PM$_{2.5}$ exposure induces age-dependent hepatic lipid metabolism disorder in female mice

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Abstract

Particulate matter exposure has been described to elevate the risk of lung and cardiovascular diseases. An increasing number of recent studies have indicated positive correlations between PM$_{2.5}$ (the fraction of airborne particles with an aerodynamic diameter less than 2.5 $\mu$m) exposure and the risk of liver diseases. However, research on the effects of PM$_{2.5}$ exposure on liver fat synthesis, secretion, and clearance mechanisms under normal diet conditions is limited, and whether these effects are age-dependent is largely unknown. Female C57BL/6 mice at different ages (4 weeks (4 w), 4 months (4 m), and 10 months (10 m)) were treated with 3 mg/kg body weight of PM$_{2.5}$ every other day for 4 weeks. Subsequently, the ultrastructural changes of liver, the expression of genes involved in oxidative damage and lipid metabolism in the liver were examined. Observation of hepatic ultrastructure showed more and larger lipid droplets in the livers of 4-week-old and 10-month-old mice exposed to PM$_{2.5}$. Further analysis showed that PM$_{2.5}$ exposure increased the expression of genes related to lipid synthesis, but decreased the expression of genes involved in lipid transport and catabolism in the livers of 10-month-old mice. Our findings suggest that exposure to PM$_{2.5}$ disrupts the normal metabolism of liver lipids and induces lipid accumulation in the liver of female mice in an age-dependent manner, with older mice being more susceptible to PM$_{2.5}$.

Introduction

Particulate matter is a major environmental pollutant and has become a public health concern worldwide. PM$_{2.5}$ (the fraction of airborne particles with an aerodynamic diameter less than 2.5 $\mu$m) is more closely associated with harmful health effects than larger-sized particles are because PM$_{2.5}$ can easily bind to toxic compounds and penetrate the respiratory barrier, transferring into the circulatory system (Feng et al., 2016).

Even at concentrations below environmental standards, the risk of damage from PM$_{2.5}$ to human health cannot be underestimated (Franklin et al., 2007; Rückerl et al., 2011). Numerous studies have documented the association of PM$_{2.5}$ exposure with morbidity and mortality (Dockery et al., 1993; Pope et al., 1995; Ranft et al., 2009; Ko and Hui, 2012; Nachman and Parker, 2012; Son et al., 2012). Recent studies have shown that PM$_{2.5}$ may also have adverse effects on systems other than the cardiopulmonary system, such as accelerating the progression of adult diabetes, neurological...
diseases and nonalcoholic fatty liver disease (NAFLD) (Rückerl et al., 2011). Among these effects, research on the relationship between NAFLD and PM2.5 is seriously ignored (Tarantino et al., 2013). The term NAFLD is a broad concept of liver disease, ranging from steatosis to nonalcoholic steatohepatitis (NASH), even encompassing hepatocirrhosis and sometimes hepatocellular carcinoma (HCC) (Angulo, 2002; Starley et al., 2010). Several epidemiological investigations have demonstrated that exposure to PM2.5 causes elevated levels of liver enzymes (Kim et al., 2015), chronic liver inflammation (Pan et al., 2016) and an increased risk of HCC (Wong et al., 2016; Deng et al., 2017; VoPham et al., 2018). Recent toxicological studies have demonstrated that PM2.5 can be transferred from lung to the liver in several ways (Kim et al., 2014), and then triggers liver inflammation, increases liver lipid accumulation, stimulates collagen expression, and promotes liver fibrosis (Ding et al., 2018; Ge et al., 2017; Tan et al., 2009; Zheng et al., 2013, 2015). In addition, Rizzo et al. (2014) found that the liver lipid content was significantly changed by increasing phosphatidylcholine and total fatty acid composition after PM2.5 exposure. Although these studies indicated that hepatic lipid accumulation and lipid metabolism disorders caused by PM2.5 are important factors in aggravating NAFLD, the details and specific mechanisms remain poorly understood.

The “two-hit” theory is proverbially accepted to explain the pathogenesis of NAFLD. Steatosis is considered to be the “first hit”, which makes the liver more susceptible to miscellaneous “second hit”, including oxidative stress, inflammatory cytokines and toxins (Day and James, 1998). Several studies have indicated that air pollution can increase obesity, insulin resistance, liver lipid content and induce oxidative stress (Tan et al., 2009; Zheng et al., 2013), implicating that air pollution represents a significant “hit” to onset and aggravation of NAFLD. Under normal physiological conditions, the liver fat content is maintained in several ways, including lipid uptake by plasma, hepatocyte synthesis, secretion of triglyceride-rich lipoproteins, fatty acid oxidation and clearance by the bile duct. Previous studies have shown that PM2.5 exposure and provision of high fat diet (HFD) caused liver fibrosis (Tan et al., 2009; Zheng et al., 2013), which may be caused, at least in part, by HFD alone. However, little is known about the effects of PM2.5 exposure on liver fat synthesis, secretion, and clearance mechanisms under normal diet conditions.

Specific populations, such as children and the elderly, are more susceptible to the harmful effects caused by PM2.5 exposure than the general population because of their different physiological conditions (Sacks et al., 2011). In general, children have greater activity levels and minute volume per unit body weight than adults; therefore, children are potentially more susceptible than adults to equivalent levels of particulate matter (PM) exposure. Evidence supports that short-term exposure to PM of all size fractions increases negative respiratory effects in children compared to adults (Peel et al., 2005; Host et al., 2008). Toxicological studies have illustrated that exposure to PM at certain developmental stages may lead to impairment of the respiratory system growth (Sacks et al., 2011). Older people are generally considered to be more susceptible than others due to the natural decline in physiological function and a high prevalence of pre-existing diseases in this population.

Epidemiological evidence indicates that exposure to PM is associated not only with an increased incidence of cardiovascular disease (Host et al., 2008) but also with increased liver enzymes, chronic hepatitis and an increased risk of liver cancer in the elderly (Kim et al., 2015; Wong et al., 2016). Tankersley et al. demonstrated altered baseline autonomic nervous function and pulmonary congestion following carbon black exposure in senescent mice (Tankersley et al., 2008). Additionally, arrhythmia cordis caused by PM2.5 exposure has been detected in aged rats, but not in younger rats (Nadziejko et al., 2004). Although many studies have shown that children and the elderly are susceptible populations to the adverse health effects of airborne particulate exposure, the hepatotoxicity of PM2.5 exposure at different developmental stages is unclear.

Therefore, it is necessary to study the effects of PM2.5 exposure on liver injury and NAFLD progression, especially in susceptible populations. The aims of the present study were to (1) clarify the effects of PM2.5 exposure on lipid synthesis, transport, and catabolism in mouse liver; and (2) understand the different hepatic physiological responses of mice at different life stages when exposed to PM2.5 to examine the existence of susceptible populations.

1. Materials and methods

1.1. PM2.5 sample collection

From November 2014 to February 2015, PM2.5 samples were collected in the urban area of Taiyuan, the capital of Shanxi Province in northern China. High temperature treated quartz filters (F90 mm, Munktell, Sweden) were used to collect the samples with a PM middle-volume air sampler (TH-150CIII, Wuhan TianHong, China) on building roof. After sampling, the PM2.5 filter was immersed in double distilled water for 30 min, vortexed for 5 min, and sonicated for 30 min. After filtration, the samples were dried using a vacuum freeze dryer. Before the animal treatment, the collected PM2.5 dry powder was diluted with sterile saline, vortexed for 10 min, and then ultrasonicated for 10 min with an ultrasound machine (Ku et al., 2017b).

1.2. Animal treatment

Female C57BL/6 mice aged 4 weeks (4 w), 4 months (4 m), and 10 months (10 m) were purchased from Junke Bioengineering Co., Ltd. Nanjing, China. The mice were housed under the standard laboratory conditions of (24 ± 2)°C, (50 ± 5)% relative humidity. Mice of each age group were randomly and equally divided into a control group and a PM2.5 treatment group. In the PM2.5 treatment group, mice were treated with 3 mg/kg body weight of PM2.5 by oropharyngeal aspiration every other day for 4 weeks. The administered dose was 1 μL of stock solution (3 μg/μL) per gram of mouse body weight, and the final dose reached 3 mg/kg bw. Animals in the control group were treated with saline that was processed with ultrasonic vibration of a blank membrane filter. Water and standard diet were provided to the animals when not being treated. The mice care and use procedures in this study were approved by the
Institutional Animal Care and Use Committee of Shanxi University and complied with guidelines set by National Institutes of Health. Mice were anesthetized with isoflurane and decapitated 24 hr after the final exposure. The livers were collected, weighted and stored at −80°C for analysis.

1.3. Transmission electron microscopy (TEM) observation
After the mice were sacrificed, liver pieces approximating 1 mm³ in size were rapidly excised from the hepatic tissue, and the pieces were fixed, stained, dehydrated and flat-embedded according to previous studies (Ji et al., 2016). Next, 70-80 nm thick embedded tissue sections were placed on copper grids, stained with uranyl acetate (15 min), and finally evaluated and imaged with a transmission electron microscope (TEM) (JEM 1400, JEOL, Japan).

1.4. Total RNA extraction and RT-PCR
Total RNA was isolated using TRIzol reagent (Invitrogen, America) and then synthesized into complementary DNA (cDNA) with a reverse transcription kit (TaKaRa, Japan) according to the manufacturer’s introductions. Specific primer RT-PCR was performed using a qTOWER 2.2 real-time PCR instrument (Analytik Jena AG, Jena, Germany) in conformity with the instructions of the miScript SYBR Green PCR kit (TaKaRa, Japan). Briefly, every 20 μL of PCR contained 2 μL of cDNA (6-fold dilution of original cDNA product), 10 μL of SYBR Premix Ex TaqII (TaKaRa, Japan), 7 μL of RNase-free H₂O, and 0.5 μL of each primer. Cytochrome P450 1A1 (Cyp1a1), superoxide dismutase 1 (Sod1), sterol regulatory element-binding proteins 1 (Srebp1), sterol regulatory element-binding proteins 2 (Srebp2), fatty acid synthase (Fasn), steraryl-CoA desaturase-1 (Sd1), ATP-binding cassette subfamily G member 5 (Abcg5), ATP-binding cassette subfamily G member 8 (Abcg8), ATP-binding cassette subfamily A member 1 (Abca1), patatin-like phospholipase domain containing 2 (Pnpla2), peroxisome proliferator activated receptor alpha (PPARα), carnitine palmitoyl transferase 1a (Cpt1a), acyl-Coenzyme A oxidase 1 (Acox1), Cytochrome P450 4A10 (Cyp4a10) and GAPDH (as an internal reference gene) genes used a three-step method, and their reaction conditions were as follows: 3 min at 95°C, 20 sec at 95°C, and 20 sec at annealing temperature, followed by 20 sec at 72°C. Primer sequence information, annealing temperatures, and cycles is provided in Table 1.

1.5. Immunoblot analysis
Proteins were extracted from the liver tissues of female mice and quantified as our previously described method (Qin et al., 2018). After boiling for 10 min, 100 μg of protein samples were loaded to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis proteins were

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transferred to nitrocellulose membranes by Bio-Rad Mini Trans-Blot Electrophoretic Transfer Cell Instruments. The membranes were blocked in 3% bovine serum albumin (BSA) and incubated overnight at 4°C with antibodies to targeted proteins (anti-GAPDH antibody, Bioss, China; anti-Cyp1a1 antibody, Bioss, China; anti-Scd1 antibody, Bioss, China; anti-Abcg5 antibody, Proteintech, China; anti-Cpt1a antibody, Bioss, China; anti-PPARα antibody, Bioss, China). The membranes were washed with PBS, exposed to IR Dye 800CW-conjugated secondary antibody (1:5000, LiCor Biosciences, USA), and then scanned and detected with a LI-COR Odyssey Infrared Fluorescent System (Odyssey Sa, LI-COR, USA).

1.6. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) of the liver tissues were measured using ELISA kits (XiTang, China), according to the manufacturer’s instructions.

1.7. Statistical analysis

The results are expressed as the mean ± standard error (SE). The data were analyzed by one-way ANOVA, followed by Tukey’s post-tests between groups to assess significant differences between exposure and control groups. The results were considered to have significant differences at \( p < 0.05 \).

2. Results

2.1. Effects of PM$_{2.5}$ on body weight and liver weight

In the initial stage of the experiment, the mean body weight of the treatment group and that of the control group were comparable for each age. After 4 weeks of treatment, the mice were reweighted, and the livers were excised and weighted. The body weights (Fig. 1a) and liver weights (Fig. 1b) of the 10-month-old mice in the treatment group were significantly lower than those in the control group following PM$_{2.5}$ exposure. Although both liver weight and body weight decreased in 10-month-old mice, there was no significant change in the ratio of liver weight to body weight (Fig. 1c). However, the body and liver weights of the 4-week-old and 4-month-old mice were not significantly different from their unexposed counterparts. These results indicate that PM$_{2.5}$ exposure might have harmful effects on the livers of aged mice.

2.2. Effects of PM$_{2.5}$ exposure on the hepatic ultrastructure

No signs of particle accumulation were detected in the liver of mice in any groups. However, electron microscopy ultrastructure detection showed more and larger lipid droplets in the livers of 4-week-old and 10-month-old mice after PM$_{2.5}$ exposure (Fig. 2).

2.3. Effects of PM$_{2.5}$ exposure on the expression of Cyp1a1 and Sod1 in mice liver

Oxidative stress, caused by an imbalance of oxidation and antioxidation in vivo, plays a “second hit” role in the development of NAFLD. In this study, the transcription levels of Cyp1a1 and Sod1 were detected, which are related to the production of reactive oxygen species and antioxidant activity, respectively. As show in Fig. 3a, the Cyp1a1 gene expression level was significantly increased in the PM$_{2.5}$-treated 4-week-old and 10-month-old mice compared to that in the corresponding control mice. The expression of Sod1 was also examined, and the results indicated that Sod1 expression was significantly depressed in mice at 4 weeks and 10 months of age following PM$_{2.5}$ exposure (Fig. 3b).

2.4. Effects of PM$_{2.5}$ exposure on the expression of genes involved in lipid synthesis

The expressions of four genes related to fat synthesis were evaluated to demonstrate the effect of exposure to PM$_{2.5}$ on fat synthesis in mice. The hepatic mRNA expression of Fasn, Scd1, Srebp1, and Srebp2 was up-regulated in the 10-month-old mice exposed to PM$_{2.5}$ (Fig. 4). The gene expression levels in the 4-week-old and 4-month-old age group were not significantly changed compared to the control group.

2.5. Effects of PM$_{2.5}$ exposure on the expression of lipid transport related genes

To investigate the ability of liver fat to metastasize from the liver following exposure to PM$_{2.5}$, we tested the gene expression of Abcg5, Abcg8, and Abca1. Proteins encoded by Abcg5 and
Abcg8 promote sterol secretion to the bile duct, and protein encoded by Abca1 functions as a pump for cholesterol efflux. As presented in Fig. 5, the expression levels of genes involved in lipid transport (Abcg5, Abcg8 and Abca1) were significantly decreased in 10-month-old mice after PM2.5 exposure. No alteration of these genes was detected in mice of other ages.

2.6. Effects of PM2.5 exposure on the expression of genes involved in lipid catabolism

Fatty acids are the core components of most lipid molecules. We examined the mRNA expression of the genes (Pnpla2, PPARα, Cpt1α, Acox1 and Cyp4a10) encoding the key enzymes and regulators involved in fatty acid oxidation and lipolysis. After exposure to PM2.5, the mRNA expression of Pnpla2 was downregulated in the liver of 4-month-old and 10-month-old mice (Fig. 6a), and the mRNA expression of PPARα, Cpt1α and Acox1 in the liver of 10-month-old mice were downregulated (Fig. 6b-d). The expression of Cyp4A10 was not changed significantly (Fig. 6e).

2.7. Effects of PM2.5 exposure on lipid metabolism related protein expression in mice liver

To confirm the effect of PM2.5 exposure on the expression of genes involved in lipid metabolism, Western blot analyses were performed to validate the expression levels of proteins. Cyp1a1 protein levels were significantly increased in the livers of 4-week-old and 10-month-old female mice after PM2.5 exposure (Fig. 7a, f). The protein levels of Cpt1a, PPARα and
Abcg5 decreased significantly in the liver of 10-month-old mice following PM2.5 exposure, while Scd1 increased significantly (Fig. 7b-f). No significant differences in the protein levels were observed in the livers of 4-month-old mice compared with the control group.

### 2.8. Effects of PM2.5 exposure on the level of HDL and LDL levels

The concentrations of HDL and LDL in the liver tissue of mice were detected using ELISA. The liver HDL concentration in mice of all ages was not significantly different between the treated and control groups (Fig. 8a). Significantly increased LDL levels were observed in the 10-month-old treatment group and were increased by 1.48-fold compared to that of the control group (Fig. 8b). There was no significant difference in liver LDL levels between the exposure and control groups of the 4-week and 4-month-old mice.

### 3. Discussion

A large number of studies have shown that atmospheric particulates have adverse effects on human health (Räckerl et al., 2011), but evidence regarding the impact on the liver is limited (Tarantino et al., 2013); nevertheless, several epidemiological studies have shown that PM2.5 exposure has adverse effects on the liver, including increasing chronic...
hepatitis and HCC, especially in the elderly (Kim et al., 2015; Wong et al., 2016). Previous studies by our research group have analyzed the chemical composition of the PM2.5 samples used in present study, and no signs of particle accumulation were detected in the liver of mice treated with the PM2.5 in the same dose and exposure method (Ku et al., 2017b). However, they indicated that exposure to PM2.5 caused systemic inflammation (Ku et al., 2017a), accumulation of metal elements (such as Mn) and lipid abnormalities (Ku et al., 2017b) in the liver of mice. Both triglyceride and total cholesterol levels were significantly elevated in the livers of 4-week-old mice, and increased total cholesterol levels were observed in the

Fig. 6 – Effects of PM2.5 exposure on the mRNA expression of the genes encoding the key enzymes or regulators involved in fatty acid oxidation and lipolysis in the livers of female mice. The mRNA expression levels of Pnpla2 (a), PPARα (b), Cpt1α (c), Acox1 (d), Cyp4A10 (e) in livers of female mice at different ages (4 weeks (4 w), 4 months (4 m), and 10 months (10 m)) after PM2.5 exposure are presented as the mean ± SE (n = 6 mice/group). *p < 0.05, **p < 0.01 vs. control group.

Fig. 7 – Effects of PM2.5 exposure on the protein expression of lipid metabolism-related genes in livers of female mice at different ages (4 weeks (4 w), 4 months (4 m), and 10 months (10 m)). (a) Protein expression of CYP1A1; (b) Protein expression of Scd1; (c) Protein expression of Abcg5; (d) Protein expression of Cpt1α; (e) Protein expression of PPARα; (f) Protein bands. Data were expressed as the means ± SE (n = 6 mice/group). *p < 0.05, **p < 0.01 vs. control group.
livers of 10-month-old animals (Ku et al., 2017b). In this study, in order to explore the possible mechanism of liver lipid abnormalities induced by PM$_{2.5}$ exposure, we examined the effects on the liver lipid metabolism after PM$_{2.5}$ treatment. We found that exposure to PM$_{2.5}$ caused changes in the expression of exogenous compound metabolic enzymes and dysfunction of lipid metabolism, as evidenced by the increase of the expression of lipid synthetase, and the decrease of the expression of the key enzymes or regulators involved in fatty acid oxidation, lipolysis and lipid transport in the livers of mice. Interestingly, we found a slight increase in fat droplets in the livers of 4-week-old and 10-month-old mice. In addition, the susceptibility of the different age groups, from highly susceptible to minimally susceptible, was 10-month, 4-week and 4-month-old mice.

The liver has a powerful detoxification function, which excretes metabolites and toxins in the body through decomposition, oxidation and binding. Therefore, the liver plays a primary role in the defense from exogenous toxic compounds that people are exposed to daily (Hasson et al., 2016). Cyp1 is a major cytochrome P450 family involved in the bioactivation of xenobiotic compounds (Ioannides, 2008). Previous studies have reported that incense smoke (Hussain et al., 2014) and cigarette smoke (Sidle et al., 2007) exposure induces the expression of Cyp1 and activates oxidative stress and inflammation in the liver. Recent studies have found that diesel exhaust particles treated epithelial cells induce Cyp1a1 expression (Saulig et al., 2003), which is known to produce reactive oxygen species (ROS) (Perret and Pompon, 1998; Kopf and Walker, 2010). Oxidative stress refers to elevated intracellular levels of ROS (Schieber and Chandel, 2014). Oxidative stress was deemed to be the “second hit” in the pathogenesis of NAFLD (Day and James, 1998). The present study has found that PM$_{2.5}$ induced significantly increased Cyp1a1 expression at 4 weeks and 10 months of age, but not at 4 months of age. As an essential antioxidant enzyme in organisms, Sod1 is responsible for counteracting and blocking the toxic effects caused by ROS. The expression of Sod1 mRNA in 4-week-old and 10-month-old mice decreased significantly after exposure to PM$_{2.5}$. These results suggested that PM$_{2.5}$ exposure induces Cyp1a1 expression and decreases Sod1 expression in the livers of mice, followed by increased oxidative stress, which is consisted with previous research (Jian et al., 2018), especially in younger and older mice.

Hepatic lipid metabolism disorders are key factors in the aggravation of NAFLD. Studies have shown that PM$_{2.5}$ exposure triggers inflammatory activity, induces collagen synthesis in hepatic stellate cells, and promotes liver fibrosis (Tan et al., 2009; Zheng et al., 2015). One study showed that chronic exposure to PM$_{10}$ increases the contents of phosphatidylcholine, total fatty acids, and docosahexaenoic acid in the liver (Rizzo et al., 2014). However, the degree and the specific cause of abnormal liver lipid metabolism induced by PM$_{2.5}$ exposure in vivo are not yet clear. To clarify this, we first analyzed the mRNA expression levels of enzymes involved in fat anabolism in the livers of mice after exposure to PM$_{2.5}$. Sterol regulatory element-binding proteins (SREBPs) are intracellular cholesterol sensors located in the endoplasmic reticulum and provide feedback regulation of cholesterol (Yuan et al., 2009). Srebp1 is involved in regulating the expression of Fasn, ACC (acetyl coenzyme-A carboxylase) and Scd1 (Yuan et al., 2009; Vesterdal et al., 2014). SREBP-2 is involved in cholesterol synthesis and uptake (Zhao et al., 2011). Fasn is a key enzyme in the de novo synthesis of fatty acids (Abdel-Magid, 2015). Scd-1 is the rate-limiting enzyme catalyzing the synthesis of monosaturated fatty acids (Amacher, 2011). Our experimental data showed that the mRNA expression levels of Srebp1, Srebp2, Fasn and Scd1 were significantly increased in the livers of 10-month-old mice that were exposed to PM$_{2.5}$. These findings indicate that PM$_{2.5}$ exposure induces hepatic lipid synthesis, and older mice are the most affected age group.

Many studies have documented that cholesterol plays a part in the development of NAFLD (Su et al., 2012). The development of NAFLD is related to the intake of free cholesterol by the liver and biliary clearance (Van Rooyen et al., 2011). We tested the expression of lipid transfer-related genes and the concentrations of HDL and LDL after exposure to PM$_{2.5}$. Abcg5 and Abcg8 encode transporters that secrete cholesterol into the bile to prevent sterol accumulation in the body and are mainly expressed in the liver and small intestine (Méndez-González et al., 2011; Vesterdal et al., 2014). Abca1 is a membrane transporter involved in cholesterol efflux in the cellular lipid removal pathway (Sticcozzi

![Fig. 8](image)
et al., 2010). In our study, the expression levels of Abcg5, Abcg8 and Abca1 in the livers of 10-month-old mice were significantly decreased following PM2.5 exposure, suggesting that exposure to environmentally equivalent doses of PM2.5 has little effect on the hepatic fat efflux in young and adult mice, but significantly reduces the expression of hepatic fat efflux-related genes in aged mice. The increase of lipid droplets observed in TEM further demonstrated the damage effect of PM exposure on lipid clearance mechanism. The metabolism of LDL and HDL occurs mainly in the liver (Lapointe et al., 2006). Lower HDL and higher LDL levels are the primary features of patients with NAFLD (Luoma et al., 1983). We found that the liver LDL levels in 10-month-old mice were significantly elevated after PM2.5 exposure, but the HDL levels were not altered.

Lipolysis is defined as the sequential hydrolysis of triacylglycerol stored in cell lipid droplets, which plays an important role in the homeostasis of lipid metabolism (Bolsoni-Lopes and Alonso-Vale, 2015). Pnplal2 is a gene encoding adipose triglyceride lipase (ATGL), which is a major hepatic triacylglycerol (TAG) hydrolase (Bolsoni-Lopes and Alonso-Vale, 2015). Fatty acids formed by hydrolysis of triglycerides are mostly metabolized by β-oxidation, which occurs mainly in mitochondria and peroxisomes (Musso et al., 2009). A small portion of fatty acids are metabolized by microsomal ω-oxidation and are mainly catalyzed by cytochrome P450 2E1, 4A10, and 4A14. Several key enzymes in these three fatty acid oxidation pathways are regulated by PPARs. Mitochondrial beta oxidation is regulated by Cpt and Malonyl-CoA. Acyl CoA oxidase 1 (Acox1), a rate-limiting enzyme, catalyzes the first step of the peroxisomal fatty acid β-oxidation (Nguyen et al., 2008). In this study, the mRNA expression of Pnplal2 was significantly decreased in the livers of 4-week-old and 10-month-old mice. The expression of Pparα, Cpt1α, and Acox1 was reduced in the 10-month-old mice liver exposed to PM2.5. The expression level of Cyp4a10 in the liver of mice remained unchanged. In addition, the significantly decreased protein levels of Pparα and Cpt1α were observed in the treated group compared to the normal counterpart. These results indicate that PM2.5 exposure down-regulates lipolysis and mitochondrial and peroxisomal fatty acid β-oxidation in mice liver, but had no effect on ω-oxidation. In addition, these effects are age-dependent.

The adverse health effects of ambient air pollution are mainly restricted to susceptible populations (O’Neill et al., 2012). Elderly individuals represent a susceptible population when compared with children and young adults. Previous research has provided biological plausibility for PM-induced systolic and diastolic dysfunction (Ying et al., 2009; Wold et al., 2012), and decreased heart rate variability in the elderly (Devlin et al., 2003; Gong et al., 2004). In addition, exposure to PM2.5 affects the systolic blood pressure and heart rate in 10-month-old mice (Qin et al., 2018) and causes arrhythmias in older rats (Nadziejko et al., 2004), but not in younger rats. Recent epidemiological investigations have shown that PM exposure is associated with not only an increased risk of cardiovascular morbidity (Host et al., 2008) but also with increased levels of liver enzymes, chronic hepatitis and an increased risk of liver cancer in the elderly (Kim et al., 2015; Wong et al., 2016). In our research, the body weight, liver weight, Oxidative stress factors, gene expression levels of liver lipid metabolic enzymes, and the liver LDL levels of aged mice exposed to PM2.5 were more severely affected than those of other age groups, and most of these indexes affected only the aged mice. Our findings indicated that exposure to PM2.5 can cause liver injury and affect liver lipid metabolism, and older mice are more susceptible to these effects.

4. Conclusions

In conclusion, PM2.5 exposure induces oxidative stress, disrupts lipids metabolism and slightly increases lipid accumulation in the livers of female mice. These effects are accompanied by increased expression of genes related to lipid synthesis and decreased expression of genes related to lipid transport, lipolysis, and fatty acid oxidation, resulting in abnormal LDL levels. The changes seen in these indicators were primarily observed in the 10-month-old mice, followed by the 4-week-old mice, and the 4-month-old mice showed few effects from exposure. These findings suggest that PM2.5 exposure at the current exposure dose disrupts normal liver metabolism and that older mice are more susceptible to adverse effects from PM2.5 exposure.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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