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慢性暴露大氣細懸浮微粒後大鼠不同器官與血清的脂質變化

Lipid Changes in Different Organs and Serum of Rats after Chronic Exposure to Ambient Fine Particulate Matter

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兩年多的碩士生涯，曾在過程中感到十分漫長，但轉眼地，也即將走到了終點。在林靖愉老師的研究室裡，學習著從未接觸過的領域，由於我沒有很多看 paper 經驗，也沒有什麼毒理學與分析化學的基礎，一開始感到很迷茫。還記得初期的 meeting 報告，每次報完我都覺得自己一團糟，連自己都無法認可自己，而且對於下一次的報告總是提心吊膽。後來隨著時間與經驗的累積，不知道何時開始，我慢慢可以在 seminar 上有良好的應答，甚至遠赴國際研討會張貼論文壁報，到最後此刻我可以完成一本自己的著作，這一路上要感謝非常多人的幫忙。

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摘要

細懸浮微粒又稱 $PM_{2.5}$，它進入人體呼吸道後能在肺泡穿越氣血屏障，進而抵達血液循環系統然後傳遞至其他器官。過去文獻指出 $PM_{2.5}$ 誘導的體內氧化壓力上升可能造成脂質的擾動，而含有磷酸膽鹼的脂質(phosphorylcholine-containing lipids)是人體構成細胞膜的最主要成分，在面對氧化攻擊時細胞膜是體內的第一道防線。本實驗室先前的研究使用脂質體學的方法探討在慢性暴露 $PM_{2.5}$ 後大鼠肺部含有磷酸膽鹼脂質的變化，發現磷脂醯膽鹼(phosphatidylcholines, PCs)的顯著改變可能與肺部表面活性劑功能的損傷有關。然而除了肺臟外，$PM_{2.5}$ 對人體其他器官的脂質效應目前仍不清楚。因此，在本研究中我們利用脂質體學的方法透過大鼠長期吸入 $PM_{2.5}$ 的動物模型，去探討慢性暴露 $PM_{2.5}$ 後不同器官與血液的脂質效應。

十隻六週大的雄性 SD 大鼠，五隻全身持續性地暴露於未經濃縮的含有 $PM_{2.5}$ 的外來一般空氣，其他五隻則吸入通過懸浮微粒過濾器的空氣。整個實驗在臺北市臺大公衛大樓進行長達八個月，實驗結束後暴露組與控制組所測得的平均 $PM_{2.5}$ 濃度分別為 16.7 ± 10.1 μg/m^3 與 0.70 ± 0.46 μg/m^3。隨後採集動物的血液樣本及其各式器官，包括心臟、肝臟、胰臟、腎臟、脾臟、睪丸及副睪丸，接著將脂質從每個器官組織及血清中萃取出來進行極致液相層析串聯式質譜儀(UPLC-MS/MS)分析，得到的圖譜經過數據前處理後，再利用偏最小平方判別分析(partial least squares discriminant analysis)搭配無母數統計方法 Wilcoxon rank sum tests 去檢驗暴露組與控制組間的脂質變異。

本研究結果指出慢性暴露 $PM_{2.5}$ 確實會在肺以外的器官造成脂質擾動的情形。偏最小平方判別分析顯示含磷酸膽鹼脂質在大鼠睪丸、胰臟、心臟、肝臟及腎臟的兩組別中有顯著差異，而無母數統計分析顯示在大鼠睪丸中發現最大量的含磷酸膽鹼脂質變化，包括多種磷脂醯膽鹼(lyso-PCs, diacyl-PCs, ether-linked PCs)及神經磷脂(sphingomyelins)，此改變推測可能與維持精子細胞膜完整性、抗氧化、抗發炎及輕微生精功能障礙有關。此外，在血液中的脂質調查發現與睪丸有一致的特定脂質 PC(16:0/18:1)下降趨勢，但其是否為 $PM_{2.5}$ 毒性的潛在生物指標物需要更進一步的研究證實。總結，脂質體學是一種有效不偏頗且靈敏的方法去探究在 $PM_{2.5}$ 造成嚴重損傷前體內的分子變化，同時幫助於潛在生物指標物的開發。

關鍵字：脂質體學、磷脂醯膽鹼、$PM_{2.5}$、慢性暴露、睪丸、毒性

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Abstract

Fine particulate matter ($PM_{2.5}$) is able to pass the respiratory barrier and further enter the circulatory system, and consequently spread to the whole body. $PM_{2.5}$-induced toxicity has been correlated with oxidative stress, which may lead to lipid perturbation. Our previous studies have applied a lipidomic platform to investigate the chronic effects of $PM_{2.5}$ exposure on the pulmonary lipids in rats inhaled ambient air, and found that significantly altered levels of phosphorylcholine-containing lipids, which might impair pulmonary surfactant functions. However, the effects of $PM_{2.5}$ on phosphorylcholine-containing lipids in other organs have not been fully elucidated yet. In this study, we examined the lipid effects of chronic $PM_{2.5}$ exposure on various organs and serum using a rat inhalation model.

Five male Sprague-Dawley rats were continually whole-body exposed to non-filtered and non-concentrated ambient air containing $PM_{2.5}$ from the outside of the Public Health building in Taipei city for 8 months, while five rats were inhaled filtered air. The mean concentrations of $PM_{2.5}$ in the exposure and control group were 16.7 ± 10.1 $\mu g/m^3$ and 0.70 ± 0.46 $\mu g/m^3$, respectively. Blood samples and various tissues, including heart, liver, kidney, pancreas, spleen, testis and epididymis were collected. Then lipids from each organ and serum were extracted for further ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) analysis. Subsequently, the partial least squares discriminant analysis (PLS-DA) and Wilcoxon rank sum tests were used to examine the variations of phosphorylcholine-containing lipids among samples.

Our results demonstrated that after the chronic and low-dose $PM_{2.5}$ exposure, the lipidome were significantly different in the certain organs. In the PLS-DA models, the patterns of phosphorylcholine-containing lipids were altered in the testis, pancreas, heart, liver and kidney of rats exposed to $PM_{2.5}$. After statistical analyses, most of significantly changed phosphorylcholine-containing lipids were discovered in the rat testis after chronic $PM_{2.5}$ exposure. The changed lipids include decreased lyso-phosphatidylcholines (PCs), increased unsaturated diacyl-PCs, a decreased ether-linked PC and increased sphingomyelins, which may be related to maintain membrane integrity of spermatozoa, play anti-oxidants and anti-inflammatory roles, and dysfunction of spermatogenesis. Additionally, our results showed decreased PC(16:0/18:1) was both observed in the serum

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and testis. Further studies to verify potential biomarkers for $PM_{2.5}$-induced toxicity are needed. We concluded lipidomics is a powerful, unbiased, and sensitive approach to study biological molecular effects in different organs after long-term and low concentration $PM_{2.5}$ exposure. Our study suggested target organs of $PM_{2.5}$ exposure and revealed the underlying possible mechanisms of PM-induced toxicity and potential biomarkers.

Key words: Lipidomics, Phosphorylcholine-containing lipids, $PM_{2.5}$, Chronic exposure, Testis, Toxicity

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Contents

doi:10.6342/NTU201904292

doi:10.6342/NTU201904292

List of figures

doi:10.6342/NTU201904292

List of tables

doi:10.6342/NTU201904292

1. Introduction

1.1 Background

In the last decades, with rapid development of industry and transportation, ambient air pollution has become a significant public health issue. Particulate matter (PM), metals, and gaseous pollutants etc. have associated with numerous adverse effects to humans [1, 2]. Especially, fine particulate matter ($PM_{2.5}$) gets more concern due to its complex composition and divergent mechanism of toxicity [3, 4]. Although several animal studies have focused on the acute effect of short-term PM exposure at high dose treatment by intratracheal instillation [5-7], the underlying knowledge on chronic inhalation of real world, non-concentrated ambient PM in animals remain unclear.

1.2 Fine particulate matter

$PM_{2.5}$ refers to particulate matter with an aerodynamic diameter less than 2.5 μm. The main source of $PM_{2.5}$ are the emissions from motor vehicles and the burning of coal [8], which cause the complex chemical properties of $PM_{2.5}$. The major components of $PM_{2.5}$ are sulfates (SO_4^{2-}), nitrates (NO_3^-), ammonium (NH_4^+) and metals. Characteristics of PM-induced toxicity have been related with particular size ranges and composition simultaneously [9]. The main route for the entry of the PM into the body is inhalation, comparing to ingestion and dermal contact [10]. Due to small sizes, $PM_{2.5}$ is able to get deep into the respiratory bronchioles and alveoli where gas exchange occurs [11].

doi:10.6342/NTU201904292

However, $PM_{2.5}$ can not only affect the lung but also multiple extrapulmonary organs of the body. The possible pathways of $PM_{2.5}$ leading to extrapulmonary effects of $PM_{2.5}$ are speculated: (1) nano-sized particulate components can go through the respiratory barrier then into circulatory system and further distributed to whole body, (2) PM-induced lung injury produce bioactive components and cytokines that subsequently enter circulation and affect extrapulmonary tissues, (3) PM interacts with pulmonary nerves and activate the autonomic nervous system resulted in systemic alterations [12-14].

1.3 Epidemiological studies on fine particulate matter

Several epidemiological studies have shown the association between $PM_{2.5}$ and numerous health effects, including increased hospital admissions, emergency room visits and mortality [15-17]. Moreover, typical pulmonary diseases have been reported, such as respiratory symptoms and lung cancer. However, more and more recent studies indicated that exposure to $PM_{2.5}$ could induce the non-pulmonary disease [18]. For example, $PM_{2.5}$ was related to adverse effects on male reproductive system [19]. Sperm morphology and motility chronologically negatively correlated with $PM_{2.5}$ levels recorded in males in Salt Lake County after two to three months exposure [20]. Comparing to rural area, exposure to higher concentrations of SO_2 and NO_2 of urban ambient air may associate with worse semen quality in Chongqing urban males [21]. Some animal studies were also indicated relationship between $PM_{2.5}$ and male reproductive dysfunction in rats [22, 23]. On the other hand, Pearson et al. suggested that ambient $PM_{2.5}$ may cause the increased

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prevalence of diabetes in the adult population in U.S. [24], and consistent results were found in animal model after $PM_{2.5}$ exposure, which revealed inflammation of adipose tissue and insulin resistance [25]. American Heart Association (AHA) has indicated that from epidemiology to toxicological studies all provided powerful evidence about $PM_{2.5}$ contributing to cardiovascular morbidity and mortality [26].

1.4 Mechanism of fine particulate matter-induced toxicity

Researchers have suggested several mechanisms about $PM_{2.5}$ induced adverse health effects. After arriving at target cells, $PM_{2.5}$ may alter the cellular physiological processes by increasing oxidative stress, inflammation, and genotoxicity, resulting in the injury on the tissues and organs, and finally develop to cardiopulmonary diseases, diabetes mellitus, adverse reproductive effects and others [4].

Oxidative stress has been considered as an important mechanism of $PM_{2.5}$-induced toxicities[27]. Oxidative stress occurs when the level of reactive oxygen species (ROS), a byproduct of energy metabolism pathway of aerobic organism, exceeds antioxidant capacity [28]. The environmentally persistent free radicals from the combustion-derived particles in $PM_{2.5}$ via reduction-oxidation cycling, or $PM_{2.5}$-mediated activation of inflammatory cells, are both capable of generating excessive ROS [29, 30]. Afterward, these ROS can readily react molecules, such as lipids, proteins and DNA, altering their structure and function, and then cause damages to the target cells and tissues [31]. A number of studies have shown that lipid peroxidation are associated with increased levels

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of $PM_{2.5}$ [32-34]. When free radicals attack lipids that contain carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFA) containing two or more double bonds, lipid peroxidation was occurred [34]. Lipids play important roles on maintaining the structure and function of cell membrane [35]. Cell culture studies showed $PM_{2.5}$ may destroy the cell membrane integrity after the bright field microscopy analysis [36, 37]. Hence, a series of ROS induced by $PM_{2.5}$ have the potential to induce a systemic oxidation of polyunsaturated phospholipids and severely impair membrane function.

As mentioned above, inflammation could prompt oxidant injury, thereby inflammation and oxidative stress are closely linked and inseparable [38]. Numerous studies have reported systemic inflammation was observed in humans and animals exposed to $PM_{2.5}$, with increased levels of inflammation biomarkers, including C-reactive protein (CRP), Interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and other markers [39-42]. Lysophospholipids and sphingolipids are bioactive lipids that could actively modulate the chronic inflammatory response and help the body to restore homeostatic balance [43]. In fact, previous studies indicated that not only certain lipid species act to regulate inflammatory responses, but inflammatory signaling also can influence lipid metabolism in biological process conversely [44].

1.5 Lipids and Lipidomics

Lipids are kind of hydrophobic small molecules, and their functions include cell membrane formation, energy storage, molecules regulating, and signaling [35]. Based on

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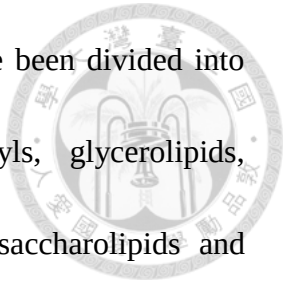

their chemical structure and biosynthetic characteristics, lipids have been divided into eight categories by LIPID MAPS [45], including fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides.

Lipidomics is a branch of the field of metabolomics. Lipidomics aims to comprehensively identify and quantify a wide range of lipids, and determine lipid roles in physiological processes. Lipidomics can characterize the biochemical changes induced by environmental stimuli by detecting alterations of lipids [46]. Thousands of lipids can be detected at a time by high-performance mass spectrometry (MS), and presenting abundant information about qualitative and quantitative analysis simultaneously. Relying on the advance of MS analysis technology, lipidomic investigations on exploring disease biomarkers and illustrating metabolic pathways become more efficient and powerful [47]. Because of high sensitivity and high throughput properties, application of MS-based lipidomics is widely to numerous fields, such as toxicology, nutrition, environmental sciences, and others [48]. In the past, numerous PM studies using metabolomics showed the changes of lipids and importance of those lipid in response to PM exposure [49-51]. However, studies focusing on PM effects on lipids are little.

1.6 Phosphorylcholine-containing lipids

Phosphorylcholine-containing lipids are abundant membrane lipids, with significant biological importance. Phosphorylcholine-containing lipids include

doi:10.6342/NTU201904292

phosphatedylcholines (PCs), a type of glycerophospholipids, and sphingomyelins (SMs), a type of sphingolipids, because their structures are both with a phosphocholine head group.

PCs are the major structural lipids in cell membranes and account for more than 50% of glycerophospholipids [52]. The basic composition of PCs is a glycerol backbone and a phosphocholine head group at the sn-3 position. Base on different fatty acyl substituents at the sn-1 and/or sn-2 position of glycerol backbone, PCs can be divided into lyso-phosphatidylcholines (lyso-PCs), diacyl-phosphatidylcholines (diacyl- PCs), plasmanylcholines (O-alkyl-acyl-PCs, O-PCs) and plasmenylcholines (O-alkenyl-acyl-PCs, P-PCs) (Figure 1). Moreover, the various numbers of carbons and double bonds on fatty acyl substituents also present different PCs, for instance, PC(16:0/18:1) means a fatty acyl substituents with 16 carbons and no double bond at the sn-1 position accompany with 18 carbons and 1 double bond at the sn-2 position.

SMs also play an important role in cell membrane and constitute 2–15% of the total phospholipid in mammal organs. Besides, certain part such as brain tissue and peripheral nervous tissue have even higher SM contents [53]. SM consists of a sphingosine backbone, a phosphocholine head group and fatty acids, which is one of the few membrane phospholipids not synthesized from glycerol. Overall, investigation of phosphorylcholine-containing lipids of various organs after chronic $PM_{2.5}$ exposure may help to characterize the PM effects at molecular level and understand the potential health

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effects.

Our laboratory has developed a liquid chromatography-tandem MS platform [54] to profile the alterations of phosphorylcholine-containing lipids in rat lungs after chronic $PM_{2.5}$ exposure [55]. The results showed that PM exposure cause decreases in lyso-PCs, surfactant PCs, unsaturated PCs and plasmenylcholines which may indicate repeated inflammatory responses, injuries of alveolar cells, and altered membrane integrity. Additionally, emphysematous and inflammatory cells were observed on the lung of PM exposed rats [55]. Although the significantly altered phosphatidylcholine levels in the lung of rats, which may impair pulmonary surfactant functions, the lipid effects after chronic exposure of $PM_{2.5}$ on other organs remain unclear.

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1.7 Study objectives

The objectives of this study is as following:

(1) To examine the effects of chronic $PM_{2.5}$ exposure on phosphorylcholine-containing lipids in various organs and blood through a rat inhalation model.

(2) To clarify and compare $PM_{2.5}$-induced lipid alteration in different organs, and suggest the target organs and potential biochemical mechanisms.

(3) To determine whether the specific lipid alteration between the target organ and blood after chronic $PM_{2.5}$ exposure which can serve as potential biomarkers.

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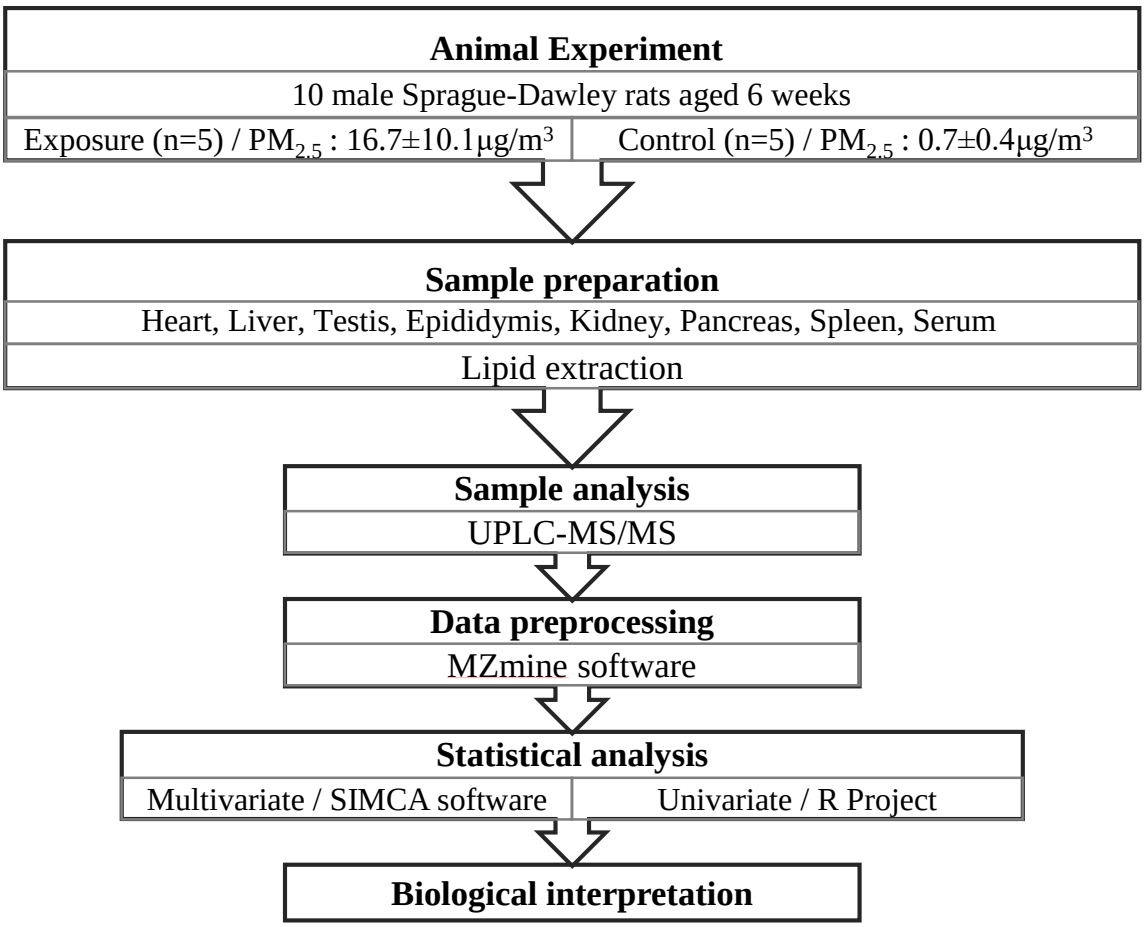
2. Materials and methods

2.1 Experiment flow chart



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2.2 Animal experiment

All the procedures of animal experiments were conducted by Dr. Tsun-Jen Cheng's laboratory [55, 56]. The animal care and experiments were approved by the National Taiwan University Institutional Animal Care and the Use Committee. Briefly, ten male Sprague-Dawley rats aged six weeks were purchased from Lasco, Charles River Technology (Yilan, Taiwan). The rats had acclimatized in cages with enough food and water for 14 days before the experiment was conducted. Exposure group (n=5) were continually whole-body exposed to non-filtered and non-concentrated ambient air containing $PM_{2.5}$ for 24 h/day, 8 months (November 2012 to June 2013) using Taipei air pollution exposure system (TAPES), which was located at the inhalation toxicology laboratory of the Public Health building. Meanwhile, control group (n=5) were inhaled filtered air through a high-efficiency particulate air (HEPA) purifier added on the inlet valve of cage.

Rats were sacrificed by overdose Zoletil, and blood samples were collected from the abdominal aorta. The tissues including heart, liver, kidney, pancreas, spleen, testis and epididymis were taken and wrapped in aluminum foil, and snap-frozen with liquid nitrogen.

2.3 Lipid extraction

All the procedures of lipid extraction from different tissues and blood were

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conducted by Chen et al. [55]. Briefly, the tissue samples were homogenized at a low temperature in liquid nitrogen, and freeze-dried for further lipid extraction. 1.0 mg tissue (or 0.01 mL serum) was spiked with the 1.0 mg/L internal standard including sphingomyelin (d18:1/17:0) in 0.2 mL of methanol. Next, the spiked sample was added 0.15 mL of 0.15 M sodium chloride$_{(aq)}$ and 0.4 mL of chloroform after thorough vortexing. The mixtures were vortexed for 10 minutes and centrifuged at 10,000 rpm and 4°C for 10 minutes. Eventually, 0.4 mL of the lower layer was collected and dried. The dried lipid extracts were reconstituted with 0.2 mL of methanol and filtered for further instrumental analysis.

2.4 UPLC-MS/MS for phosphorylcholine-containing lipids profiling

LC-MS method was based on our previous publication by Tang et al. [54] and the analysis was conducted by Chen et al. [55]. Three duplicate lipid samples from each tissue and serum of each rat were analyzed by Waters ACQUITY Ultra-performance liquid chromatography system coupled with Waters Quattro Premier XE triple quadrupole mass spectrometry (UPLC-MS/MS) (Waters, Milford, MA, USA).

Reversed-phase liquid chromatography with Waters BEH C_{18} column (1.7 μm, 2.1 mm x 100 mm) was conducted in the binary solvent system, including A: 10 mM ammonium acetate in water and B: acetonitrile/methanol (65/35, v/v) containing 10 mM ammonium acetate. In MS system, the positive ion of the precursor ions of the mass-to-charge ratio (*m/z*) 184 was scanned, which was the signal of phosphorylcholine head

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group in $[M+H]^+$ scan.

2.5 Data preprocessing

The raw MS spectra was transformed into NetCDF by Masslynx V4.1 software (Waters, CA, USA). Afterward MZmine 2.33. software [57] was applied to process and analyze mass spectrometry based molecular profile data. Briefly, we performed mass detection, chromatogram building, chromatogram deconvolution, deisotoping and alignment to develop a preliminary data list. The minimal signal intensity was 3000 and *m/z* tolerance was 1000 ppm. Next, we executed gap-filling check manually to filter out unstable signals and fill the correct integral areas. The *m/z* tolerance was 1000 ppm and RT tolerance was 0.1 min. Finally, the peak area of each detected lipid was normalized by the total spectra area using Excel (Office 2016, Microsoft, USA) to balance the variation of instrument sensitivity.

2.6 Multivariate analysis

The statistical analysis we used were divided into two methods, multivariate combine with univariate analysis. The processed data were imported into SIMCA 13.0.1 (Umetrics, Umeå, Sweden) for multivariate analysis. In SIMCA 13.0.1, after logarithm transformation and unit variance scaling, we performed partial least squares discriminant analysis (PLS-DA), a common supervised multivariate analysis which was applied in highly-dimensional dataset, such as metabolomics data [58]. The score plot of PLS-DA

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model could visualize the clustering of samples, and the variable importance in projection (VIP) score indicated the contribution of each lipid for the difference between two groups. The PLS-DA model was evaluated using R^2Y and Q^2, which represent the model performance and prediction, respectively. The levels of R^2Y were higher than 0.9 indicated that the model was powerful; Q^2 values were not less than 0 indicated that the model was qualified [58]. In addition, permutation test, a statistical tool which rearranges the labels on the samples to calculate distribution of all possible statistic values, could confirm whether the model was over-fitting or not. We performed 500 randomly assigning samples to the two groups, and all of the 500 Q^2 levels in the randomly grouped models should be lower than the Q^2 in the original model or the intercept of random Q^2 regression line and Y axis should be below 0 [58].

2.7 Univariate analysis

The fold changes of identified lipid features were present by the median of peak area ratio between two groups. The univariate analysis was used to evaluate the statistical significance between two groups by independent variable. Owing to the sample size, Wilcoxon rank sum tests, the nonparametric statistics was conducted to examine the statistical significance between $PM_{2.5}$ treatment and control groups (p-value < 0.05) by R 3.3.1 project. Furthermore, false discovery rate (FDR) method was also used to compare two group strictly for avoiding false-positive findings caused by multiple testing (p-value < 0.2) [59]. In metabolomics, especially for detecting environmental impacts, often

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tolerant FDR thresholds are used in order to capture as many features as possible, because environmental chemicals effects in the human metabolites may be slight [60]. However, only a few lipid features in the testis and serum were qualified FDR correction. Therefore, combining with multivariate analysis, we identified the significant changed lipid features that had *p*-values lower than 0.05 in Wilcoxon rank sum tests and VIP scores higher than 1.00 in the PLS-DA model.

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3. Results

3.1 $PM_{2.5}$ exposure data

The concentrations of $PM_{2.5}$ in the exposure and control cages throughout the experiment were 16.7 ± 10.1 μg/m3 and 0.70 ± 0.46 μg/m3 (mean ± standard deviation), respectively. $PM_{2.5}$ is accounted for 99.2% of total PM less than 10 μm in diameter (PM_{10}) in the whole TAPES, which demonstrated the majority of the particles rats exposed to were $PM_{2.5}$. Furthermore, the major components of particles in TAPES were organic carbon (30.9%), sulfate (30.4%), ammonium (14.8%) and nitrate (11.4%), which showed typical traffic-related air pollution in Taipei City [55].

3.2 Phosphorylcholine-containing lipids composition in various organs of rat

After MS analysis and data preprocessing, the numbers of phosphorylcholine-containing lipid signals detected in each organ of rat were following: kidney was 128, pancreas was 126, testis was 123, spleen was 118, epididymis was 100, liver was 92, heart was 82, and lung from Chen et al. was 110 [55]. Furthermore, there were 68 lipid signals in serum of rat. Then, the lipid signals were identified by our in-house library with *m/z* and retention time. The numbers of lipids and percentages of each lipid class among total lipids in each organ and serum were shown in Figure 2.

The diacyl-PCs were the major lipid class, which almost accounted for half

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percentage among total phosphorylcholine-containing lipids in all organs. SMs were second largest lipid class in each organ, and they were most abundant in the kidney than other organs. Besides, ether-linked PCs displayed slightly higher percentage in the epididymis (Figure 2).

3.3 Effects of $PM_{2.5}$ on patterns of phosphorylcholine-containing lipids in the organs and serum of rats

In the PLS-DA model, each spot represents a sample, and the distance between two samples are closer which represents they are more similar. In this study, chronic ambient $PM_{2.5}$ exposure mostly induced more phosphorylcholine-containing lipid changes in the testis, pancreas, and serum of rats, than those in the heart, liver and kidney of rats (Figure 3). No obvious changes on patterns of phosphorylcholine-containing lipids were found in the spleen and epididymis of rats after exposure.

In the testis, phosphorylcholine-containing lipids profile of the treatment group differed from that in the control group in PLS-DA model (Figure 3A). Besides, the total explained variance R^2Y was 0.962 and the predictive ability Q^2 was 0.825. The PLS-DA model also satisfied the permutation test, because original Q^2 value was higher than randomized Q^2 values (Figure 4A).

In the pancreas, one sample in the exposure group of pancreas was excluded from following analysis as a result of its weak MS signal intensity which was approximately 10 times lower than other samples. We recorded this sample was not completely dried

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after lyophilisation, which may cause PC degradation. In the PLS-DA model, phosphorylcholine-containing lipids profile of the pancreas in the treatment group also differed from that in the control group (Figure 3B). Besides, the total explained variance R^2Y was 0.972 and the predictive ability Q^2 was 0.833. The permutation test was also qualified (Figure 4B).

In the heart, the phosphorylcholine-containing lipids profile of the treatment group and control group presented a clear separation in PLS-DA model (Figure 3C). The total explained variance R^2Y was 0.933 and the predictive ability Q^2 was 0.627, and the model passed the permutation test absolutely (Figure 4C).

Liver samples have different phosphorylcholine-containing lipids pattern between treatment group and control group in PLS-DA model (Figure 3D). The acceptable total explained variance R^2Y and predictive ability Q^2 was 0.955 and 0.498, respectively. It also qualified permutation test result (Figure 4D).

In the PLS-DA model for the analysis of kidney, the pattern of treatment group seems to differed from that in the control group (Figure 3E), and the total explained variance R^2Y was 0.928, but the predictive ability Q^2 was 0.272. However, it was still considered that $PM_{2.5}$ exposure caused the significant alteration of phosphorylcholine-containing lipids in kidney of rat, due to its eligible permutation test result (Figure 4E).

Serum can reflect the systemic effect of exposure. The phosphorylcholine-containing lipids profile of $PM_{2.5}$ treatment group was quite different from that of control

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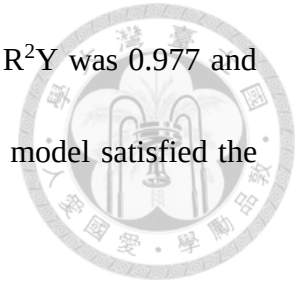
group in PLS-DA model (Figure 3F). The total explained variance R^2Y was 0.977 and the predictive ability Q^2 was 0.885, and Figure 4F showed that the model satisfied the permutation test totally.



3.4 Effects of $PM_{2.5}$ on individual phosphorylcholine-containing lipids in the organs and serum of rats

3.4.1 Testis

After Wilcoxon rank sum tests combined with the VIP scores of the PLS-DA model, 24 significantly changed testis phosphorylcholine-containing lipids were picked (Table 1). In the treatment group, the lyso-PCs including PC(16:1/0:0) + PC(0:0/16:1), PC(20:4/0:0), PC(0:0/18:1), PC(20:2/0:0) and PC(22:5/0:0), were significantly lower than those in control group. A few saturated and monounsaturated diacyl-PCs such as PC(16:0/16:0) and PC(16:0/18:1), and one ether-linked PC, PC(P-36:3), were also decreased in the rat testis after chronic $PM_{2.5}$ treatment.

On the other hand, among nine changed diacyl-PCs, the levels of six polyunsaturated diacyl-PCs, including PC(18:2/20:4), PC(16:0/22:6), PC(34:4), PC(38:2), PC(42:6) and PC(42:4) had the same trend which is higher in the $PM_{2.5}$ exposure group compared with those in the control group. Lastly, SMs such as SM(d18:2/23:0), SM(d18:2/16:0) and other unknown-SMs except SM(d34:1), were all higher than those in the control group. Noticeably, all the significantly changed phosphorylcholine-containing lipids of testis

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passed the FDR correction (p-value < 0.2).

3.4.2 Pancreas

In the pancreas, five significantly changed phosphorylcholine-containing lipids were recognized after rats exposing to $PM_{2.5}$ (Table 2). Comparing to that in the control group, the level of lyso-PC, PC(0:0/18:2) was higher in ambient $PM_{2.5}$ treatment group. Besides, among four changed diacyl-PCs, three diacyl-PCs including PC(16:0/22:6), PC(18:0/22:6) and PC(44:4), were lower in treatment group than those in the control group. However, a diacyl-PC, PC(40:2), was increased after chronic $PM_{2.5}$ treatment.

3.4.3 Other organs

In the heart and liver, only two phosphorylcholine-containing lipids satisfied both criteria of multivariate analysis (VIP score >1.00) and univariate analysis (p-value < 0.05) (Table 3), even though there were approximately 30 lipids with VIP scores higher than 1.00. The level of diacyl-PC(20:4/20:4) was increased, and the level of ether-linked PC, PC(O-18:0/16:0), was decreased in the heart of rat after chronic $PM_{2.5}$ exposure (Table 3). In the liver, diacyl-PC(34:4) was 0.5-fold lower in treatment group than that in the control group (Table 3).

On the other hand, although four significantly changed phosphorylcholine-containing lipids were detected in the kidney, only an ether-linked PC, PC(O-18:0/16:0) was identified to decrease in the kidney of rat after chronic $PM_{2.5}$ exposure (Table 3).

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Interestingly, the increasing trend of unknown-SM was consistent with that in the testis.

3.4.4 Serum

After statistical analyses, seven significantly changed phosphorylcholine-containing lipids were recognized in the serum (Table 4), and four of them even passed the FDR correction (p-value < 0.2). The level of lyso-PC(18:0/0:0) was higher in the $PM_{2.5}$ exposure group than that in the control group, moreover, unsaturated diacyl-PCs, including PC(16:0/16:1), PC(16:0/17:1), PC(16:0/18:1) and PC(16:0/20:4) were lower in the $PM_{2.5}$ exposure group compared with those in the control group.

In addition, phosphorylcholine-containing lipids profiles in the serum were also used to find out the potential biomarkers for specific organs after $PM_{2.5}$ exposure in the present study. After comparison of the changed lipids between serum and organs, decrease PC(16:0/18:1) was both found in the serum and testis in the $PM_{2.5}$ treatment group compared with control group (Table 1 & 4).

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4. Discussion

A previous literature has demonstrated that lung PC alterations of the rat that were exposed to ambient $PM_{2.5}$ chronically, which were associated with inflammation, oxidative stress, and alveolar cell injuries [55]. However, The trend towards atypical respiratory-related disease was reported by epidemiological and toxicological studies on $PM_{2.5}$ health effect [18]. Although there were few lipidomic studies about extrapulmonary effects of $PM_{2.5}$ exposure, the perturbations of lipidome were found in the testis, pancreas, heart, liver and kidney of rat after chronic $PM_{2.5}$ exposure in the present study. In this study, we applied an MS-based lipidomics approach to examine the changes in phosphorylcholine-containing lipids after rat inhalation of ambient air to further identify potential health effects of $PM_{2.5}$.

4.1 Lipidome comparison among various organs of rat

In this study, we established a foundational phosphorylcholine-containing lipid library of various rat organs through MS-based lipid profiling. This is the first study comparing phosphorylcholine-containing lipids in various organs. Our result showed that diacyl-PCs almost accounted for half percentage among total phosphorylcholine-containing lipids in all organs (Figure 2), and SMs account for 30% in the phosphorylcholine-containing lipids of rat kidney, which is most abundant among all organs (Figure 2A). Previous studies showed that SMs abound in phospholipid class

doi:10.6342/NTU201904292

distribution of rat kidney comparing to other organs such as liver, spleen and heart [61-64]. Moreover, O-PC (alkyl ether bond) and P-PC (alkenyl ether bond), are richer in the rat epididymis than other organs (Figure 2E). In fact, spermatozoa from mammal animal such as human, ram and rat contain high levels of ether-linked PCs [65-68], and epididymis play a role in storage and maturation of spermatozoa [69, 70].

In addition, we also found that the lipid effects followed by chronic inhalation of $PM_{2.5}$ particles. Our results suggested that testis was the most sensitive organ for $PM_{2.5}$ through the results of PLS-DA model combined with Wilcoxon rank sum tests, which both present more significant alterations of lipidome than those in the other organs. Consequently, the potential mechanism underlying the rat sensitive organs after chronic $PM_{2.5}$ exposure were discussed in the following sections.

4.2. Significantly changed phosphorylcholine-containing lipids in the rat testis after chronic $PM_{2.5}$ treatment

In this study, we observed the significant decreased lyso-PCs, increased unsaturated diacyl-PCs, a decreased ether-linked PC and increased SMs in the testis of rat exposed to $PM_{2.5}$. The decreased levels of lyso-PCs and increased levels of unsaturated diacyl-PCs may be associated with membrane function. Lyso-PCs could influence the permeability and flexibility of membrane, due to their micelle property in lipid aggregated structures [71]. Thus, decreased lyso-PCs could be regarded as protective roles to promote membrane curvature to maintain stability [72], and to avoid forming the pores on

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membrane [73]. In addition, the double bond on unsaturated fatty acyl chains of diacyl-PCs increases the inter-chain distance and forms space of membrane by creating the rigid kink in the hydrocarbon chain [74]. Comparing with the saturated diacyl-PCs, unsaturated diacyl-PCs can prevent fatty acyl chains from packing closely together and provide more fluidity and flexibility of membrane [74, 75]. Previous metabolomic study has also demonstrated increasing trends of unsaturated diacyl-PCs in testis of rats exposed to $PM_{2.5}$, the outcomes may affect sperm morphology and thus impaired the reproductive function of rats [76]. In this study, the decreased lyso-PCs and increased unsaturated diacyl-PCs in the testis may relate to promote the integrity of cell membrane.

On the other hand, most of up-regulation diacyl-PCs contained PUFA chains, including PC(18:2/20:4), PC(16:0/22:6), PC(34:4), PC(42:6) and PC(42:4). The increased unsaturated diacyl-PCs could not only bend membrane and enhance flexibility, but also supply capacity for surface area expansion and external force resistance, such as oxidative stress induced cell swelling [77, 78]. Therefore, increase diacyl-PCs contained PUFA chains may act as important roles to strengthen cellular function as well as resist further $PM_{2.5}$-induced oxidative stress.

In present study, we observed the decreased P-PC, PC(P-16:0/20:3), which may be associated with anti-oxidants. Ether-linked PCs are PCs with an ether bond at the sn-1 position which is more susceptible to oxidative attack than the ester-linked PCs [79, 80], however, they are also prone to autoxidation and thus inhibit peroxidation of PUFA [81].

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Because the excessive oxidative stress may lead to cellular damage and aging, and even apoptosis [82, 83]. Therefore, the consumption of ether-linked PCs might play an anti-oxidant role to trigger self-protection mechanism, and protect other membrane constituents from oxidative conditions.

Five increased SMs were discovered in the testis after rat exposed to $PM_{2.5}$ chronically. Although a few SMs with large molecular weight were unidentified due to shortage of database and insufficient MS signal intensity for structural identification, previous studies showed SMs indeed contain very long chain-fatty acid with at least 28 carbons in certain tissues such as testis and sperm [84, 85]. Through sphingomyelinases, hydrolysis of SMs generate simpler sphingolipids such as ceramides, which is involved in cell signaling events [86, 87], cell survival regulation and apoptosis [88, 89]. The accumulation of SMs may result from suppression of sphingomyelinase activity, importantly, SMs were demonstrated to serve as an endogenous anti-inflammatory molecule [90]. SMs helps maintain the integrity of the plasma membrane by protecting diacyl-PCs against phospholipase degradation. Previous study indicated that lecithin-cholesterol acyltransferase (LCAT) action is inhibited by SMs [91] and thereby regulate the eicosanoid synthesis and the inflammatory reactions [90, 92]. Thus, the increasing trend of testis SMs in this study may protect cell/tissue from $PM_{2.5}$-induced inflammation.

Furthermore, besides the general functions of phosphorylcholine-containing lipid species, the certain activities of those in the testis are also essential. Lyso-PCs, which

doi:10.6342/NTU201904292

possessed a hydroxyl substituent at either the sn-1 or sn-2 position instead of the esterified linking fatty acyl substituent, can be converted back to PCs by the re-acylation of lysophosphatidylcholine acyltransferase (LPCAT) [93]. The perturbations in lyso-PCs could have resulted from abnormal enzyme activities [94], and previous study indicated overexpression of the LPCAT1 gene may contribute to the progression of human cancers [95], such as prostate cancer [96]. Thus, decreased lyso-PCs may correlate with some form of reproductive disease. In addition, dihydroxyacetonephosphate acyltransferase (DHAPAT) are enzymes involved in the synthesis of the ether bond on lipids in mammals, previous study described the DHAPAT-deficient mouse model with the lack of ether-linked lipids presented early arrest of spermatogenesis [97, 98]. It also be mention in the chapter 4.5. The SMs contain very long chain-fatty acid, particularly in testis, is related to maintain membrane integrity of spermatozoa, sperm capacitation and survive in the female reproductive tract [99, 100].

In conclusion of testis lipidome changes after $PM_{2.5}$ exposure, the decreased lyso-PCs and increased unsaturated diacyl-PCs could strengthen the physical properties of cell membrane to resist membrane morphological alterations. Besides, the increased diacyl-PCs consisting of PUFAs and decreased ether-linked PCs were regarded as anti-oxidants, and increased SMs also act a protective role against inflammation induced by $PM_{2.5}$.

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4.3 Contrary trend of changes of phosphorylcholine-containing lipid in the rat pancreas after chronic $PM_{2.5}$ treatment

In this study, comparing to the results in the testis, we found the contrary trend of lipidome alterations such as an increased lyso-PC and decreased unsaturated diacyl-PCs in the pancreas after chronic $PM_{2.5}$ treatment. The down-regulation of diacyl-PCs with unsaturated fatty acyl chains included PC(16:0/22:6), PC(18:0/22:6) and PC(44:4). Based on physical function of unsaturated diacyl-PCs on the cell membrane, it may be inferred a phenomenon of poor fluidity and flexibility of the membrane [74].

On the other hand, The position of double bonds on fatty acyl chains of diacyl-PCs is highly vulnerable to oxidative attack from free radicals, due to its much reactive attribute [101]. Under sustained attack, the diacyl-PCs become degraded through their fatty acid tails [102]. Thus, decreased unsaturated diacyl-PCs in the pancreas could result from damaged membrane integrity induced by elevating oxidative stress.

As we know, pancreas is an important organ about our blood glucose balance, and the pancreas dysfunction involves in each type of diabetes [103]. Besides, diabetes is one of the major risk factors for cardiovascular disease, and at least a half percent of people with diabetes die from some form of hypertension and cardiovascular disease [104]. Accordingly, the trend of changed lipids in the pancreas may be observed in the serum meanwhile.

Our results displayed only two increased lyso-PCs, PC(0:0/18:2) and PC(18:0/0:0),

doi:10.6342/NTU201904292

after $PM_{2.5}$ treatment, one is in the rat pancreas and the other in the serum. A lot of studies suggested the increased level of lyso-PCs in the plasma of diabetes mellitus and cardiovascular disease patients [105-108]. Lyso-PCs could be involved in the pathogenesis of type II diabetes by inducing insulin resistance and islet cell injury [109]. Martina Wallace et al. indicated that PC(0:0/18:2) and PC(18:0/0:0) are specific lipids highly associated with insulin resistance after lipidomic analysis on 39 human blood samples [110]. Besides, they also reported that, in the blood of human with insulin resistance, PC(34:1) and PC(36:4) have a negative association with CRP, which is a major marker of inflammation [110]. Interestingly, we also observed decreased diacyl-PCs, PC(16:0/18:1) and PC(16:0/20:4) in the serum after chronic $PM_{2.5}$ treatment.

Additionally, the perturbations in lyso-PCs could have resulted from abnormal enzyme activities, lyso-PCs are produced by the cutting of PCs via the phospholipase A_2 (PLA_2) [111] or by the LCAT action on plasma lipoproteins [112]. Higher LCAT activity is correlated with insulin resistance and promotes metabolic syndrome [113]. Overproduction of lyso-PCs induced by enhanced activity of PLA_2 in endothelial cells [114], which lead to oxidative stress elevations as well as development of atherosclerosis [115, 116].

In conclusion of pancreas lipidome changes after $PM_{2.5}$ exposure, the decreased unsaturated diacyl-PCs and increased lyso-PCs may indicate elevating oxidative stress and inflammation, which may associated with insulin resistance, early stage of diabetes

doi:10.6342/NTU201904292

and cardiovascular disease.

4.4 Less changes of phosphorylcholine-containing lipids in other organs of rats after chronic $PM_{2.5}$ treatment

Unlike the testis and pancreas, less changes of phosphorylcholine-containing lipids in the heart, liver, and kidney of rats exposed to $PM_{2.5}$ chronically. It may attribute to the long-term and low-dose $PM_{2.5}$ exposure, which provided the recovery period for slightly injury of those organs. Particularly in the liver, containing higher levels of glutathione which is an antioxidant to protect from $PM_{2.5}$-induced damages [117, 118]. However, it did not explain the totally changeless lipidome in the heart and kidney after chronic $PM_{2.5}$ treatment, the $PM_{2.5}$-induced effects may reflect on the other classes of lipids.

4.5 Lipid alterations in blood may reflect to organ toxicity

The circulatory system is a network by circulating blood to transport molecular like nutrients, hormones, and metabolites to and from the different organs in the body. The alterations of phosphorylcholine-containing lipids in the rat serum after exposing to $PM_{2.5}$ could reflect the $PM_{2.5}$-induced systemic effects from various organs by compare the results from serum with target organs, it may determine the potential exposure biomarkers of target organs for application in the future.

After chronic $PM_{2.5}$ exposure, the overall alteration trend of subclass of phosphorylcholine-containing lipids in the serum was similar to those in the pancreas.

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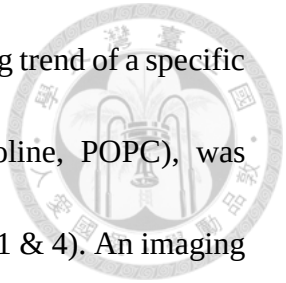

However, on the alteration of individual lipid, the consistent decreasing trend of a specific lipid, PC(16:0/18:1) (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC), was observed in the testis and serum after chronic $PM_{2.5}$ treatment (Table 1 & 4). An imaging MS research indicated POPC located in the peripheral and the middle layer of the seminiferous tubules but not inner layer, which include spermatogonium and spermatocyte [119]. Based on the reduction of POPC, this result was consistent with our above inference about disturbance of early spermatogenesis [97, 98].

Remodeling of POPC is one of pathway to generate PC(16:0/16:0) (Dipalmitoylphosphatidylcholine, DPPC) which accounts for 60% in the pulmonary surfactant system [120, 121]. Previous study supported exogenous DPPC was able to protect a maintenance of spermatogenesis from chemotherapeutic agents-induced cytotoxicity, especially on morphological aspects [122]. Therefore, in the current study, the lower trend of testis DPPC attributed to insufficient POPC may cause dysfunction of spermatogenesis in $PM_{2.5}$ treated rats. Besides, another study suggested palmitic acid (C16:0) and stearic acid (C18:0), substrates for POPC, could induce apoptosis in testicular Leydig cell [123], a type of interstitial cells is in charge of testosterone biosynthesis [124].

POPC was proved to serve as a component of the pulmonary surfactant system about alveolar surface tension in the past [120, 121], but the functions of POPC in the testis remain unclear now. Our previous study did not observe significantly changed POPC in the lung of rat from our identical $PM_{2.5}$ treatment batch [55]. However, we revealed that

doi:10.6342/NTU201904292

changes of POPC was simultaneously observed in the mouse lung and serum after naphthalene treatment [125, 126], which may relate to the PPAR α pathway in the pulmonary system [126]. Hence, PC(16:0/18:1) might be a unique and specific lipid in different organs under different environmental stimuli.

The alterations of blood lipids may be contributed from various organs, as a result, further studies to confirm whether PC(16:0/18:1) would be a potential biomarker of $PM_{2.5}$ in the serum to measure testis effects are needed.

4.6 Limitation and future work

In this study, we focused on the phosphorylcholine-containing lipid effects after chronic $PM_{2.5}$ exposure. However, the perturbations of other lipids are unknown. Additionally, in some organs such as pancreas, the levels of lipidome difference between two groups in PLS-DA model was not consistent with those in Wilcoxon rank sum tests; we speculate the univariate analysis was easily influenced by outlier sample, due to our small sample sizes (*n*). On the other hand, the lipid changes may occur in certain cell types of the organ; however, we are unable to confirm, due to an entire organ was used for analysis.

To our knowledge, this is the first study to examine the changes of phosphorylcholine-containing lipids in various organs and serum of rats after chronic exposure to low-dose $PM_{2.5}$ by MS-based lipidomics. Although few researches demonstrated that the direct relation between $PM_{2.5}$ and changes of lipids in

doi:10.6342/NTU201904292

extrapulmonary organs, the underlying mechanisms is not clear. The results of this study provided the direction and foundation for future studies. In the future, further examination such as histopathological analysis focusing on the target organ—testis, and insulin resistance test on the pancreas will help us to clarify the potential important health effects of $PM_{2.5}$ exposure on the male reproductive system and pancreas function.

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Figures

doi:10.6342/NTU201904292

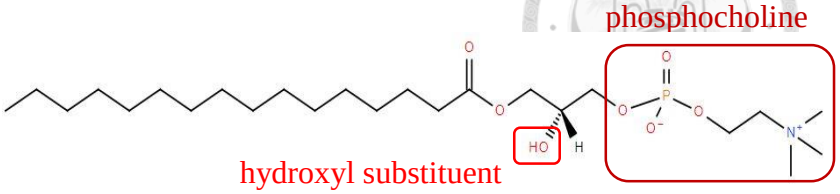
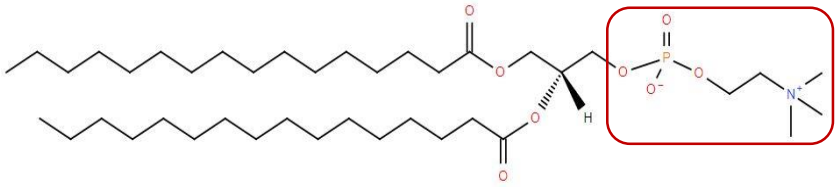
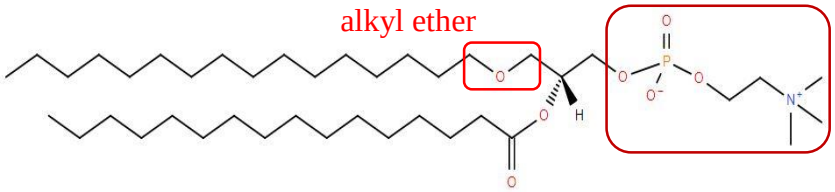
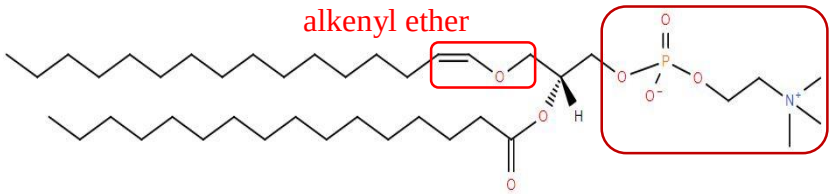
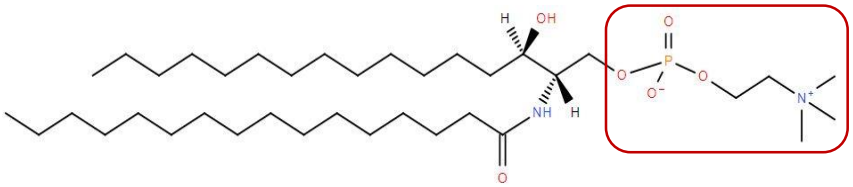
Phosphorylcholine-containing lipids	Representative structures
Lyso-PCs (Lyso-phosphatidylcholines)	
Diacyl-PCs (Diacyl-phosphatidylcholines)	
O-PCs (O-alkyl-acyl-PCs, Plasmanylcholines)	
P-PCs (O-alkenyl-acyl-PCs, Plasmenylcholines)	
SMs (Sphingomyelins)	

Figure 1. Representative structures of various phosphorylcholine-containing lipids.

doi:10.6342/NTU201904292

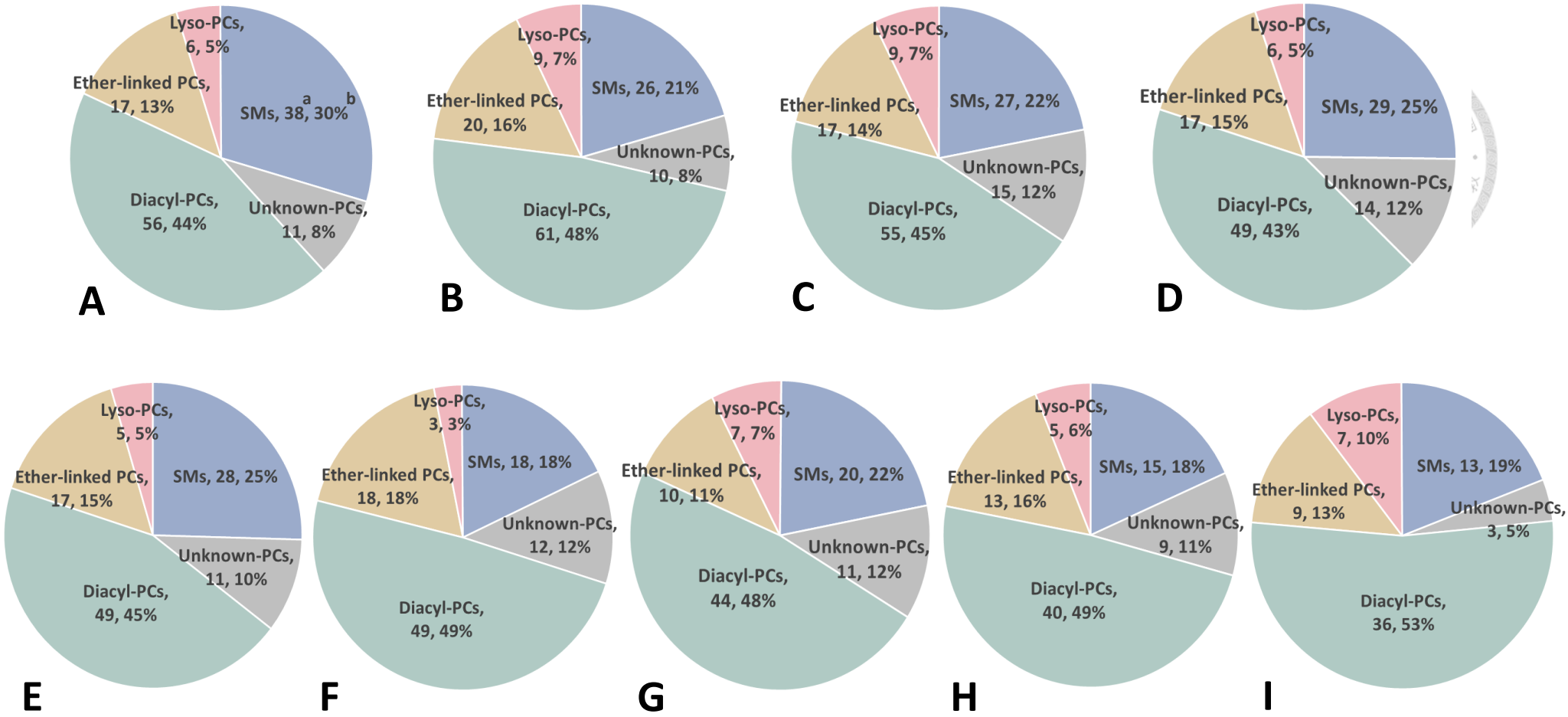


Figure 2. Phosphorylcholine-containing lipids composition in each organ of rat. (A) Kidney (B) Pancreas (C) Testis (D) Spleen (E) Lung (F) Epididymis (G) Liver (H) Heart (I) Serum. [a] Number of identified lipids for each lipid subclass; [b] Percentage of identified lipids for each lipid subclass.
Lyso-PCs: lyso-phosphatidylcholines; Diacyl-PCs: diacyl-phosphatidylcholines; O-PCs: O-alkyl-acyl-PC (plasmanylcholines); P-PCs: O-alkenyl-acyl-PC (plasmenylcholines); Unknown-PCs: unknown-phosphatidylcholines; SMs: sphingomyelins.

doi:10.6342/NTU201904292

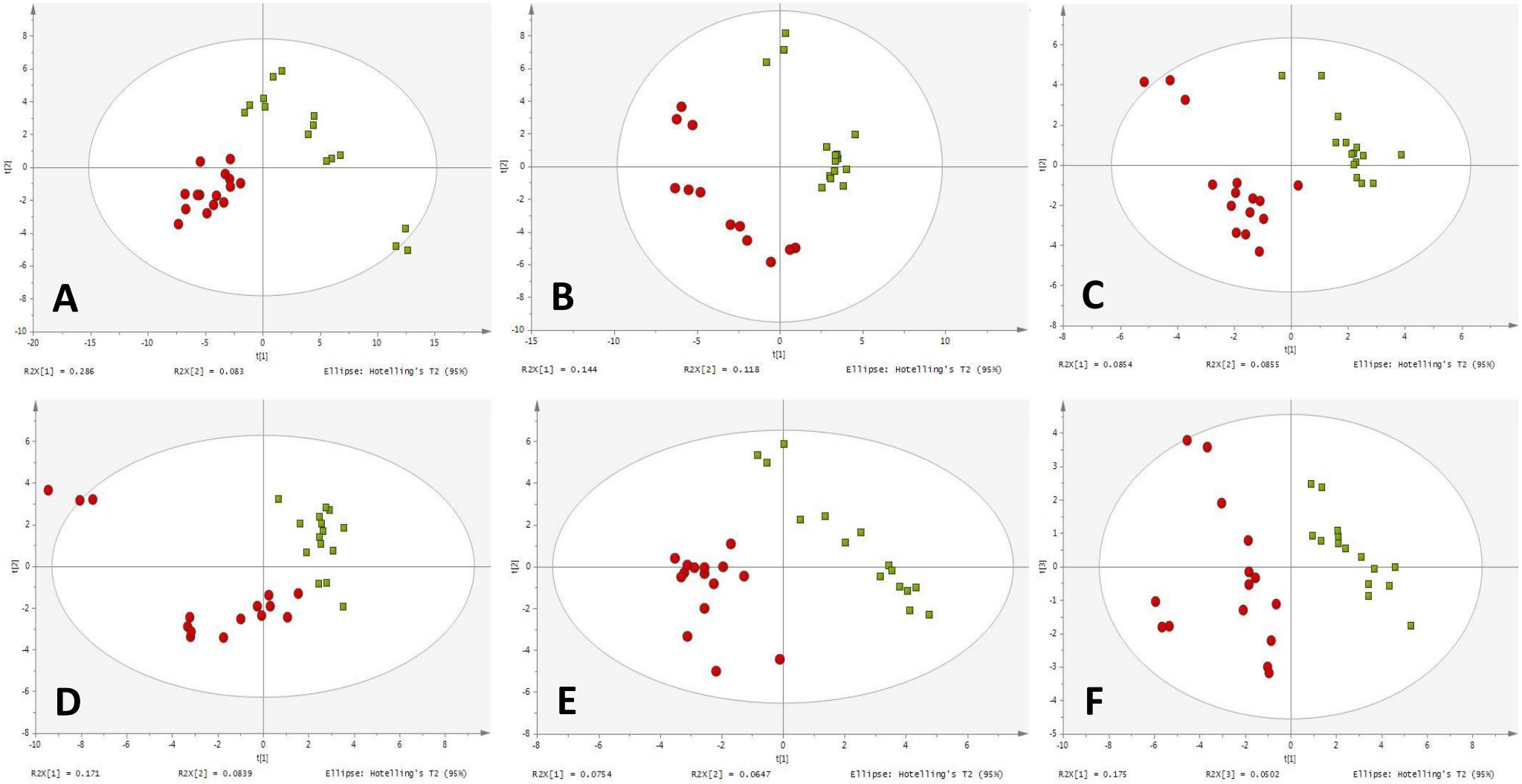


Figure 3. The PLS-DA score plots from the analysis of UPLC-MS/MS spectra of phosphorylcholine-containing lipids in each organ of rats after chronic ambient $PM_{2.5}$ exposure. (A) Testis, R^2Y: 0.962, Q^2: 0.825 (B) Pancreas, R^2Y: 0.972, Q^2: 0.833 (C) Heart, R^2Y: 0.933, Q^2: 0.627 (D) Liver, R^2Y: 0.955, Q^2: 0.498 (E) Kidney, R^2Y: 0.928, Q^2: 0.272 (F) Serum, R^2Y: 0.977, Q^2: 0.885. Rad circle: exposure $PM_{2.5}$ group; Green square: control group.

doi:10.6342/NTU201904292

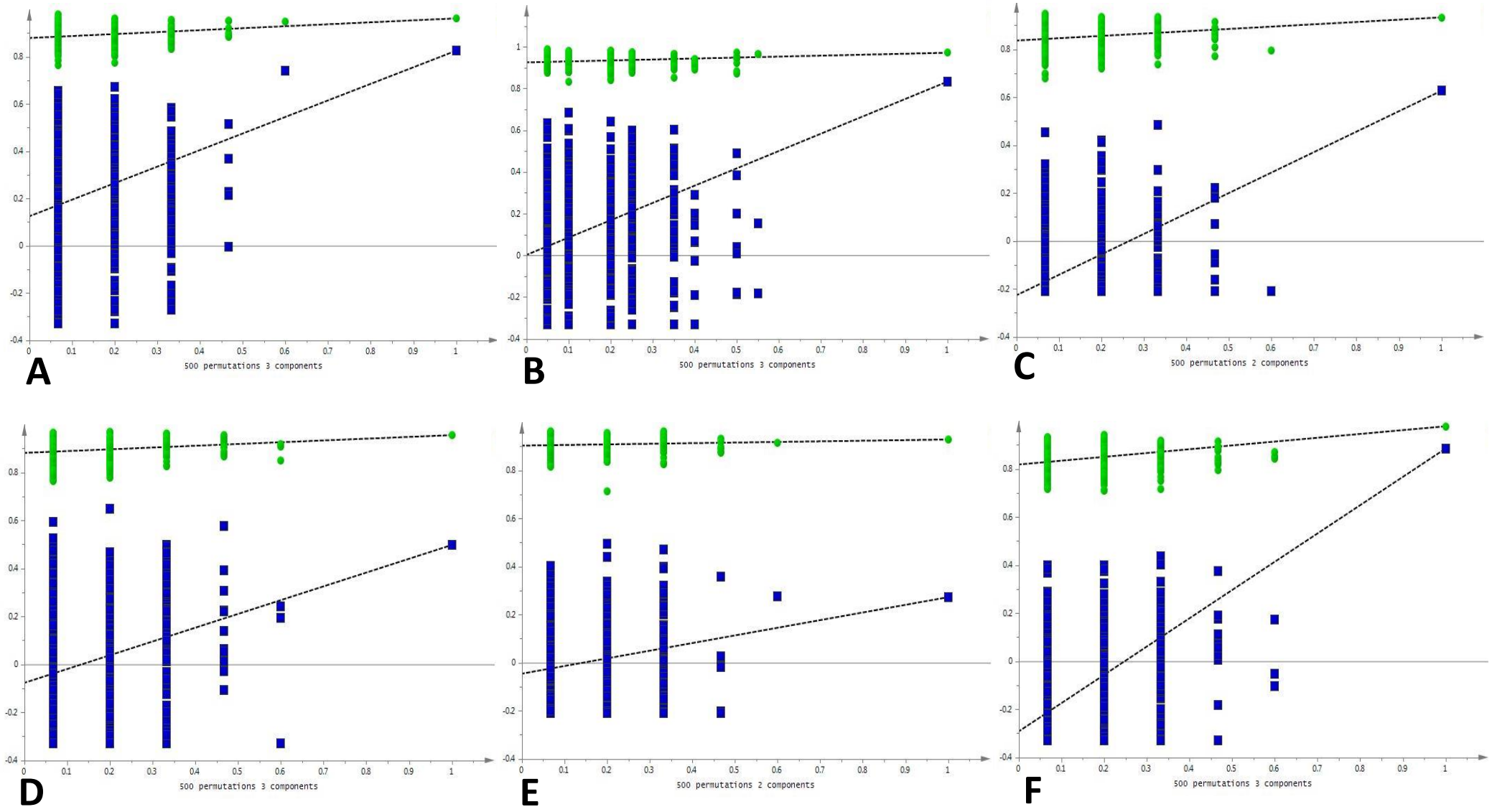


Figure 4. Permutation test of PLS-DA model for phosphorylcholine-containing lipids in each organ of rats after chronic ambient $PM_{2.5}$ exposure. (A) Testis (B) Pancreas (C) Heart (D) Liver (E) Kidney (F) Serum. 500 times permutation tests were used in the study. All groups accorded with the requirement of the standard. R^2 presented on green circles and Q^2 presented on blue boxes.

doi:10.6342/NTU201904292

Tables

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Table 1. Significant changed phosphorylcholine-containing lipids in the testis of rats after chronic ambient $PM_{2.5}$ exposure.

Subclass		Lipid name	VIP [a]	Fold change [b]	Wilcoxon rank sum tests (*p*-value) [c]
Lyso-PCs		PC(16:1/0:0) + PC(0:0/16:1)	1.62	0.12	*
		PC(20:4/0:0)	1.94	0.04	**
		PC(0:0/18:1)	1.91	0.06	**
		PC(20:2/0:0)	1.59	0.08	**
		PC(22:5/0:0)	1.89	0.03	**
Diacyl-PCs	Saturated	PC(16:0/16:0)	1.76	0.72	**
	Monounsaturated	PC(16:0/18:1)	1.54	0.95	*
	Polyunsaturated	PC(22:5/18:0)	1.66	0.55	*
		PC(18:2/20:4)	1.63	2.58	*
		PC(16:0/22:6)	1.11	1.45	*
		PC(34:4)	1.32	1.54	*
		PC(38:2)	1.39	1.58	*
		PC(42:6)	1.52	2.09	**
		PC(42:4)	1.34	1.43	*

[a] Variable Importance Projection of PLS-DA component 1; [b] Fold change calculated by the median peak area ratio of treatment group and control group; [c] All the features were qualified false discovery rate correction. * *p*-value < 0.05; ** *p*-value < 0.01. Lyso-PCs: lyso-phosphatidylcholines; Diacyl-PCs: diacyl-phosphatidylcholines.

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Table 1. Significant changed phosphorylcholine-containing lipids in the testis of rats after chronic ambient $PM_{2.5}$ exposure. (continued)

Subclass	Lipid name	VIP [a]	Fold change [b]	Wilcoxon rank sum tests (*p*-value) [c]
P-PCs	PC(P-16:0/20:3)	1.69	0.66	*
Sphingomyelins	SM(d18:2/23:0)	1.37	2.01	*
	SM(d18:2/16:0)	1.49	2.18	*
	SM(d34:1)	1.29	0.42	*
Unknown	PC(509)	2.03	0.68	**
	PC(597)	1.99	0.05	**
	PC(599)	1.78	0.08	**
	SM(862)	1.95	1.88	**
	SM(878)	1.82	1.84	*
	SM(888)	1.72	2.33	*

[a] Variable Importance Projection of PLS-DA component 1; [b] Fold change calculated by the median peak area ratio of treatment group and control group; [c] All the features qualified false discovery rate correction. * *p*-value < 0.05; ** *p*-value < 0.01. P-PCs: O-alkenyl-acyl-PC (plasmenylcholines).

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Table 2. Significant changed phosphorylcholine-containing lipids in the pancreas of rats after chronic ambient $PM_{2.5}$ exposure.

Subclass	Lipid name	VIP [a]	Fold change [b]	Wilcoxon rank sum tests (*p*-value)
Lyso-PCs	PC(0:0/18:2)	2.49	1.55	*
Diacyl-PCs	PC(40:2)	1.32	1.24	*
	PC(16:0/22:6)	2.50	0.67	*
	PC(18:0/22:6)	2.62	0.74	*
	PC(44:4)	1.99	0.46	*

[a] Variable Importance Projection of PLS-DA component 1; [b] Fold change calculated by the median peak area ratio of treatment group and control group. * *p*-value < 0.05; ** *p*-value < 0.01. Lyso-PCs: lyso-phosphatidylcholines; Diacyl-PCs: diacyl-phosphatidylcholines.

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Table 3. Significant changed phosphorylcholine-containing lipids in the heart, liver, and kidney of rats after chronic ambient $PM_{2.5}$ exposure.

Organs	Subclass	Lipid name	VIP [a]	Fold change [b]	Wilcoxon rank sum tests (*p*-value)
Heart	Polyunsaturated diacyl-PCs	PC(20:4/20:4)	2.83	1.23	*
	O-PCs	PC(O-18:0/16:0)	1.96	0.75	*
Liver	Polyunsaturated diacyl-PCs	PC(34:4)	2.50	0.53	*
	Unknown	PC(827)	1.31	0.77	*
Kidney	O-PCs	PC(O-18:0/16:0)	1.66	0.64	*
	Unknown	PC(843)	1.81	0.80	*
		PC(863)	1.04	0.78	*
		SM(872)	2.69	1.46	*

[a] Variable Importance Projection of PLS-DA component 1; [b] Fold change calculated by the median peak area ratio of treatment group and control group. * *p*-value < 0.05; ** *p*-value < 0.01. Diacyl-PCs: diacyl-phosphatidylcholines; O-PC: O-alkyl-acyl-PCs (Plasmanylcholines).

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Table 4. Significant changed phosphorylcholine-containing lipids in the serum of rats after chronic ambient $PM_{2.5}$ exposure.

Subclass		Lipid name	VIP [a]	Fold change [b]	Wilcoxon rank sum tests (*p*-value)
Lyso-PCs		PC(18:0/0:0)	2.30	1.34	** [c]
Diacyl-PCs	Monounsaturated	PC(16:0/16:1)	2.19	0.60	** [c]
		PC(16:0/17:1)	1.52	0.74	*
		PC(16:0/18:1)	1.69	0.83	*
	Polyunsaturated	PC(16:0/20:4)	2.31	0.83	** [c]
		PC(22:5/20:3)	1.52	1.30	** [c]
Unknown		PC(843)	1.37	1.25	*

[a] Variable Importance Projection of PLS-DA component 1; [b] Fold change calculated by the median peak area ratio of treatment group and control group; [c] Qualified false discovery rate correction. * *p*-value < 0.05; ** *p*-value < 0.01. Lyso-PCs: lyso-phosphatidylcholines; Diacyl-PCs: diacyl-phosphatidylcholines.

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