(11):2073-2087.
23. Ng TW, Ooi EM, Watts GF, Chan DC, Weir JM, Meikle PJ, Barrett PH. Dose-dependent effects of rosuvastatin on the plasma sphingolipidome and phospholipidome in the metabolic syndrome. J Clin Endocrinol Metab. 2014; 99(11):E2335-E2340.
24. Zoeller RA, Grazia TJ, LaCamera P, Park J, Gaposchkin DP, Farber HW. Increasing plasmalogen levels protects human endothelial cells during hypoxia. Am J Physiol. 2002;283(2):H671-H679.
25. van der Veen JN, Kennelly JP, Wan S, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta. 2017;1859(9):1558-1572.
26. Edsfeldt A, Dunér P, Ståhlman M, Mollet IG, Asciutto G, Grufman H, Nitulescu M, Persson AF, Fisher RM, Melander O, Ortho-Melander M, Borén J, Nilsson J,

Gonçalves I. Sphingolipids contribute to human atherosclerotic plaque inflammation. Arterioscler Thromb Vasc Biol. 2016;36(6):1132-1140.
27. Mou D, Yang H, Qu C, Chen J, Zhang C: Pharmacological activation of peroxisome proliferator-activated receptor δ increases sphingomyelin synthase activity in THP-1 macrophage-derived foam cell. Inflammation. 2016;39(4):1538-1546.
28. Daida H, Miyauchi K, Ogawa H, Yokoi H, Matsumoto M, Kitakaze M, Kimura T, Matsubara T, Ikari Y, Kimura K, Tsukahara K, Origasa H, Morino Y, Tsutsui H, Kobayashi M, Isshiki T; PACIFIC investigators. Management and two-year long-term clinical outcome of acute coronary syndrome in Japan: prevention of atherothrombotic incidents following ischemic coronary attack (PACIFIC) registry. Circ J. 2013;77(4):934-943.
29. Oma S, Mawatari S, Saito K, Wakana C, Tsuboi Y, Yamada T, Fujino T. Changes in phospholipid composition of erythrocyte membrane in Alzheimer's disease. Dement Geriatr Cogn Dis Extra. 2012; 2(1):298-303.
30. Mawatari S, Hazeyama S, Morisaki T, Fujino T. Enzymatic measurement of ether phospholipids in human plasma after hydrolysis of plasma with phospholipase A_1. Pract Lab Med. 2018; 10(1):44-51.
31. Ebisawa S, Kashima Y, Miyashita Y, Yamazaki S, Abe N, Saigusa T, Miura T, Motoki H, Izawa A, Ikeda U. Impact of endovascular therapy on oxidative stress in patients with peripheral artery disease. Circ J. 2014;78(6):1445-1450.
32. Mawatari S, Katafuchi T, Miake K, Fujino T. Dietary plasmalogen increases erythrocyte membrane plasmalogen in rats. Lipids Health Dis. 2012;11:161. DOI: 10.1186/1476-511X-11-161.
33. Katafuchi T, Ifuku M, Mawatari S, Noda M, Miake K, Sugiyama M, Fujino T. Effects of plasmalogens on systemic lipopolysaccharide-induced glial activation and β-amyloid accumulation in adult mice. Ann NY Acad Sci. 2012;1262(1):85-92.
34. Fujino T, Yamada T, Asada T, Tsuboi Y, Wakana C, Mawatari S, Kono S. Efficacy and blood plasmalogen changes by oral administration of plasmalogen in patients with mild Alzheimer's disease and mild cognitive impairment: a multicenter, randomized, double-blind, placebo-controlled trial. EBioMedicine. 2017;17(5): 199-205.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26742