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# Identification of Lysophosphatidylcholines and Sphingolipids as Potential Biomarkers for Acute Aortic Dissection via Serum Metabolomics

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**WHAT THIS PAPER ADDS**

Acute aortic dissection (AAD) has a high mortality rate, and may be difficult to diagnose at an early stage. In this study, a metabolomics approach was used to identify serum biomarkers of two types of AAD: Stanford types A and B. Differences were found in the levels of lysophosphatidylcholines and sphingolipids between AAD patients and controls. Furthermore, the two subtypes of AAD could be distinguished according to various levels of sphingolipids, in which sphinganine, phytosphingosine, and ceramide were remarkably reduced in the Stanford type A group, but not in the Stanford type B group.

**Objectives:** Acute aortic dissection (AAD) is a severe clinical emergency with a high mortality, and is easily misdiagnosed in its early stage. This study aimed at discovering serum metabolomic markers with the potential to diagnose AAD and distinguish between two subtypes of AAD.

**Methods:** Thirty-five patients with AAD, including 20 with Stanford type A and 15 with Stanford type B were enrolled in this study, together with 20 healthy controls. All patients with AAD were admitted within 72 h of onset. Serum metabolomics profiles were determined by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and the data were analysed by principal component analysis and partial least squares discriminant analysis.

**Results:** A total of 17 metabolites differing between the control and AAD groups were finally screened and identified as lysophosphatidylcholines (LPC) and sphingolipids including sphinganine, phytosphingosine, sphingomyelin, and ceramide. Compared with those in the healthy control group, LPC levels were significantly lower in both the Stanford type A and type B AAD groups. Interestingly, sphingolipids, including sphinganine, phytosphingosine, and ceramide, were remarkably reduced in the Stanford type A AAD group, but not in the Stanford type B AAD group. Subgroup analysis showed that the changes in LPC and sphingolipid levels were unrelated to hypertension or gender.

**Conclusions:** The present results indicate that LPCs and sphingolipids are significantly altered in patients with AAD, and several sphingolipids, such as sphinganine, phytosphingosine, and ceramide, were dramatically decreased in patients with Stanford type A AAD. A combination of these two families of metabolites could serve as a potential biomarker for the diagnosis of AAD and distinguishing between Stanford type A and Stanford type B.

**Keywords:** Acute aortic dissection, Biomarker, Metabolomics, Lysophosphatidylcholine, Sphingolipid
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## INTRODUCTION

Acute aortic dissection (AAD), representing separation (dissection) between layers of the aortic wall, is a life threatening vascular disease with a mortality of about 20% on day one and 30% in 48 h as a result of potentially fatal complications.[1] Currently, AAD can be diagnosed using

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computed tomography angiography (CTA), magnetic resonance angiography (MRA), and transesophageal echocardiography (TEE). Some biomarkers, such as D-dimer,[2] matrix metalloproteinases,[3,4] soluble elastin fragments,[5] smooth muscle myosin heavy chain,[6] calponin,[7] and soluble ST2[8] have been reported as useful in the diagnosis and detection of AAD, and may find their optimum use in the emergency department to differentiate different causes of chest pain. However, other than D-dimer, most of these biomarkers are not widely applied in clinical practice.

Compared with other high throughput omics techniques, metabolomics approaches specifically characterise small molecules (molecular weight $<1000$) produced by active cells during their lifecycles to cope with environmental alterations, representing the endpoints of the omics cascade, and could be useful for screening effective diagnostic markers and unravelling pathological mechanisms.[9,10] Few studies have examined AAD using a metabolomics approach. However, a recent metabolomics analysis of patients with aortic dissection discovered that a number of amino acids were significantly changed in comparison with coronary heart disease;[11] another report characterised metabolomic alterations in patients with ascending thoracic aortic aneurysms or dissections.[12] To the best of the present authors' knowledge, metabolomic differences between Stanford type A and Stanford type B AAD have not been evaluated, and comprehensive metabolomics studies are still needed to identify other valuable biomarkers for AAD. In the present study, serum samples of patients with Stanford type A AAD, Stanford type B AAD, and controls were analysed using metabolomics.

## MATERIALS AND METHODS

### *Study design and subjects*

A total of 20 healthy individuals and 35 patients with AAD (20 with Stanford type A and 15 with Stanford type B) were enrolled in this study. All patients were selected consecutively from July 2015 to November 2016 from Fudan University affiliated Zhongshan Hospital (Shanghai, China). All patients with AAD were admitted within 72 h of an episode of chest and/or back pain lasting 5 min or longer. The diagnosis of AAD was confirmed by CTA and classified according to the Stanford standard. Patients with Marfan syndrome or other connective tissue disorders or on haemodialysis were excluded. The healthy controls were enrolled consecutively from the medical examination centre. Participants with aneurysms, atherosclerotic diseases, or valvular diseases were excluded from the control group. Baseline characteristics of subjects were collected from medical records. Cardiac troponin T (cTnT), D-dimer levels, and electrocardiogram (ECG) results on admission were obtained from the medical history. Following the manufacturer's instructions, cTnT was detected using Cobas E analysers (Roche, Basel, Switzerland), and D-dimer levels were detected using VIDAS PC (Biomerieux, Marcy-l'Étoile, France). Reference values for cTnT and D-dimer are less than 0.03 ng/mL and 0.8 mg/L, respectively, and cTnT levels exceeding the reference value were considered positive. Hypertension was defined as systolic pressure $\geq$140 mmHg and/or diastolic pressure $\geq$90 mmHg, or the use of antihypertensive drugs. The protocol was approved by the ethics committee of Zhongshan Hospital, and conformed to the ethical guidelines of the 1975 revision of the Declaration of Helsinki. Informed consent was obtained from each of the participants prior to inclusion in the study. For each AAD patient, a blood sample was collected in BD Vacutainer SST tubes (BD Diagnostics, Plymouth, UK) immediately after diagnosis in the emergency room and then centrifuged at 4000 rpm for 10 min at room temperature. All collected serum samples were stored at $-80$ °C until analysis.

### *Chemicals and reagents*

Lipid markers were obtained from Avanti Polar Lipids (Alabaster, AL, USA). All authentic standards were purchased from Sigma—Aldrich (St. Louis, MO, USA). Methanol, isopropanol, and acetonitrile were purchased from TEDIA (Fairfield, OH, USA).

### *Metabolomics analysis*

**Sample preparation.** Serum (20 μL) was mixed with 380 μL of 67% acetonitrile. The solution was vortexed for 30 s and then maintained for 5 min at room temperature. The mixture was centrifuged for 15 min at 13,000 rpm to obtain the supernatant for ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UPLC-MS). Quality control (QC) samples were prepared by pooling equal volumes of each analysed sample.

**UPLC-MS analysis.** Ultra-performance liquid chromatography coupled with quadrupole/time-of-flight mass spectrometer (Agilent 1290 Infinity LC system and Agilent 6538 UHD Accurate-Mass Q-TOF spectrometer, Agilent Technologies, Santa Clara, CA, USA) was used for the metabolomics analysis. A Waters ACQUITY HSS T3 column (2.1 $\times$ 100 mm, 1.8 μm; Waters, Milford, MA, USA) was used as the stationary phase at 50 °C. The mobile phase consisted of water containing 0.1% formic acid (Phase A) and acetonitrile containing 0.1% formic acid (Phase B). Gradient elution conditions were as follows: 5% B was held for 2 min, 5% B was increased to 95% B from 2 min to 17 min, and finally 95% B was held for 2 min. The column equilibration time was 5 min. The flow rate was 0.3 mL/min. The Q-TOF mass spectrometer was operated with a capillary voltage of 4.0 kV, drying gas flow of 11 L $min^{-1}$, and a gas temperature of 350 °C. The nebuliser pressure was set at 45 psig. The fragmentor voltage was 120 V and skimmer voltage was 60 V. The mass range was set at m/z 100—1100. The positive ionisation mode was used in this study because the positive mode was found to give many more ions of the metabolites than the negative mode.

**Data processing and multivariable data analysis.** The acquired LC-MS data were converted into mzdata format using Agilent Masshunter Qualitative Analysis B.06.00 (Agilent Technologies). Data deconvolution was performed using

XCMS Online (https://xcmsonline.scripps.edu/). Centroiding, deisotoping, filtering, peak recognition, and integration were performed to generate a multivariable data matrix of sample identity, ion identity (retention time and m/z), and ion abundance. Variables available for at least 80% of the samples were kept. SIMCA-P11 (Umetrics, Kinnelon, NJ, USA) was used to perform the multi-dimensional statistical analysis. The major latent variables were modelled using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA).

Ions with differences among groups with variable influence on projection (VIP) values greater than 1.5 and $p$ values less than .05 were screened. The potential biomarkers were then identified by comparisons with the authentic standards based on their MS/MS fragmentation patterns and retention times. Relative levels of metabolites in various groups were represented as fold changes to evaluate how these selected differential metabolites varied between the AAD and healthy control groups.

**Metabolic pathway analysis.** A metabolic pathway analysis was performed using MBRole[13] based on various databases, including KEGG (http://www.genome.jp/kegg/), Human Metabolome Database (http://www.hmdb.ca/), and PubChem (http://www.ncbi.nlm.nih.gov/pccompound/), to identify the affected metabolic pathways and facilitate further biological interpretation.

### *Statistical analysis*

Demographic and medical information for patients with AAD and the control group are summarised by mean ± SD or percentages for categorical data. The chi-square test was used to compare the distributions of categorical data. Continuous variables were analysed using one way analysis of variance (ANOVA) with multiple comparison test (Bonferroni adjusted) and two tailed $t$ tests for subject characteristics. Differences between groups in metabolite levels were assessed using the non-parametric Mann—Whitney test. A heat map analysis was conducted using MeV version 4.9.0. All data were analysed using SPSS Statistics 24 (IBM, Armonk, NY, USA). Two sided $p$ values and 95% CI were used. A $p$ value $< .05$ was considered statistically significant.

## RESULTS

### *Patient characteristics*

Thirty-five patients with AAD were enrolled in this study (Table 1). Baseline clinical data showed that the average ages in the control and AAD group were not different (57.25 ± 8.07 years vs. 58.1 ± 14.8 years). The incidence of hypertension was higher in both AAD groups than in the control group; however, these differences were not statistically significant ($p > .05$). All 35 patients were diagnosed with AAD according to the findings of CTA imaging of the true and false aortic lumens and approximate entry tear sites. In total, 20 cases were classified as Stanford type A and 15 as Stanford type B. Cardiac troponin T levels and ECG findings were checked for myocardial infarction. Cardiac troponin T levels were increased in patients with type A AAD. However, no new pathological Q waves or ST segment and T wave changes were found in all patients, especially in patients who were cTnT positive. The D-dimer concentrations did not differ significantly between the type A and type B aortic dissection groups.

### *Metabolic profiles of patients with AAD*

To identify serum markers of AAD, a metabolomics approach was used to characterise the metabolic profiles of subjects in various groups. PCA and PLS-DA score plots showed clear clustering of the three groups, the control group, patients with Stanford type A AAD, and patients with Stanford type B AAD (Fig. 1). The two groups of patients with AAD were close to each other and separated from the control group, consistent with the clinical phenotypes. Based on the PLS-DA loading plot, 17 metabolites discriminating the control and AAD groups with VIP >1.5 and $p < .05$ were finally obtained. These were identified as LPC, sphinganine (dihydrosphingosine, DHS), sphingomyelin (SM), and ceramide (CER). The altered metabolites in the three groups are listed in Table S1. The average levels of the 17 differential metabolites in the corresponding groups are shown using a heat map, a graphical representation of data where the individual values are represented as colours to help visualise relative differences between groups (Fig. 2).[14]

**Table 1. Characteristics of 35 patients with acute aortic dissection (AAD)**

| Parameters | Control group (n = 20) | AAD group | | $p$ - value |
|---|---|---|---|---|
| | | Stanford type A (n = 20) | Stanford type B (n = 15) | |
| Age, years | 57.25 ± 8.07 | 56.4 ± 15.75 | 60.20 ± 13.88 | .676 |
| Male gender, n (%) | 11 (55) | 14 (70) | 8 (53) | .517 |
| Time elapsed between onset and admission, hours | / | 26.63 ± 29.88 | 28.29 ± 31.38 | |
| Hypertension, n (%) | 7 (35) | 12 (60) | 10 (67) | .128 |
| cTnT, ng/mL | 0.017 ± 0.010 | 0.060 ± 0.087 | 0.018 ± 0.022 | .028 |
| cTnT > 0.03 ng/mL, n (%) | 1 | 5 | 2 | .100 |
| D-dimer, mg/L | Not detected | 5.93 ± 5.99 | 11.37 ± 11.57 | .186[a] |

Values are presented a mean ± standard deviation (SD) unless stated otherwise. One way analysis of variance (ANOVA) with multiple comparison test (Bonferroni adjusted) for continuous variables or chi-square test for categorical variables were used for calculating significance across three groups (except D-dimer). AAD = acute aortic dissection; cTnT = cardiac troponin T.

[a] Two tailed $t$ test was used for calculating significance between Stanford type A and Stanford type B AAD groups.

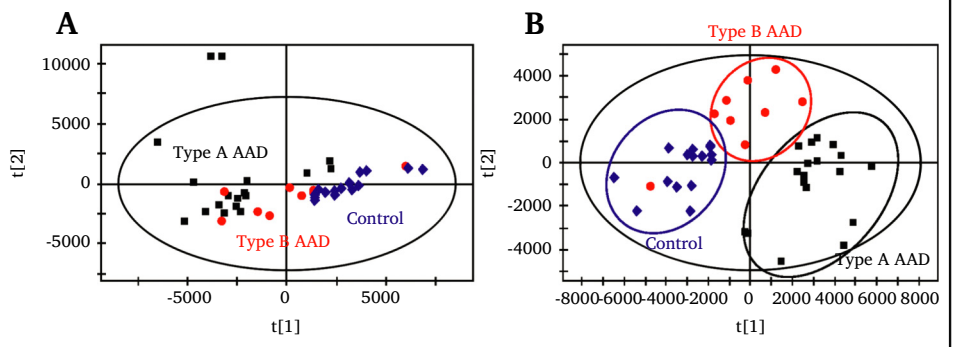


**Figure 1.** Score plots show that the two groups of patients with AAD were separated from the control group. (A) PCA score plot of the three groups, as labelled in the figure. The cumulative fitness ($R^2$ value) of the PCA model was 0.64. (B) PLS-DA score plot of the three groups, as labelled in the figure. The cumulative fitness ($R^2$ value) and prediction power ($Q^2$ value) of the PLS-DA model were 0.604 and 0.395, respectively. The t [1] and t [2] values in the figures represent the scores of each sample in principal components 1 and 2, respectively. Each dot/square/diamond on the plot represents a sample in the corresponding group. AAD = acute aortic dissection; PCA = principal component analysis; PLS-DA = partial least square discriminant analysis.

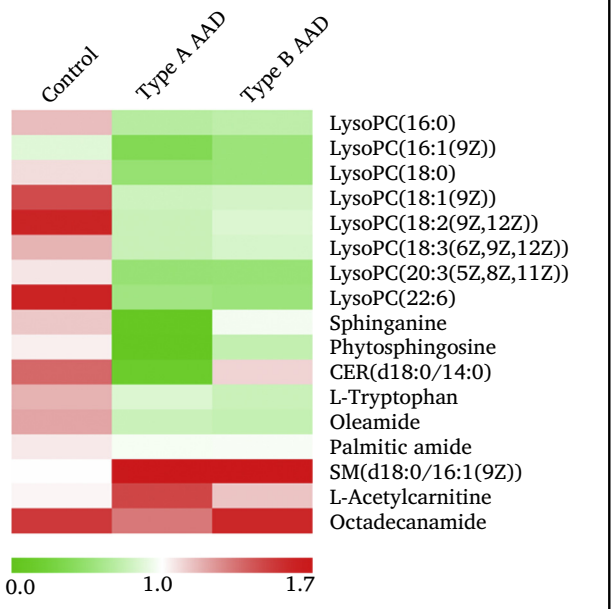


**Figure 2.** Heat map of the average levels of the 17 differential metabolites in the Stanford type A and type B aortic dissection and control group. Each colour cell on the map corresponds to a metabolite in a certain group, and the average level of the metabolite in the group is graphically represented as a colour. Shades of red or green represent an increase or decrease of a metabolite level, respectively. To a certain metabolite, colours changing from green to red across various groups indicate that average levels of the metabolite are increasing, and vice versa. AAD = acute aortic dissection; lysoPC = lysophosphatidylcholine; CER = ceramide; SM = sphingomyelin.

### *LPC levels decreased significantly in both AAD groups*

Levels of the LPCs, as shown in Fig. 2 and Table S1, were significantly lower in both the Stanford type A and Stanford type B AAD groups than in the control group (all $p < .01$, Fig. 3A), and there were no significant differences between the Stanford type A and Stanford type B AAD groups. When the subjects with or without hypertension were analysed separately, decreased LPC levels could still be observed in both of the AAD groups, demonstrating that the change in LPC levels was unrelated to hypertension (Fig. 3B and C). Similarly, the decrease in LPCs was not correlated with the gender of patients with AAD (Fig. 3D and E).

### *Sphingolipids decreased significantly in the Stanford type A group*

In Stanford type A, the sphinganine, phytosphingosine, and ceramide levels were much lower than those in the control or Stanford type B AAD groups (Figs. 2 and 4A where all $p < .005$). Similar trends were observed in subgroup analyses considering hypertension or gender (Fig. 4B—E). Interestingly, SM levels were elevated in both AAD groups (Fig. 4) and were higher in the Stanford type B AAD group than in the Stanford type A AAD group. In contrast, the level of L-tryptophan was higher in the control group than in both AAD groups, and L-tryptophan levels were also independent of hypertension or gender.

### *The sphingolipid pathway was identified in a metabolic pathway analysis*

Based on the identified metabolites, a functional enrichment analysis was performed to determine the most relevant pathways involved in AAD. Four metabolites; sphinganine, phytosphingosine, SM(d18:0/16:1), and CER(d18:0/14:0), were located in the same sphingolipid pathway (Table S2). The disturbed sphingolipid pathway network of the regulated metabolites is summarised in Fig. 5.

## DISCUSSION

The onset of AAD is not usually accompanied by specific symptoms. Although acute chest pain is commonly present

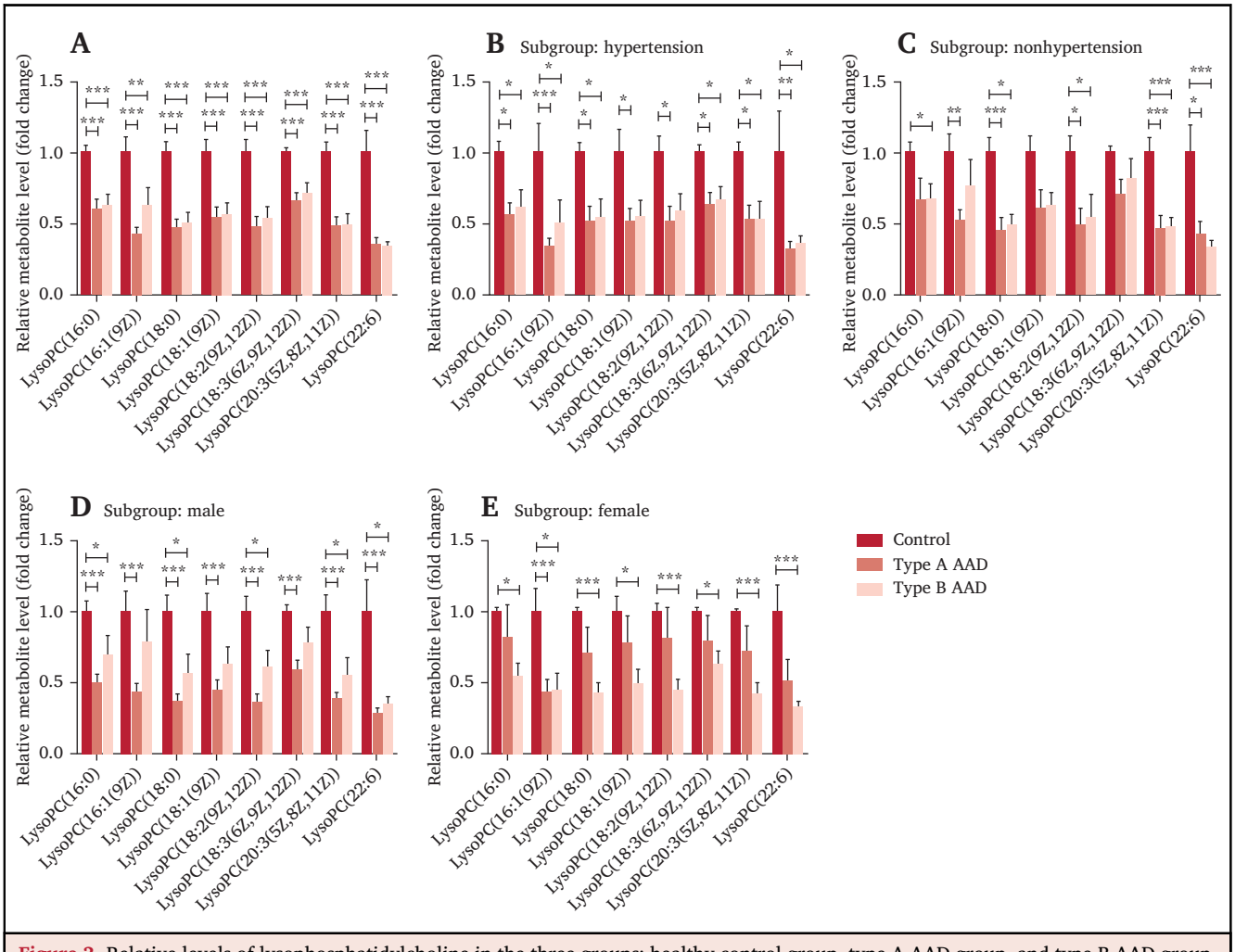


**Figure 3.** Relative levels of lysophosphatidylcholine in the three groups; healthy control group, type A AAD group, and type B AAD group. (A) Lysophosphatidylcholine levels for all subjects in the three groups. Subgroup analyses according to hypertension (B) or non-hypertension (C) of relative lysophosphatidylcholine levels in the three groups. Lysophosphatidylcholine levels in three groups of males (D) or females (E). Data are expressed as means ± SEM. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .005$. AAD = acute aortic dissection; lysoPC = lysophosphatidylcholine. Non-parametric Mann–Whitney test for comparisons between groups.

in the majority of the patients, this can be easily misdiagnosed as acute myocardial infarction or myocardial ischaemia.[15] In recent years, high throughput omics techniques have been used for identification of biomarkers associated with the pathogenesis of diseases. Using quantitative proteomics assays, proteins involved in cell matrix interactions, ECM remodelling, and inflammation have been identified as potential AAD related serum biomarkers, which may be useful for the diagnosis and determination of disease stage and progression. In the present study, using a metabolomics approach, it was found that LPCs and sphingolipids could serve as biomarkers for the diagnosis of AAD. Moreover, two types of AAD, Stanford types A and B, could be discriminated by a combination of LPCs and sphingolipids. The working model of this study for the diagnosis of AAD is summarised in Fig. 6.

LPC is a major plasma lipid and an important cell signalling molecule via the action of phospholipase A2 on phosphatidylcholine (PC).[16] A metabolomic profile analysis revealed a reduction in serum LPC levels in patients with type 2 diabetes mellitus with cardiovascular disease (CVD).[17] Another large scale metabolomic profiling analysis also identified several LPCs as risk factors for coronary heart disease.[18] Fernandez et al. reported that CVD development is preceded by reduced levels of LPC16:0 and LPC20:4.[19] The decrease in LPCs in CVD is consistent with their role in the regulation of peroxisome proliferator activated receptor alpha and may be related to the uptake and oxidation of fatty acids.[20] Therefore, the present findings that patients with both Stanford type A and Stanford type B AAD have decreased LPC levels are in accordance with previous results for various CVDs. Furthermore, in subgroup analyses, the decreased LPC levels in patients with AAD were unrelated to hypertension or gender.

In this study, sphinganine (dihydrosphingosine, DHS) and phytosphingosine levels were dramatically lower in the serum of patients with Stanford type A than in patients with Stanford type B and the control group. Accordingly, patients with Stanford type A had a more substantial decrease in CER(d18:0/14:0) levels than those in the Stanford type B

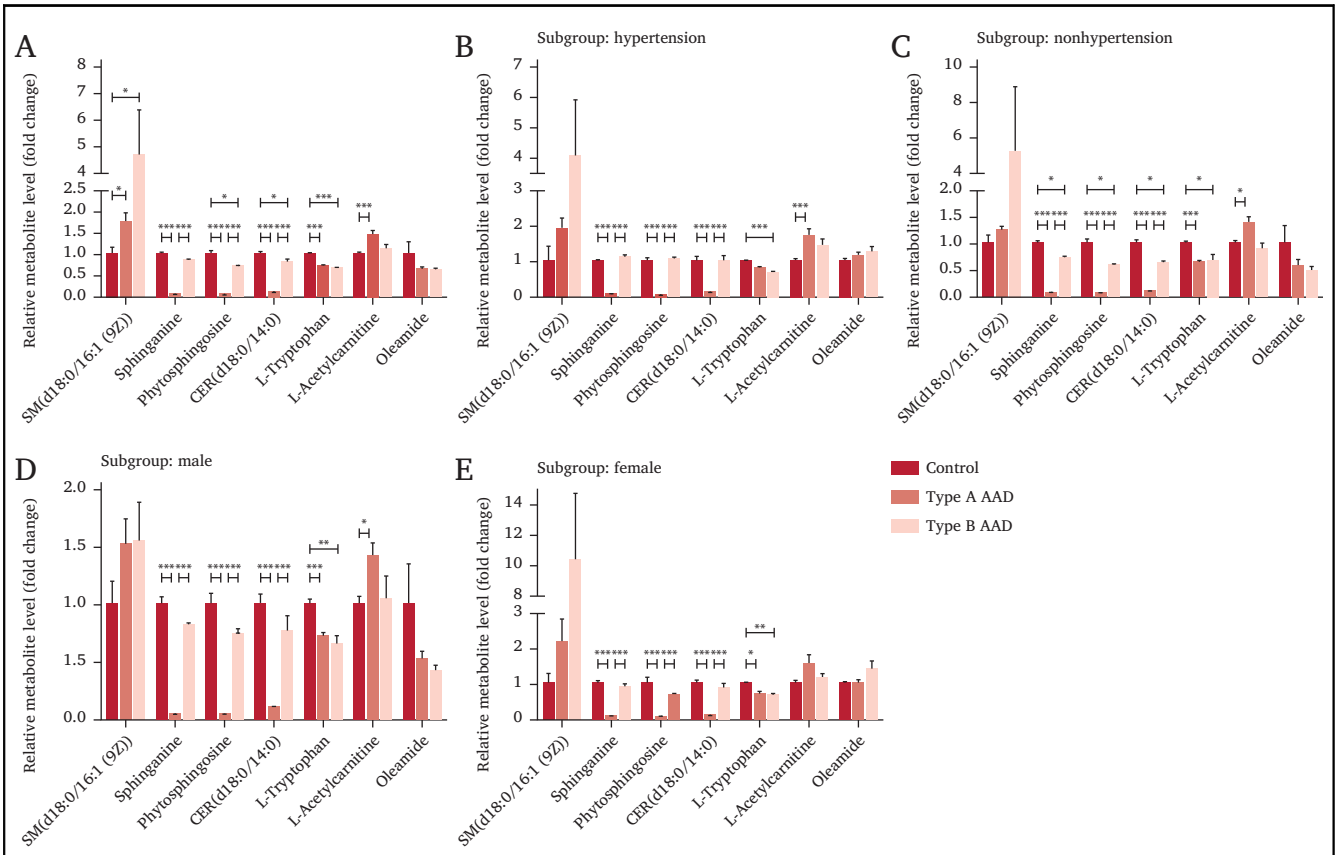


**Figure 4.** Relative levels of sphingolipid and other metabolites in the three groups; the healthy control group, type A AAD group, and type B AAD group. (A) Sphingolipid and other metabolite levels for all subjects in the three groups. Subgroup analyses according to hypertension (B) or non-hypertension (C) of relative levels of sphingolipid and other metabolites in the three groups. Sphingolipid and other metabolite levels in three groups of males (D) or females (E). Data are expressed as means ± SEM. *$p < .05$, **$p < .01$, and ***$p < .005$. AAD = acute aortic dissection; CER = ceramide; SEM = standard error of the mean; SM = sphingomyelin. Non-parametric Mann–Whitney test for comparisons between groups.

group and the control group. SM(d18:0/16:1) levels were increased in both AAD groups compared with those in the control group. According to the metabolic pathway analysis, these four metabolites were in the same sphingolipid pathway, which was highlighted as the potentially intervened metabolic pathway for patients with AAD.

Sphingolipids are biologically active lipids with important roles in various cellular processes. Accumulating evidence suggests an important role for sphingolipids in the cardiovascular system under physiological and pathophysiological conditions.[21–23] In sphingolipid metabolism, CER is a key compound formed from SM by sphingomyelinase.[24] Importantly, the activation of the sphingomyelinase-ceramide pathway promotes pro-inflammatory and pro-oxidative activities (e.g. via oxidised low density lipoprotein), mediating vascular smooth muscle cell calcification, in which matrix metalloproteinase 2 may play a role, resulting in atherosclerosis and other cardiovascular events.[25,26] However, CER, sphinganine, and phytosphingosine levels were lower in the Stanford type A AAD group than in the control and Stanford type B AAD groups, along with elevated SM levels, suggesting that sphingomyelinase activity might be inhibited in Stanford type A AAD. Therefore, it was hypothesised that insufficient sphingomyelinase-ceramide pathway activity may play an important role in Stanford type A AAD and classical pro-atherogenic processes may not get involved, which is consistent with the results of a recent study[12] demonstrating that SM levels are significantly higher in thoracic aortic wall tissues of tricuspid aortic valve associated aortic dissections than in healthy controls.

In addition, according to the present metabolomics findings and subgroup analysis, Stanford type A and type B groups had distinct patterns of sphingolipid metabolism, unrelated to hypertension history or gender. In combination with LPC, this sphingolipid metabolism pattern could be used to discriminate these two types of AAD. However, differences in the roles of the sphingomyelinase-ceramide pathway between Stanford type A and Stanford type B AAD disease progression need further validation. As there was no statistically significant difference in D-dimer levels between Stanford type A and Stanford type B aortic dissection, the observed disturbance of the sphingomyelinase-ceramide pathway provides a basis for effective discrimination between these two AAD types.

At present, D-dimer is the most promising biomarker in suspected acute aortic dissection and is widely used in clinical practice. A recent study showed that soluble ST2 (sST2) was elevated in patients with AAD, showing a

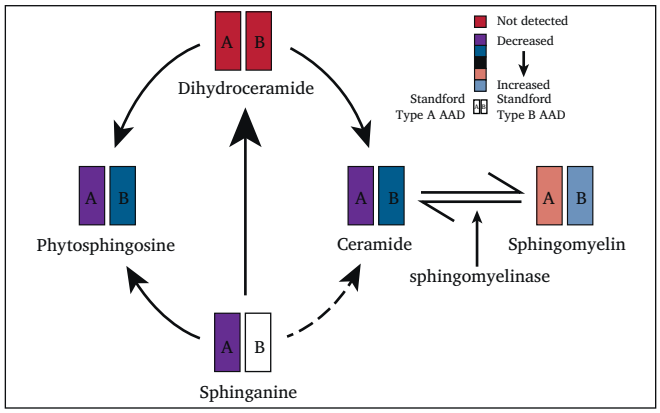


**Figure 5.** Schematic overview of perturbed sphingolipid metabolism in patients with acute aortic dissection. Each box in the figure was equally divided into left and right halves (except the top grey one). Colours in the left halves show the level changes of the metabolites in the Stanford type A AAD group compared with control, and colours in the right halves show the level changes of the metabolites in the Stanford type B AAD group compared with control, colours changing from blue to red indicate decreased to increased levels of the metabolites in AAD groups compared with control. Ceramide, sphinganine, and phytosphingosine were significantly decreased, especially in the Stanford type A group. Sphingomyelin was increased in AAD patients, suggesting possible inhibition of sphingomyelinase, a hydrolase breaking sphingomyelin down into ceramide. Dihydroceramide was not detected using the current method. AAD = acute aortic dissection.

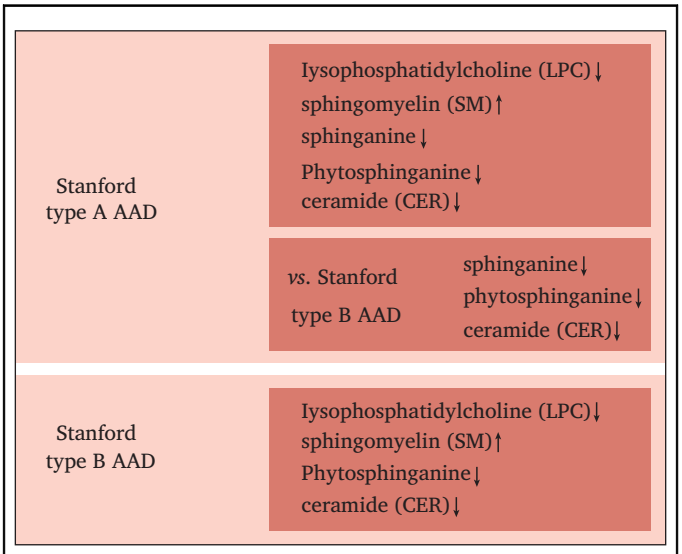


**Figure 6.** Metabolites that were increased (upward arrows) or reduced (downward arrows) in the aortic dissection group compared with the control group. AAD = acute aortic dissection.

superior "rule out" diagnostic performance over D-dimer.[8] Other potential roles of biomarkers might be limited by its time window, which is 3—6 h and 12 h in smooth muscle myosin heavy chain and calponin, respectively.[27] The present study included participants within the first 72 h of the onset of symptoms which may widen the time window.

Recently, Ren et al. conducted a metabolomics analysis on AAD and highlighted eight metabolites as potential biomarkers for AAD, including N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), glycerophosphocholine, and ergothioneine.[28] However, they simply enrolled AD patients without using the Stanford standard for further classification and divided participants into control, hypertension, and AD groups, different to the groups included in the present study and perhaps contributing to different results. Moreover, the present study design could potentially reveal some different mechanisms between Stanford type A AAD and type B AAD. The time interval between the initial onset and sampling was less than or equal to 14 days in Ren et al.'s study, but restricted to 72 h in the present study. Hence, the present study reflected the alteration of metabolite levels shortly after the onset of AAD and might be able to reveal the metabolites that are closely related to AAD pathology. However, further investigations are still required to examine the dynamic level changes of the metabolites reported in this work, especially in the acute stage.

In clinical practice, acute coronary malperfusion is frequently complicated by AAD, particularly type A, which has an approximate incidence of 6—19%.[29] To eliminate differences in metabolite levels caused by possible myocardial damage, cTnT levels and ECG findings on admission were reviewed. Initial ECG showed no obvious acute coronary syndrome like patterns, especially in patients with positive cTnT tests, suggesting that there was no evidence of myocardial ischaemia or infarction.

According to the instructions for use of iopamidol (the contrast media used) and previous studies, there were no signs of metabolism of iopamidol in plasma and it was excreted unmetabolised by the kidney, indicating that iopamidol might not affect metabolomics analysis directly.[30,31] Besides, blood samples were collected immediately after the confirmed diagnosis to avoid indirect potential metabolic changes.

The present study was not without limitations. First, the sample size was relatively small, and a larger population is required for further validation. Second, only healthy individuals were used as controls. Acute diseases involving acute chest pain, such as acute coronary syndrome and pulmonary embolism, should be included to enable the use of this metabolic approach for differential diagnostic testing in the future.

## CONCLUSIONS

A comprehensive metabolomic evaluation was performed to identify clinically relevant disturbances in circulating metabolites in AAD. LPCs and sphingolipids were significantly altered in patients with AAD, and some sphingolipids, such as sphinganine, phytosphingosine, and ceramide, were dramatically decreased in patients with Stanford type A AAD. The combination of these two families of metabolites could potentially serve as a biomarker for the diagnosis of AAD and for distinguishing between Stanford type A and Stanford type B AAD. This altered metabolomic pattern might provide a fast and cost-effective diagnostic method for AAD. However, these results need to be validated using

a larger population, and further research on the underlying mechanisms is required to improve the management of this life threatening condition in clinical practice.

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## CONFLICTS OF INTEREST

None.

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## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejvs.2018.07.004.

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