

DOCTORAL THESIS

PM_{2.5} Exposure and Its Association with Respiratory, Cardiac and Hepatic Diseases

SONG, Yuanyuan

Date of Award:
2021

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

ABSTRACT

Airborne particulate matter, especially with an aerodynamic diameter less than $2.5 \mu\text{m}$ ($\text{PM}_{2.5}$), can go directly to the respiratory bronchioles of the lung, and result in respiratory diseases. More recently, the epidemiological studies indicated that $\text{PM}_{2.5}$ exposure was also associated with cardiovascular diseases, hepatic diseases and obesity. However, the underlying molecular mechanisms involved in $\text{PM}_{2.5}$ -elicited damages are not well elucidated. In this thesis, the effects of $\text{PM}_{2.5}$ on respiratory, cardiac and hepatic systems were investigated in both *in vitro* and *in vivo* models by using multi omics and biological analyses.

As an industrial city of China, Taiyuan has suffered from serious $\text{PM}_{2.5}$ pollution for years due to large coal combustion and active industrial activities. An ambient exposure system was established in Taiyuan, which enabled to mimic real atmospheric conditions of $\text{PM}_{2.5}$ exposure. Compared with traditional intratracheal instillation method, the installed $\text{PM}_{2.5}$ exposure system not only eliminated the possible artificial effects on animals but also maintained the real physiochemical characteristics of $\text{PM}_{2.5}$. The case studies indicated that real-ambient $\text{PM}_{2.5}$ exposure induced a variety of physiological damages in aged mice and Sprague-Dawley (SD) rats. Some previously uncharacterized effects were firstly found to be associated with the real-ambient $\text{PM}_{2.5}$ exposure. In this thesis, both conventional intratracheal instillation method and established $\text{PM}_{2.5}$ exposure system were applied in animal studies and the efficiency were

compared in these two methods.

In *in vitro* studies, PM_{2.5} collected from Taiyuan were exposed to lung BEAS-2B cells, liver L02 cells and heart neonatal rat cardiomyocytes (NRCMs) cells. The results indicated that PM_{2.5} exposure greatly decreased the cell viability and induced oxidative stress in all three kinds of cells. The metabolomic analysis in BEAS-2B cells showed that tricarboxylic acid (TCA) cycle and glycolysis were significantly affected by PM_{2.5} exposure. In addition, obvious metabolic phenotype remodeling between oxidative phosphorylation and glycolysis were found in BEAS-2B cells, NRCMs and L02 cells after treated with PM_{2.5}. Notably, high fat was found to enhance the effects of PM_{2.5} on the BEAS-2B cells in energy metabolic reprogramming. These *in vitro* studies suggested that energy metabolism disorder might be a potential mechanism of PM_{2.5}-induced toxicities.

To investigate the effects of PM_{2.5} exposure on the lung, heart and liver, high-fat diet (HFD)-induced obese mice model and Sirtuin3 knockout (Sirt3 KO) mice model were used. In the obese mice model, though the body weight and blood glucose were not increased, the respiratory rate, blood pressure, heart rate, heart weight and liver weight were significantly elevated in the obese mice after exposure to PM_{2.5} through intratracheal instillation method, suggesting that 4-week PM_{2.5} exposure did not exacerbate obesity but obesity increased the vulnerability to the harmful effects of PM_{2.5}. In the lung, the results from

metabolomic and gene expression analysis indicated that cotreatment of PM_{2.5} and HFD promoted glycolysis but inhibited fatty acid oxidation, glutamine metabolism and TCA cycle, resulting in the reduction of oxidative phosphorylation and adenosine triphosphate (ATP) production. In the heart, the metabolomic and gene expression analysis indicated that cotreatment of PM_{2.5} and HFD elevated the metabolites and genes related to oxidative stress and inflammation and altered substrate preference from glycolysis to fatty acid oxidation. The metabolic reprogramming in the lung and heart indicated that energy metabolism was disturbed by PM_{2.5} combined with obesity. Considering that mitochondria were the major sources of endogenous reactive oxygen species (ROS), the disorder of energy metabolism would aggravate oxidative stress and inflammation to induce more severe adverse effects on the lung and heart, further proving that energy metabolism dysfunction might be an important contributing factor to PM_{2.5}-induced cardiopulmonary toxicities. In the liver, PM_{2.5} showed nonalcoholic steatohepatitis (NASH) symptom in the obese mice. The multi-omics analysis showed that the purine metabolism, glutathione (GSH) metabolism, cysteine and methionine metabolism related to oxidative stress, arginine and proline metabolism and tryptophan metabolism associated with inflammation as well as lipid metabolism related to hepatic steatosis were significantly disturbed by combined effects of PM_{2.5} and HFD.

In the Sirt3 KO mice model, the deficiency of Sirt3, which was one

nicotinamide adenine dinucleotide (NAD⁺)-dependent protein deacetylase, might affect many important cellular processes, including redox balance, immunity and energy production. To clarify the roles of Sirt3 in PM_{2.5}-induced energy metabolism disorder, oxidative stress and inflammation, the wild type (WT) and Sirt3 KO mice were exposed to the real-ambient PM_{2.5} exposure system for 15 weeks. The physiological results suggested that PM_{2.5} decreased the heart rate and catecholamines to induce heart failure and increased ROS and pro-inflammatory cytokines to induce liver dysfunction in the Sirt3 KO mice. The results from metabolomic, lipidomic and proteomic analysis showed that the increase of fatty acid oxidation and decrease of glycolysis in the heart as well as decrease of arginine and proline metabolism, purine metabolism and cysteine and methionine metabolism in the liver of Sirt3 KO mice were consistent with the results found in the obese mice model. The similar results found in Sirt3 KO mice model and obese mice model proved that the installed PM_{2.5} exposure system was not only efficient in establishing animal models for PM_{2.5} toxicological studies but also more representative than traditional intratracheal instillation method. Furthermore, the overexpression of Sirt3 in L02 cells alleviated PM_{2.5}-induced cell death, oxidative stress and inflammation, suggesting that PM_{2.5}-induced energy metabolism disorder in the heart and oxidative stress and inflammation in the liver were regulated by Sirt3.

In summary, our results found that, beyond oxidative stress and inflammation,

energy metabolism dysfunction was also an important contributing factor to PM_{2.5}-elicited toxicities. Of note, Sirt3 gene was found to play critical role in mediation of PM_{2.5}-induced adverse effects. The results of this thesis may provide new insights into the health risk of PM_{2.5} to pulmonary, cardiac and hepatic diseases.

Table of Contents

DECLARATION	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS	vii
Table of Contents	ix
List of Tables	xviii
List of Figures.....	xx
List of Abbreviations	xxix
Chapter 1 General introduction	1
1.1 Basic information of PM _{2.5}	1
1.2 PM _{2.5} composition and its toxicity	2
1.3 Human diseases associated with PM _{2.5} pollution.....	5
1.3.1 PM _{2.5} exposure as a risk factor for human health.....	5
1.3.2 Preexisting diseases increase vulnerability to the harmful effects of PM _{2.5} exposure.....	7
1.4 PM _{2.5} Exposure methods for cells and animals.....	10
1.5 Potential mechanisms of PM _{2.5} -induced toxicities.....	13
1.5.1 Oxidative stress	13

1.5.2 Inflammation	14
1.5.3 Mitochondrial dysfunction and energy metabolism disorder	15
1.6 Omics studies on PM _{2.5} toxicities	16
1.6.1 Metabolomics, lipidomics and proteomics	16
1.6.2 Application of multi omics in the studies of PM _{2.5} toxicities	18
1.7 Objectives	19
Chapter 2 Construction of real-ambient PM_{2.5} exposure system and its applications in toxicity studies with different animal models	23
2.1 Introduction	23
2.2 Materials and methods	24
2.2.1 Construction of the real-ambient PM _{2.5} exposure system	24
2.2.2 Exposure protocol	25
2.2.3 Statistical analysis	30
2.3 Results and discussion	30
2.3.1 Installation of real-ambient PM _{2.5} exposure system	30
2.3.2 Real-ambient PM _{2.5} exposure of aged mice	33
2.3.3 Real-ambient PM _{2.5} exposure of LPS-induced acute lung injury mice	34
2.3.4 Real-ambient PM _{2.5} exposure of <i>db/db</i> mice	36
2.3.5 Real-ambient PM _{2.5} exposure of SD rats	37

2.4 Chapter summary	38
Chapter 3 Effects of PM_{2.5} on the lung of high fat-induced cell and animal models	40
3.1 Introduction	40
3.2 Materials and methods	41
3.2.1 Chemicals and reagents	41
3.2.2 Taiyuan winter PM _{2.5} collection and determination	42
3.2.3 Cell culture and exposure to PM _{2.5}	42
3.2.4 Animal experiments.....	44
3.2.5 Sample preparation for metabolomic analysis	45
3.2.6 Nontargeted and targeted metabolomics	46
3.2.7 Data analysis.....	51
3.2.8 Measurement of SOD, ROS, MDA and NO levels	52
3.2.9 Pro-inflammatory cytokines measurement.....	52
3.2.10 RNA extraction and quantitative real-time PCR	52
3.2.11 XF Cell Mito stress, glycolytic stress, substrate oxidation stress and Mito fuel flex tests.....	55
3.2.12 Statistical analysis	57
3.3 Results	57

3.3.1 Characterization of PM _{2.5}	57
3.3.2 PM _{2.5} decreased the cell viability in BEAS-2B cells.....	58
3.3.3 Multivariate statistical analysis of metabolic profiles	61
3.3.4 Metabolites screening and pathway analysis.....	62
3.3.5 PM _{2.5} induced energy metabolism reprogramming in BEAS-2B cells .	68
3.3.6 Oxidative stress and inflammation in PM _{2.5} exposed BEAS-2B cells ..	70
3.3.7 PM _{2.5} decreased the cell viability and induced oxidative stress and membrane damage in BEAS-2B cells after cotreated with oleic acid.....	72
3.3.8 Cotreatment of oleic acid and PM _{2.5} changed substrates preference in BEAS-2B cells.....	75
3.3.9 Cotreatment of oleic acid and PM _{2.5} increased glycolysis and inhibited oxidative phosphorylation in BEAS-2B cells.....	79
3.3.10 PM _{2.5} increased the respiratory rate in the lung of the obese mice	81
3.3.11 Global metabolic changes in the lung of the obese mice model after exposed to PM _{2.5}	83
3.3.12 PM _{2.5} induced oxidative stress and inflammation in the lung of the obese mice model	90
3.3.13 PM _{2.5} promoted glycolysis and inhibited fatty acid oxidation in the lung of the obese mice	91

3.3.14 PM _{2.5} inhibited glutamine metabolism and BCAA catabolism in the lung of the obese mice	95
3.3.15 PM _{2.5} changed the mRNA levels of genes involved in glucose and fatty acid oxidation in the lung of the obese mice.....	97
3.4 Discussion	98
3.5 Chapter summary	106
Chapter 4 Combined effects of PM_{2.5} and obesity on the heart and liver dysfunction	107
4.1 Introduction	107
4.2 Materials and methods	108
4.2.1 Chemicals and reagents	108
4.2.2 Winter PM _{2.5} samples from Taiyuan, China and animal experiments.	108
4.2.3 Cell culture and exposure to PM _{2.5}	109
4.2.4 XF Cell Mito stress and glycolytic stress test	109
4.2.5 Histopathology analysis.....	109
4.2.6 Determination of AST and ALT.....	110
4.2.7 Determination of dopamine, epinephrine and norepinephrine in serum and pro-inflammatory cytokines in the liver	110
4.2.8 Determination of MDA	110

4.2.9 RNA extraction and qPCR	110
4.2.10 Sample preparation and LC-MS based global metabolomics and lipidomics	112
4.2.11 iTRAQ-based proteomic study.....	113
4.2.12 Data processing	115
4.2.13 Statistical analysis	116
4.3 Results	117
4.3.1 PM _{2.5} exposure increased blood pressure, heart rate and liver weight in the obese mice	117
4.3.2 PM _{2.5} exposure induced heart and liver inflammation in the obese mice model	120
4.3.3 PM _{2.5} exposure activated phenylalanine metabolism pathway in serum, pantothenate and CoA biosynthesis pathway in the heart of the obese mice	121
4.3.4 Targeted metabolomics showed that PM _{2.5} exposure disturbed energy metabolism in the heart of the obese mice	132
4.3.5 PM _{2.5} exposure disturbed lipid metabolism in the heart of the obese mice.	135
4.3.6 PM _{2.5} exposure changed the expression of genes involved in inflammation, oxidative stress and fatty acid oxidation in the obese mice..	141

4.3.7 Non-targeted and targeted metabolic analysis of the liver after exposure to PM _{2.5} in the obese mice model.....	144
4.3.8 PM _{2.5} exposure disturbed lipid metabolism in the liver of the obese mice model	155
4.2.9 Protein changes in the liver after exposed to PM _{2.5} in the obese mice	165
4.3.10 PM _{2.5} exposure induced energy metabolism remodeling in NRCMs	174
4.3.11 PM _{2.5} exposure increased glycolysis but not significantly affected oxidative phosphorylation in L02 cells	176
4.4 Discussion	178
4.5 Chapter summary	190
Chapter 5 Effects of Sirt3 on the PM_{2.5}-induced heart and liver diseases....	191
5.1 Introduction	191
5.2 Materials and methods	192
5.2.1 Chemicals and reagents	192
5.2.2 Animal experiments.....	192
5.2.3 Culture of L02 cells	193
5.2.4 siRNA and plasmid transfection.....	193
5.2.5 Determination of catecholamines in serum	194

5.2.6 Determination of pro-inflammatory cytokines in the liver of both cell and animal models	194
5.2.7 Determination of MDA in L02 cells and the liver of Sirt3 KO mice model	195
5.2.8 Sample preparation and LC-MS based metabolomics, lipidomics and proteomics	195
5.2.9 RNA extraction and qPCR	195
5.2.10 Western blot.....	196
5.2.11 Statistical analysis	197
5.3 Results and discussion.....	197
5.3.1 PM _{2.5} exposure decreased the expression of Sirt3 in cells and animal models.....	197
5.3.2 Changes of physiological indices in PM _{2.5} -exposed Sirt3 KO mice ...	199
5.3.3 Targeted metabolomic analysis revealed that PM _{2.5} disturbed energy metabolism in the heart through Sirt3 signaling pathway	202
5.3.4 Targeted proteomics revealed that energy metabolism was disrupted in the heart of PM _{2.5} exposed Sirt3 KO mice	205
5.3.5 PM _{2.5} exposure disturbed lipid metabolism in the heart of the Sirt3 KO mice.....	214

5.3.6 Targeted metabolomic revealed that Sirt3 deficiency aggravated PM _{2.5} -disturbed oxidative stress and inflammation in the liver	222
5.3.7 Sirt3 deficiency enhanced PM _{2.5} -disturbed lipid metabolism disorder in the liver.....	224
5.3.8 Development of overexpression and knockdown cell models	232
5.3.9 Overexpressed Sirt3 alleviated oxidative stress and inflammation in L02 cells after exposed to PM _{2.5}	235
5.4 Chapter summary	237
Chapter 6 Conclusions and future work.....	239
References.....	245
Publication List	280
CURRICULAM VITAE.....	282

List of Tables

Table 3.1 SRM transitions of metabolites in targeted metabolomic analysis by LC-QqQ-MS.	49
Table 3.2 Primer sequences used for real-time qPCR analysis.	54
Table 3.3 Concentrations of metals and ions in Taiyuan winter PM _{2.5}	59
Table 3.4 Concentrations of PAHs in Taiyuan winter PM _{2.5}	60
Table 3.5 Identification of metabolites detected using HPLC-MS when cells were exposed to PM _{2.5}	64
Table 3.6 Identified metabolites in the lung of the obese mice model after exposed to PM _{2.5}	85
Table 4.1 Primer sequences used for real-time qPCR analysis.	111
Table 4.2 Identified metabolites in serum of the obese mice model after exposed to PM _{2.5}	124
Table 4.3 Identified metabolites in the heart of the obese mice model after exposed to PM _{2.5}	127
Table 4.4 Identified lipids in the heart of the obese mice model after exposed to PM _{2.5}	137
Table 4.5 Identified metabolites in the liver of the obese mice model after exposed to PM _{2.5}	150

Table 4.6 Identified lipids in the liver of the obese mice model after exposed to PM _{2.5}	157
Table 4.7 Identified proteins in the liver of the obese mice model after exposed to PM _{2.5}	169
Table 5.1 Primer sequences used for real-time qPCR analysis.	196
Table 5.2 Identified proteins in the heart of the Sirt3 KO mice model after exposure to PM _{2.5}	208
Table 5.3 Identified lipids in the heart of the Sirt3 KO mice model after exposure to PM _{2.5}	216
Table 5.4 Identified lipids in the liver of the Sirt3 KO mice model after exposure to PM _{2.5}	226

List of Figures

Figure 1.1 Size comparison of PM _{2.5} and PM ₁₀	2
Figure 1.2 Potential mechanisms of obesity-induced increases of susceptibility to air pollution.....	10
Figure 1.3 Different methods for PM _{2.5} exposure.....	12
Figure 1.4 Illustration of PM _{2.5} -induced toxicological mechanisms.	16
Figure 1.5 Schematic overview of the development of the omics fields.....	18
Figure 1.6 Proposed research plan.....	22
Scheme 2.1 Schematic diagram of the exposure system.	25
Scheme 2.2 Development of aged mice model.....	27
Scheme 2.3 Development of LPS-induced acute lung injury mice model.	28
Figure 2.1 Characterization of PM _{2.5} : the concentration of PM _{2.5} during exposure time (A); the efficiency of PM _{2.5} exposure system (B).	33
Figure 2.2 Physiological indices changes in the PM _{2.5} exposed aged mice: body weight (A); heart rate (B) and blood pressure (C)	35
Figure 2.3 Physiological indices changes in PM _{2.5} exposed <i>db/db</i> mice: body weight (A); blood glucose (B) and heart rate (C)	37
Figure 2.4 Physiological indices changes in PM _{2.5} exposed rats	38
Scheme 3.1 Exposure procedure of oleic acid-induced high fat cell model.....	43

Scheme 3.2 Schematic overview of the obese mice model development.	45
Figure 3.1 Cell viability of BEAS-2B cells after PM _{2.5} exposure.	61
Figure 3.2 PLS-DA plots and volcano plots of BEAS-2B cells after exposure to PM _{2.5} in negative ionization mode (A) and positive ionization mode (B) conducted by global metabolomics.....	63
Figure 3.3 Metabolic pathways analysis.....	65
Figure 3.4 Heatmap analysis of identified metabolites between control (Ctrl) and treatment (L, M, H) group	67
Figure 3.5 Fold changes of identified metabolites after total PM _{2.5} exposure in purine metabolism (A); arginine and proline metabolism (B); TCA cycle (C) and glycolysis (D).....	68
Figure 3.6 Mitochondrial stress test after cells exposed to different doses of PM _{2.5} (A); fold changes of four major indices indicating the mitochondrial respiration capacity change after cells exposed to different doses of PM _{2.5} (B); glycolytic stress test after cells exposed to different doses of PM _{2.5} (C); fold changes of four major indices indicating the glycolytic capacity change after cells exposed to different doses of PM _{2.5} (D)	70
Figure 3.7 Fold changes of ROS, MDA, SOD and NO in BEAS-2B cells upon PM _{2.5} exposure.....	71

Figure 3.8 Fold changes of pro-inflammatory cytokines in BEAS-2B cells upon PM _{2.5} exposure.....	72
Figure 3.9 The level of TG after exposed to oleic acid	73
Figure 3.10 Effects of PM _{2.5} and oleic acid on BEAS-2B cells: cell viability (A); LDH release (B) and ROS production (C).....	74
Figure 3.11 Changes of dependency, capacity and flexibility of glucose, glutamine and LCFAs in BEAS-2B cells after exposed to oleic acid and PM _{2.5} ..	76
Figure 3.12 Substrate oxidation stress test after cells exposed to PBS (A); substrate oxidation stress test after cells exposed to combination of PM _{2.5} and oleic acid (B); fold changes of four major indices indicating the mitochondrial respiration capacity change after cells exposed to PBS (C); fold changes of four major indices indicating mitochondrial respiration capacity after cells exposed to combination of PM _{2.5} and oleic acid (D).	78
Figure 3.13 Mitochondrial stress test after cells exposed to PM _{2.5} , oleic acid and combination of PM _{2.5} and oleic acid (A); glycolytic stress test after cells exposed to PM _{2.5} , oleic acid and combination of PM _{2.5} and oleic acid (B); fold changes of four major indices indicating the mitochondrial respiration capacity change after cells exposed to PM _{2.5} , oleic acid and combination of PM _{2.5} and oleic acid (C); fold changes of three major indices indicating the glycolytic capacity change after cells exposed to PM _{2.5} , oleic acid and combination of PM _{2.5} and oleic acid (D).....	80

Figure 3.14 Changes of body weight (A) and blood glucose (B) in the obese mice model.....	81
Figure 3.15 Changes of respiratory rate in the obese mice model.....	82
Figure 3.16 Global metabolomic analysis of lung in the obese mice model: PLS-DA plot of lung in negative ionization mode (A); PLS-DA plot in positive ionization mode (B); pathway analysis (C); volcano plot of ND-PBS vs ND-PM _{2.5} (D); volcano plot of ND-PBS vs HFD-PBS (E); volcano plots of ND-PBS vs HFD-PM _{2.5} (F)	84
Figure 3.17 Contents of MDA (A) and fold changes of mRNA levels of pro-inflammatory cytokines in the lung (B).	91
Figure 3.18 Fold changes of metabolites in glycolysis (A) and TCA cycle (B)....	93
Figure 3.19 Fold changes of free fatty acids (A) and CNs (B).....	94
Figure 3.20 Fold changes of metabolites in arginine and proline metabolism (A) and other amino acids (B).....	96
Figure 3.21 Fold changes of mRNA levels of energy metabolism related genes..	98
Figure 3.22 Schematic overview of the disturbed metabolic pathways in the lung of cell and mice models upon PM _{2.5} exposure.....	105
Figure 4.1 Effects of PM _{2.5} and HFD on physiological indices changes in the heart: heart weight (A); heart-body weight ratio (B); blood pressure (C) and heart rate (D).....	118

Figure 4.2 Effects of PM _{2.5} and HFD in the liver: liver weight (A) and liver-body weight ratio (B).	119
Figure 4.3 Fold changes of AST (A) and ALT (B) in serum of obese mice model	119
Figure 4.4 H&E of representative heart sections of the four groups: ND-PBS (A); ND-PM _{2.5} (B); HFD-PBS (C) and HFD-PM _{2.5} (D)	120
Figure 4.5 H&E of representative liver sections of the four groups. ND-PBS (A); ND-PM _{2.5} (B); HFD-PBS (C) and HFD-PM _{2.5} (D)	121
Figure 4.6 PLS-DA plots of serum in negative ionization mode (A) and positive ionization mode (B) and heart in negative ionization mode (C) and positive ionization mode (D) conducted by global metabolomics.	122
Figure 4.7 Pathway analysis of serum (A) and heart (B).....	123
Figure 4.8 Fold changes of metabolites in phenylalanine pathways in serum	130
Figure 4.9 Fold changes of identified metabolites in pantothenate and CoA biosynthesis pathway in serum and heart	131
Figure 4.10 Fold changes of identified metabolites in purine metabolism.....	132
Figure 4.11 Overall abundance of LCFAs in serum (A) and heart (B) and CNs in serum (C) and heart (D).	133
Figure 4.12 The heat map of LCFAs in serum (A) and heart (B); CNs in serum (C) and heart (D) and other energy metabolism related metabolites in the heart (E)	134

Figure 4.13 PLS-DA plots of heart in negative ionization mode (A) and positive ionization mode (B) conducted by lipidomics.....	136
Figure 4.14 Fold changes of overall abundance of lipids.....	141
Figure 4.15 Fold changes of mRNA expression of pro-inflammatory cytokines in the heart (A) and hypothalamus (B), oxidative stress related genes (C) and fatty acid metabolism related genes (D)	144
Figure 4.16 PLS-DA plots of liver in negative ionization mode (A) and positive ionization mode (B) in metabolomic analysis and PLS-DA plots in negative ionization mode (C) and positive ionization mode (D) in lipidomic analysis.	145
Figure 4.17 Volcano plot of ND-PBS vs ND-PM _{2.5} (A); volcano plot of ND-PBS vs HFD-PBS (B) and volcano plots of ND-PBS vs HFD PM _{2.5} (C)....	146
Figure 4.18 Pathway analysis of metabolomic analysis in the liver.....	147
Figure 4.19 Fold changes of metabolites in purine metabolism (A); arginine and proline metabolism (B) and cysteine and methionine metabolism (C)	148
Figure 4.20 Fold changes of MDA (A) and pro-inflammatory cytokines (B) in the liver of the obese mice model.	149
Figure 4.21 Fold changes of lipids in four groups: glycerophospholipids (A); total abundance of LCFAs and CNs (B); sphingolipids (C) and glycerolipids (D)	156

Figure 4.22 Proteomic analysis of the liver in four groups: venn diagram of identified proteins (A); keywords analysis (B); molecular function analysis (C); KEGG pathway analysis (D) and biological process analysis (E).....	166
Figure 4.23 Fold changes of specific proteins related to pathways identified in metabolomic analysis.....	167
Figure 4.24 Fold changes of metabolites in glycolysis (A) and tryptophan metabolism (B) after cotreated with PM _{2.5} and HFD	168
Figure 4.25 Cell viability (A); LDH release (B) and ROS generation (C) in NRCMs after exposure to PM _{2.5}	175
Figure 4.26 Mitochondrial stress test after NRCMs exposed to PM _{2.5} (A); fold changes of five major indices indicating the mitochondrial respiration capacity change after NRCMs exposed to PM _{2.5} (B); glycolytic stress test after NRCMs exposed to PM _{2.5} (C); fold changes of three major indices indicating the glycolytic capacity change after NRCMs exposed to PM _{2.5} (D)	176
Figure 4.27 The cell viability (A) and ROS release (B) in L02 cells after exposed to different concentrations of PM _{2.5}	177
Figure 4.28 Mitochondrial stress test after L02 cells exposed to PM _{2.5} (A); fold changes of three major indices indicating the mitochondrial respiration capacity change after L02 cells exposed to PM _{2.5} (B); glycolytic stress test after L02 cells	

exposed to PM _{2.5} (C); fold changes of three major indices indicating the glycolytic capacity change after L02 cells exposed to PM _{2.5} (D).....	178
Figure 4.29 The relationship between heart and other organs.....	181
Figure 4.30 Proposed energy metabolism pathway disturbed by cotreatment of PM _{2.5} and HFD.....	184
Figure 4.31 Schematic diagram of identified pathways in the liver after exposure to PM _{2.5} and HFD.	189
Scheme 5.1 The development of Sirt3 KO mice model.	193
Figure 5.1 Fold changes of mRNA expression of Sirt3 in BEAS-2B cells (A) and C57BL/6 mice (B).....	198
Figure 5.2 The level of Sirt3 in the liver of the obese mice model	198
Figure 5.3 Fold changes of heart rate (A) and catecholamines (B) in the PM _{2.5} exposed Sirt3 KO mice model.....	200
Figure 5.4 Fold changes of AST (A) and ALT (B) in serum of PM _{2.5} exposed Sirt3 KO mice model.....	201
Figure 5.5 Fold changes of pro-inflammatory cytokines (A) and MDA (B) in PM _{2.5} exposed Sirt3 KO mice model.....	202
Figure 5.6 Fold changes of metabolites in pantothenate and CoA biosynthesis pathway in the heart of PM _{2.5} exposed Sirt3 KO mice model.....	203

Figure 5.7 Fold changes of metabolites in glycolysis (A) and TCA cycle in the heart of PM _{2.5} exposed Sirt3 KO mice model (B)	204
Figure 5.8 Proteomic analysis of the heart in four groups; reactome pathway (A); KEGG pathway (B); molecular function analysis (C) and biological process analysis (D).....	206
Figure 5.9 Fold changes of proteins in the heart of PM _{2.5} exposed Sirt3 KO mice model	207
Figure 5.10 Heap map of amino acids in the heart of Sirt3 KO mice after exposure to PM _{2.5}	213
Figure 5.11 Heat maps of lipids in the heart of PM _{2.5} exposed Sirt3 KO mice model; FAs (A); DG (B); TG (C); PC and PE (D) and LPC and LPE (E).....	215
Figure 5.12 Fold changes of metabolites in arginine and proline metabolism (A), cysteine and methionine metabolism (B) and purine metabolism (C).....	223
Figure 5.13 Fold changes of lipids in four groups: total abundance of FAs and CNs (A); sphingolipids (B) and glycerophospholipids (C).	225
Figure 5.14 The transfection efficiency of lipofectamine 3000 in L02 cells.....	234
Figure 5.15 mRNA expression of Sirt3 in L02 cells after transfection.	235
Figure 5.16 Cell viability of cells exposed to different doses of PM _{2.5} after transfected with NC, plasmid DNA and siRNA	236
Figure 5.17 Fold changes of MDA (A) and IL-6 (B) after transfection.	237