

Exposure to ambient particulate matter induces a NASH-like phenotype and impairs hepatic glucose metabolism in an animal model

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Background & Aims: Air pollution is a global challenge to public health. Epidemiological studies have linked exposure to ambient particulate matter with aerodynamic diameters <2.5 μm (PM_{2.5}) to the development of metabolic diseases. In this study, we investigated the effect of PM_{2.5} exposure on liver pathogenesis and the mechanism by which ambient PM_{2.5} modulates hepatic pathways and glucose homeostasis.

Methods: Using “Ohio’s Air Pollution Exposure System for the Interrogation of Systemic Effects (OASIS)-1”, we performed whole-body exposure of mice to concentrated ambient PM_{2.5} for 3 or 10 weeks. Histological analyses, metabolic studies, as well as gene expression and molecular signal transduction analyses were performed to determine the effects and mechanisms by which PM_{2.5} exposure promotes liver pathogenesis.

Results: Mice exposed to PM_{2.5} for 10 weeks developed a non-alcoholic steatohepatitis (NASH)-like phenotype, characterized by hepatic steatosis, inflammation, and fibrosis. After PM_{2.5} exposure, mice displayed impaired hepatic glycogen storage, glucose intolerance, and insulin resistance. Further investigation revealed that exposure to PM_{2.5} led to activation of inflammatory response pathways mediated through c-Jun N-terminal kinase (JNK), nuclear factor kappa B (NF- κ B), and Toll-like receptor 4 (TLR4), but suppression of the insulin receptor substrate 1

(IRS1)-mediated signaling. Moreover, PM_{2.5} exposure repressed expression of the peroxisome proliferator-activated receptor (PPAR) γ and PPAR α in the liver.

Conclusions: Our study suggests that PM_{2.5} exposure represents a significant “hit” that triggers a NASH-like phenotype and impairs hepatic glucose metabolism. The information from this work has important implications in our understanding of air pollution-associated metabolic disorders.

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Introduction

Recent studies have indicated that exposure to fine ambient particulate matter (aerodynamic diameter <2.5 μm , PM_{2.5}) is closely associated with the pathogenesis of cardiovascular disease and metabolic syndrome [1–4]. Studies from our group and others suggested that PM_{2.5}-triggered systemic and pulmonary inflammation (low grade) promotes a variety of maladaptive signaling pathways that may lead to insulin resistance [1,5,6]. Consistent with these findings, we have recently demonstrated an important interaction of PM_{2.5} exposure with a high-fat diet in promoting metabolic syndrome [1,6]. These prior studies were focused on lung or adipose pathways in mediating inflammatory responses but had not pronounced effects of PM_{2.5} exposure on modulating hepatic pathways associated with metabolic disease.

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases ranging from simple non-alcoholic fatty liver (NAFL), to non-alcoholic steatohepatitis (NASH), to irreversible cirrhosis [7]. NAFLD is considered a precursor or hepatic manifestation of cardiovascular disease and metabolic syndrome. The progression of NASH is explained by a “two-hit” working model [8]. According to this model, steatosis represents the “first hit,” which increases the vulnerability of the liver to various “second hits” induced by endotoxin, saturated fatty acids,

Keywords: Air pollution; NASH; Glucose metabolism; Liver disease.

Received 17 January 2012; received in revised form 1 August 2012; accepted 6 August 2012; available online 15 August 2012

* DOI of original article: <http://dx.doi.org/10.1016/j.jhep.2012.10.008>.

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Abbreviations: PM, ambient particulate matter; PM_{2.5}, PM with aerodynamic diameter less than 2.5 μm ; FA, filtered air; OASIS-1, Ohio’s Air Pollution Exposure System for the Interrogation of Systemic Effects; NASH, non-alcoholic steatohepatitis; HOMA-IR, homeostasis model assessment-insulin resistance; PPAR, peroxisome proliferator-activated receptor; JNK, the c-JUN N-terminal kinase; IRS1, insulin receptor substrate 1; NF- κ B, Nuclear Factor-Kappa B; TLRs, Toll-like receptors.



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inflammatory cytokines, oxidative stress, or other liver injuries. The “second hit” in turn leads to hepatic inflammation and fibrosis, the key features of NASH. Recent works have shown that PM_{2.5} exposure can activate Kupffer cells in murine liver tissue, which suggests that PM_{2.5} may represent a risk factor for NAFLD progression [9,10].

In this study, we used a “real-world” PM_{2.5} exposure system to perform whole-body exposure of mice to environmentally relevant PM_{2.5}. We demonstrate that exposure to PM_{2.5} causes a NASH-like phenotype and impairs hepatic glycogen storage in animals. Through both *in vivo* and *in vitro* analyses, we reveal the signaling pathways through which PM_{2.5} exposure promotes NASH-associated activities and impairment of hepatic glucose metabolism.

Materials and methods

Exposure of animals to ambient PM_{2.5}

Mice were exposed to concentrated ambient PM_{2.5} or filtered air (FA) in a mobile trailer “Ohio’s Air Pollution Exposure System for the Interrogation of Systemic Effects (OASIS)-1” in Columbus, OH, where most of the PM_{2.5} components are attributed to long-range transport (Supplementary Fig. 1) [11]. The concentrated PM_{2.5} was generated using a versatile aerosol concentration enrichment system (VACES) as described previously [1,11]. Mice were exposed to concentrated PM_{2.5} at nominal 10× ambient concentrations 6 h per day, 5 days per week for a total of 3 or 10 weeks, as detailed previously [1,9]. The control (FA) mice in the experiment were exposed to an identical protocol with the exception of a high-efficiency particulate-air filter positioned in the inlet valve position to remove PM_{2.5} particles in the filtered air stream.

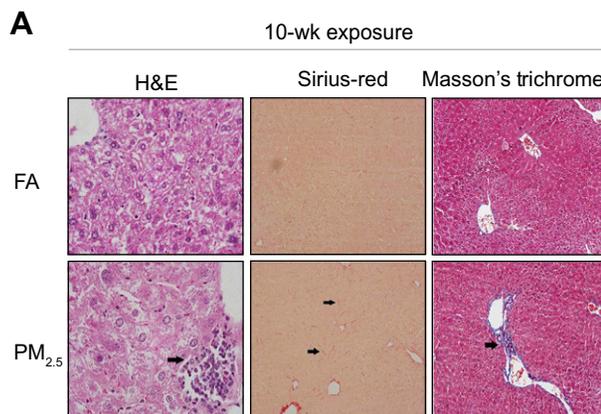
For a full description of Materials and Methods, see Supplementary information.

Results

Mice after inhalation exposure to PM_{2.5} develop a NASH-like phenotype

To elucidate *in vivo* effects of PM_{2.5} exposure, male C57BL/6 mice were exposed to concentrated ambient PM_{2.5} or filtered air (FA) for 3 or 10 weeks (5 days/week, 6 h/day) in exposure chambers of “OASIS-1”, which was composed of the Midwestern regional background in Columbus, USA, where most of the PM_{2.5} is attributed to long-range transport (Supplementary Fig. 1) [9]. It has been demonstrated that the distribution and size of concentrated PM_{2.5} in the OASIS-1 exposure chamber air truly reflect that of non-concentrated PM_{2.5} present in the ambient air [11,12]. The “OASIS-1” system enables studies on animal models that recapitulate personal, short- or long-term exposure to environmentally relevant PM_{2.5}.

During the exposure time period, the mean daily ambient PM_{2.5} concentration was 6.5 µg/m³, while the mean concentration of PM_{2.5} in the exposure chamber was 74.6 µg/m³ [9]. Mice were exposed to concentrated PM_{2.5} or FA for 6 h per day, 5 days per week. After taking into account the unexposed time and weekends, the calculated average daily PM_{2.5} concentration to which mice were exposed was 11.6 µg/m³ [the annual average PM_{2.5} National Ambient Air Quality Standard is 15.0 µg/m³ (epa.gov/air/criteria.html)]. Previously, our X-ray fluorescence spectroscopic analysis of PM_{2.5} composition in the exposure chambers revealed that the major components of PM_{2.5} complex



B

| | Steatosis | Ballooning | Inflammation | | Mallory bodies | Grade (0-3) | Stage (0-4) |
|-----------------------|-------------|-------------|--------------|-------------|----------------|-------------|-------------|
| | | | Lobular | Portal | | | |
| FA (4) | 0.25 ± 0.25 | 0.00 ± 0.00 | 0.25 ± 0.25 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.25 ± 0.25 | 0.25 ± 0.25 |
| PM _{2.5} (5) | 1.2 ± 0.20 | 0.60 ± 0.40 | 0.80 ± 0.20 | 0.40 ± 0.24 | 0.20 ± 0.20 | 1.60 ± 0.24 | 1.40 ± 0.24 |
| <i>p</i> value | 0.024 | 0.208 | 0.135 | 0.178 | 0.374 | 0.007 | 0.014 |

Fig. 1. PM_{2.5} exposure induces a NASH-like phenotype in the mouse liver. (A) Histological analysis of liver cellular structure (H&E staining, 600×), collagen deposition (Sirius-Red staining, 200×), and collagen fiber (Masson’s trichrome staining, 600×) in liver tissue sections from mice exposed to FA or PM_{2.5} for 10 weeks. The arrows point out areas of hepatic inflammation or fibrosis. (B) Histological scoring for NASH activities in the livers of C57BL/6J mice exposed to PM_{2.5} or FA for 10 weeks. The grade scores were calculated based on the scores of steatosis, hepatocyte ballooning, lobular and portal inflammation, and Mallory bodies. The stage scores were based on liver fibrosis. Mean ± SEM values are shown (n = 4 mice for the FA-exposed group or 5 mice for the PM_{2.5}-exposed group). *p* values were calculated by Mann–Whitney U-test. wk, week.

are alkali metals, alkaline earth metals, transition metals, poor metals, non-metals, metalloid, and halogens [9].

To evaluate the impact of PM_{2.5} exposure on liver pathology, we performed histological analyses with liver tissue sections of mice exposed to PM_{2.5} or FA for 3 or 10 weeks. Based on hematoxylin and eosin (H&E) staining of the liver cellular structure as well as Sirius-red and Masson’s trichrome staining of the hepatic collagen deposition, we identified hepatic steatosis, lobular and portal inflammation, and perisinusoidal fibrosis in the liver of mice exposed to PM_{2.5} for 10 weeks (Fig. 1A). In comparison, mice exposed to PM_{2.5} for 3 weeks displayed non-significant, subtle hepatic steatosis, lobular inflammation, and hepatocyte ballooning (Supplementary Fig. 2). Using the NAFLD grading and staging score system, we confirmed that mice exposed to PM_{2.5} for 10 weeks developed modest NASH, characterized by steatosis, hepatic inflammation, and perisinusoidal fibrosis (Fig. 1B).

Exposure to PM_{2.5} reduces hepatic glycogen storage and impairs glucose and insulin homeostasis

To assess the impact of PM_{2.5} exposure on liver metabolism, we examined glucose and insulin homeostasis in mice exposed to PM_{2.5} or FA. Under normal conditions, glycogen storage is located in the area close to central veins in the liver tissue [13]. Mice exposed to PM_{2.5}, but not FA, for 10 weeks, lost the normal distribution of glycogen storage around central veins, as

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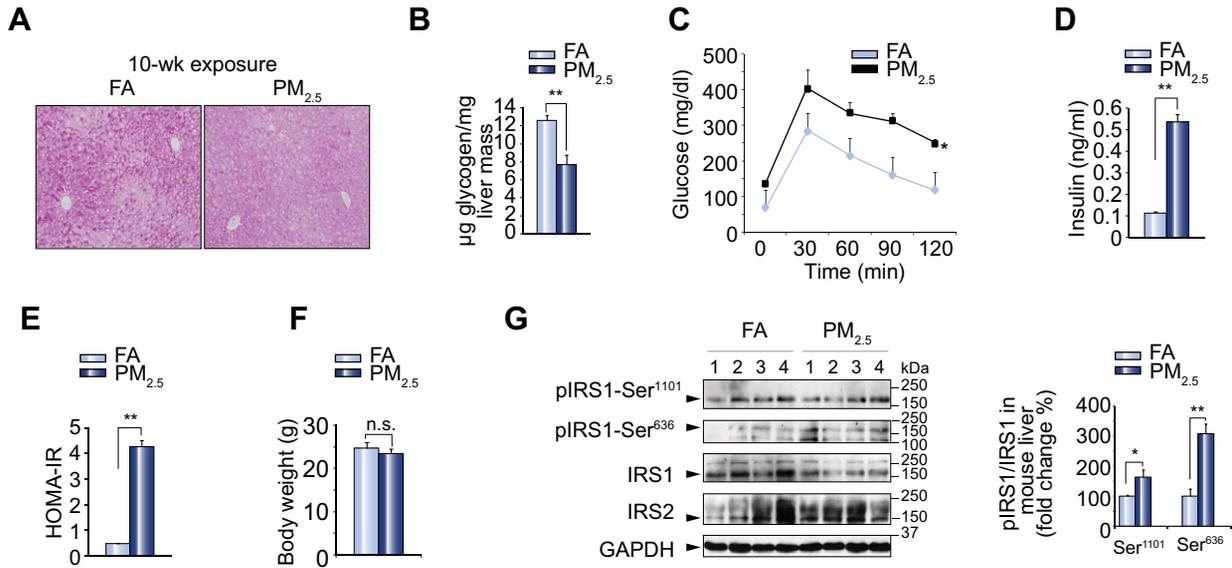


Fig. 2. Exposure to PM_{2.5} leads to impaired hepatic glycogen storage, glucose intolerance, and insulin resistance. (A) Periodic-acid Schiff's staining of hepatic glycogen in the livers of mice exposed to PM_{2.5} or FA for 10 weeks (magnification: 200×). (B) Levels of hepatic glycogen in liver tissues of mice exposed to PM_{2.5} or FA for 10 weeks. (C) IPGTT analysis with mice exposed to PM_{2.5} or FA for 68 days. Blood glucose levels were measured after i.p. injection of 2 mg glucose/g body weight into animals fasted for 12 h. Measurement of fast blood glucose and insulin levels as well as IPGTT analysis were performed 2 days before the animals were euthanized. (D) Fast blood insulin levels in mice exposed to PM_{2.5} or FA. Blood insulin levels were determined in mice after 12 h of fasting. (E) Homeostasis model of assessment of insulin resistance (HOMA-IR) for mice exposed to PM_{2.5} or FA. (F) Body weights of mice exposed to PM_{2.5} or FA for 10 weeks. (G) Immunoblotting analysis of phosphorylated and total IRS1 as well as IRS2 in liver tissues of mice exposed to PM_{2.5} or FA. The graph shows fold changes of the ratios of phosphorylated vs. total IRS1 in liver tissues. For panels B–G, each bar or point denotes mean ± SEM (n = 4 mice). *p < 0.05; **p < 0.01; n.s., not significant. wk, week.

indicated by periodic-acid Schiff (PAS) staining of glycogens in liver tissue sections (Fig. 2A). Both glycogen staining and enzymatic assessment of hepatic glycogen indicated that levels of glycogen in the PM_{2.5}-exposed mouse liver were reduced, compared to those in the FA-exposed mouse liver (Fig. 2A and B), suggesting a significant negative effect of PM_{2.5} exposure on hepatic glycogen storage. In comparison, the amounts and distribution of hepatic glycogen in mice exposed to PM_{2.5} for 3 weeks were similar to those in FA-exposed control mice (Supplementary Fig. 3A and F).

To determine whether PM_{2.5} exposure can affect glucose homeostasis, we performed an intraperitoneal glucose tolerance test (IPGTT) with the animals after exposure to PM_{2.5} or FA. Upon administration of glucose, mice exposed to PM_{2.5} for 10 weeks, but not 3 weeks, had higher levels of glucose in the blood (Fig. 2C; Supplementary Fig. 3G). Moreover, fasting blood glucose and insulin levels in mice exposed to PM_{2.5} for 10 weeks were much higher than those in FA-exposed mice (Fig. 2C and D). Homeostasis model assessment of insulin resistance (HOMA-IR) data indicated that mice exposed to PM_{2.5}, but not FA, displayed significant insulin resistance (Fig. 2E). Additionally, body weights of mice after PM_{2.5} exposure were slightly reduced, compared to those of FA-exposed mice (Fig. 2F; Supplementary Fig. 3H).

Exposure to PM_{2.5} represses the signaling pathway mediated through the insulin receptor substrate 1 (IRS1)

We investigated potential signal transduction pathways in the liver, through which PM_{2.5} exposure disrupts glucose and insulin homeostasis. Glycogen synthesis is stimulated by the insulin receptor via activation of IRS1 [14]. It is known that phosphorylation of IRS1 at residues Ser636 and/or Ser1101 inhibits

IRS1-mediated insulin signaling through Akt kinase in regulating glycogen synthesis and blood glucose levels [14,15]. Immunoblotting analysis indicated that phosphorylation of IRS1 at both Ser636 and Ser1101 was increased in the liver tissue of mice exposed to PM_{2.5}, compared to that of FA-exposed mice (Fig. 2G), suggesting a PM_{2.5}-triggered inhibition of the IRS1 signaling. Consistently, phosphorylation of Akt, the downstream of IRS1 in regulating glycogen synthesis [16], was decreased in the liver of mice exposed to PM_{2.5}, thus confirming the effect of PM_{2.5} exposure on suppressing the IRS1-Akt signaling pathway (Supplementary Fig. 4A).

We assessed the effect of PM_{2.5} on IRS1 signaling in hepatic stellate cells (HSC), a cell type in the liver that interacts with resident macrophages to play critical roles in liver metabolism and fibrosis [17]. LX-2 is a human HSC cell line that retains key features of HSC and has been used as an experimental tool for the study of liver metabolism and fibrosis [18]. We cultured LX-2 cells in conditioned medium from the mouse macrophage cell line RAW264.7 exposed to 5 µg/ml of PM_{2.5}, a justified PM_{2.5} concentration that can induce intracellular stress response and macrophage activation *in vitro* (Supplementary Fig. 5). Immunoblotting analysis showed that phosphorylation of IRS1 at both Ser1101 and Ser636 was increased in LX-2 cells exposed to the PM_{2.5}-containing conditioned medium in a time-dependent manner (Supplementary Fig. 4B), thus confirming the suppression effect of PM_{2.5} on the IRS1-mediated signaling.

PM_{2.5} exposure leads to dysregulated lipid homeostasis and reduced expression of PPAR γ and PPAR α in the liver

We next examined the impact of PM_{2.5} exposure on lipid metabolism. Consistent with the hepatic steatosis phenotype

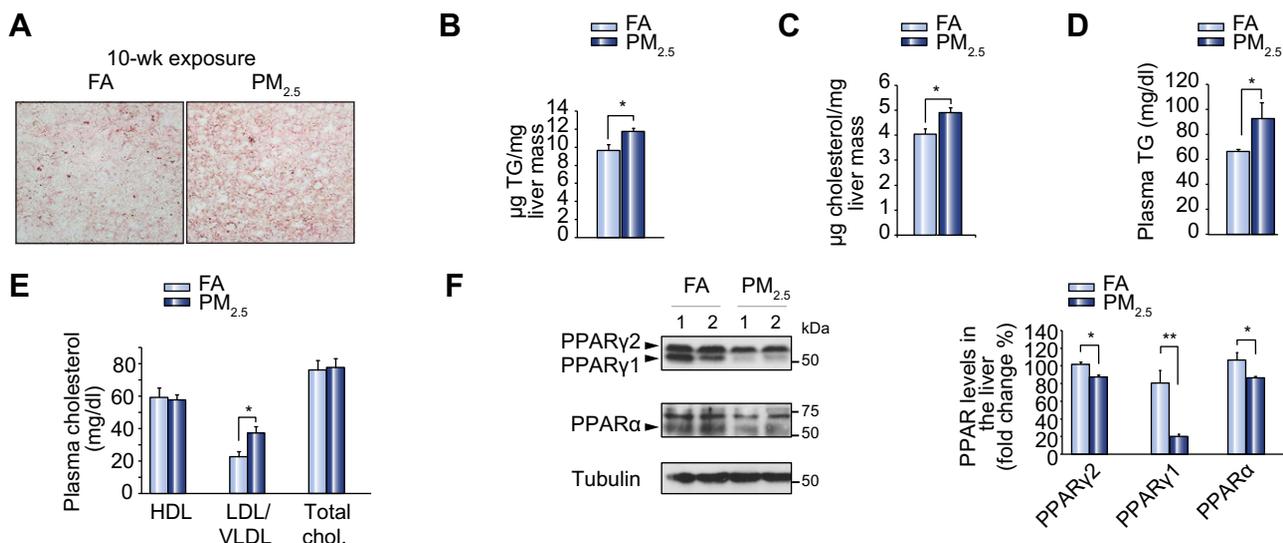


Fig. 3. PM_{2.5} exposure leads to hepatic steatosis and downregulates expression of PPARs. (A) Oil-Red O staining of lipid droplets in the livers of mice exposed to PM_{2.5} or FA for 10 weeks (magnification: 600×). (B–E) Levels of hepatic TG (B), hepatic cholesterol (C), plasma TG (D), and plasma cholesterol (E) of mice exposed to PM_{2.5} or FA for 10 weeks. Total chol, total plasma cholesterol. (F) Immunoblotting analysis of PPARγ and PPARα in the liver tissue from mice exposed to PM_{2.5} or FA for 10 weeks. The graph alongside the images shows fold changes of PPARγ1, PPARγ2, and PPARα levels in the liver of PM_{2.5}- or FA-exposed mice. For panels B–F, each bar or point denotes mean ± SEM (n = 4 mice). *p < 0.05; **p < 0.01. wk, week.

(Fig. 1), accumulation of hepatic lipid droplets, as indicated by Oil-Red O staining, was increased in the liver of mice exposed to PM_{2.5} for 10 weeks (Fig. 3A). The accumulation of hepatic lipid contents was further confirmed by the increased levels of hepatic triglycerides (TG) and cholesterol detected in the liver of PM_{2.5}-exposed mice (Fig. 3B and C). Moreover, levels of plasma TG and low/very low-density lipoproteins (LDL/VLDL) were elevated, while levels of plasma high-density lipoproteins (HDL) were not significantly changed in mice exposed to PM_{2.5} for 10 weeks (Fig. 3D and E). However, no significant induction of liver enzymes aspartate amino transferase (AST) and alanine aminotransferase (ALT), the indicators of liver damage [19], was detected in PM_{2.5}-exposed mice (Supplementary Fig. 6). Additionally, mice exposed to PM_{2.5} for 3 weeks only displayed marginal increase in hepatic steatosis (Supplementary Figs. 2 and 3A–C) and plasma TG levels (Supplementary Fig. 3D).

PPARs play important roles in the regulation of cellular differentiation, lipid and glucose metabolism, as well as inflammation [20]. In particular, levels of PPARγ and PPARα are inversely correlated with hepatic steatosis and glycogen storage in the progression of metabolic disease. Immunoblotting analysis showed that expression of two PPARγ isoforms, PPARγ1 and PPARγ2, was decreased in the livers of mice exposed to PM_{2.5} for 10 weeks, compared to that in the FA-exposed mice (Fig. 3F). Expression of PPARα, a key regulator of fatty acid oxidation [20], was also decreased in the liver of PM_{2.5}-exposed mice (Fig. 3F). Decreased expression of PPARα may contribute to hepatic steatosis in PM_{2.5}-exposed animals via downregulation of fatty acid oxidation, an interesting question to be further investigated. Likely, downregulation of PPARγ and PPARα, two key regulators of hepatic inflammation and metabolism, may partially account for the NASH-like phenotype observed in PM_{2.5}-exposed animals.

PM_{2.5} triggers the inflammatory pathways mediated through JNK, NF-κB, and TLR4

We assessed inflammatory responses in the livers of animals exposed to PM_{2.5}. Immunohistochemical (IHC) staining of the macrophage activation marker F4/80 indicated that numbers of activated Kupffer cells (hepatic macrophages) and alveolar macrophages (pulmonary macrophages) were increased in the liver and lung of mice exposed to PM_{2.5} for 10 weeks, compared to those in the FA-exposed mice (Fig. 4A). Expression of the mRNAs encoding pro-inflammatory cytokines IL1β, IL6, and TNFα was increased in the liver of mice exposed to PM_{2.5} (Fig. 4B). PM_{2.5}-induced expression of the *Il1β*, *Il6*, and *Tnfα* genes was confirmed via an *in vitro* experiment with RAW264.7 cells treated with PM_{2.5} particles collected from the filters retrieved from OASIS-1 (Supplementary Fig. 7). Enzyme-linked immunosorbent assay (ELISA) indicated that plasma levels of TNFα, the major pro-inflammatory cytokine that is closely associated with NASH [21], were significantly increased in mice exposed to PM_{2.5} for 10 weeks (Fig. 4C). Interestingly, although mice exposed to PM_{2.5} for 3 weeks exhibited marginal hepatic inflammation (Supplementary Fig. 2), plasma levels of TNFα were significantly increased in these mice (Supplementary Fig. 8B). Additionally, F4/80 IHC staining indicated a non-significant increase in the number of activated alveolar macrophages in the lung of mice after 3 weeks of PM_{2.5} exposure (Supplementary Fig. 8D). Combining the results obtained from mice exposed to PM_{2.5} for 10 weeks, our studies suggest that systemic inflammation occurs from early on and is followed by lung and hepatic inflammation in animals under PM_{2.5} exposure.

To elucidate signal transduction pathways by which PM_{2.5} stimulates expression of pro-inflammatory cytokines in the liver, we first examined JNK, a major inflammatory stress mediator that promotes pro-inflammatory cytokine expression by activating

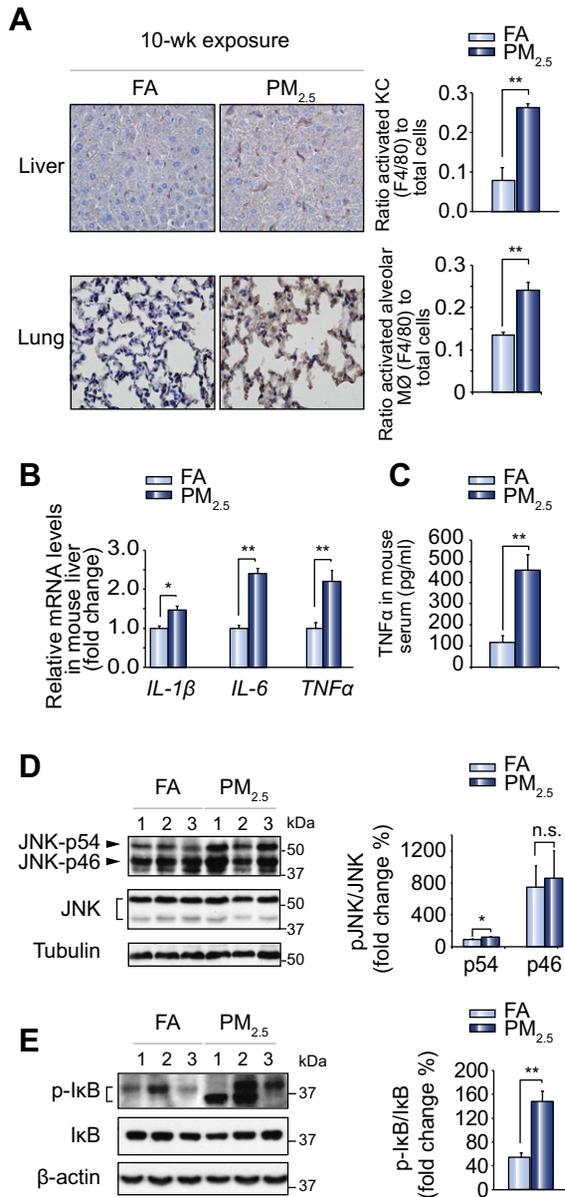


Fig. 4. Exposure to PM_{2.5} activates the inflammatory pathways mediated through JNK and NF- κ B in the liver. (A) Immunohistochemical staining of macrophage cell surface marker F4/80 in liver and lung tissues of mice exposed to PM_{2.5} or FA for 10 weeks (magnification: 600 \times). The graph alongside the image shows the ratios of F4/80-positive Kupffer cells or alveolar cells to total liver or lung cells. The ratios were determined by counting the F4/80-positive and total cells in 3 random fields per sample. Data are shown as mean \pm SEM (n = 3 mice per group). (B) Quantitative real-time RT-PCR analysis of the mRNAs encoding *IL-1 β* , *IL-6*, and *TNF α* in the liver of mice exposed to PM_{2.5} or FA for 10 weeks. Fold changes of mRNA levels were determined after normalization to internal control β -actin RNA levels. Each bar denotes mean \pm SEM (n = 5 mice). (C) Plasma levels of TNF α , determined by ELISA, in mice exposed to PM_{2.5} or FA for 10 weeks (n = 4 mice for FA or 5 mice for PM_{2.5} group). (D–E) Immunoblotting analysis of phosphorylated and total JNK (D) and I κ B (E) in the liver of mice exposed to PM_{2.5} or FA for 10 weeks. Levels of tubulin or β -actin were determined as loading controls. The graphs beside the images show fold changes of the ratios of phosphorylated vs. total JNK or I κ B in the liver of PM_{2.5}- or FA-exposed mice (n = 3 mice). MØ, macrophage; KC, Kupffer cells. *p < 0.05; **p < 0.01; n.s., not significant. wk, week.

active-protein 1 (AP-1) [22]. JNK phosphorylation was increased in the liver of mice exposed to PM_{2.5} for 10 weeks (Fig. 4D). Supporting the effect of PM_{2.5} on JNK activation, levels of phosphorylated JNK were increased in RAW264.7 cells cultured with PM_{2.5} (Supplementary Fig. 9A). Since AP-1 is the downstream transactivator in regulating the expression of pro-inflammatory cytokines under the JNK-mediated inflammatory response, we determined PM_{2.5}-triggered transcriptional activation of AP-1 in RAW264.7 cells. Upon PM_{2.5} challenge, transactivation of the AP-1 promoter, indicated by luciferase reporter analysis, was significantly increased in RAW264.7 cells (Supplementary Fig. 9B). Further, we tested whether JNK/AP-1 signaling is solely responsible for PM_{2.5}-triggered inflammation by using the JNK inhibitor SP600125 [23]. Suppression of JNK pathway in RAW264.7 macrophages by SP600125 did not significantly reduce induction of the *IL6* gene triggered by PM_{2.5} (Supplementary Fig. 9C), suggesting that PM_{2.5} may trigger other inflammatory pathways to induce production of pro-inflammatory cytokines. Indeed, we found that PM_{2.5} exposure can also activate NF- κ B, a key mediator of the inflammatory response, in the liver. Activation of the NF- κ B is initiated by signal-induced phosphorylation and degradation of NF- κ B inhibitor (I κ B) [24]. Western blot analysis demonstrated that levels of phosphorylated I κ B were increased in the liver of PM_{2.5}-exposed mice (Fig. 4E), indicating activation of NF- κ B pathway in the liver under PM_{2.5} exposure. Furthermore, induction of Toll-like receptors (TLRs), the specific pattern recognition receptors that recognize structurally conserved molecules, can lead to activation of NF- κ B and subsequent pro-inflammatory cytokine production [25]. Expression levels of *TLR2* and *TLR4* mRNAs were increased in the liver of mice exposed to PM_{2.5} for 10 weeks (Supplementary Fig. 10A). Pre-treatment of the TLR4 signaling antagonist RP105 reduced expression levels of the pro-inflammatory cytokine genes *IL6* and *TNF α* in RAW264.7 cells in response to PM_{2.5} challenge (Supplementary Fig. 10B–D) [26], thus confirming the involvement of TLR4-mediated signaling in PM_{2.5}-triggered macrophage inflammation.

We previously showed that reactive oxygen species (ROS) production is required for PM_{2.5}-induced ER-stress response in macrophages [9]. To further delineate the mechanism underlying PM_{2.5}-triggered inflammation, we tested whether PM_{2.5}-triggered inflammation depended on intracellular ROS. ROS production from NADPH oxidase or mitochondria in macrophages was blocked by overexpressing dominant negative N17Rac1, the small GTPase component of NADPH oxidase, or manganese superoxide dismutase (Mn-SOD) using adenovirus-based expression system [9,27]. Upon PM_{2.5} challenge, expression levels of the genes encoding proinflammatory cytokines *IL-6* and *TNF α* were significantly reduced in RAW264.7 macrophages expressing dominant negative N17Rac1 or Mn-SOD (Supplementary Fig. 11). These results indicate that ROS produced through NADPH oxidase or mitochondria is critical for PM_{2.5}-triggered inflammation.

Discussion

In this study, we demonstrate that environmentally relevant PM_{2.5} exposure induces a “NASH-like” phenotype and alters glucose/insulin signaling pathways in the murine liver. This work extends our prior observations on the link between air pollution exposure and abnormalities in glucose homeostasis [1,6]. An

important finding in this study is the reduction of hepatic glycogen storage upon PM_{2.5} exposure (Fig. 2C–E). Given the central role of hepatic glycogen storage in whole body glucose homeostasis, PM_{2.5}-induced glucose intolerance and insulin resistance may be a direct consequence of hepatic glycogen depletion. Our study revealed that PM_{2.5} exposure suppressed the function of IRS1 in glycogen synthesis by increasing phosphorylation of IRS1 at residues Ser636 and Ser1101, which can impair IRS1-mediated insulin signaling through Akt and subsequent glycogen synthesis in the liver (Fig. 2G; Supplementary Fig. 4) [16]. Previously, we demonstrated impaired insulin signaling through Akt in skeletal muscle and adipose tissues of PM_{2.5}-exposed mice [1,28]. Therefore, impaired insulin signaling through IRS1-Akt in skeletal muscle, adipose tissue, and liver is likely crucial to insulin resistance and glucose intolerance observed in mice after PM_{2.5} exposure.

Our study demonstrated that exposure to PM_{2.5} for 10 weeks triggered inflammation pathways mediated through JNK-AP1, NF-κB, and TLR4 in the liver (Fig. 4; Supplementary Figs. 7–10). However, at the 3-week exposure stage, lung and liver inflammation has not been significantly elevated, although systemic inflammation, reflected by increased pro-inflammatory cytokine levels in the plasma, was observed (Supplementary Figs. 2 and 8). The organs/tissues vulnerable to PM_{2.5} exposure, such as blood vessels, circulating leukocytes, and adipose tissue [1,29], may contribute to systemic inflammation at the early stage of PM_{2.5} exposure. As PM_{2.5} exposure gets prolonged to 10 weeks, PM_{2.5} particles are delivered to the liver to activate Kupffer cells [9,10]. The presence of PM_{2.5} in the liver may act in synergy with systemic inflammation to exacerbate hepatic inflammation and dysregulation of lipid and glucose metabolism, an interesting question to be further investigated in the future.

The finding of PM_{2.5}-mediated repression of PPARγ and PPARα in the liver was quite unexpected (Fig. 3F). The reduction of PPARγ is consistent with PM_{2.5}-mediated JNK activation (Fig. 4D), which has been reported to downregulate transcriptional activity of PPARγ [30]. In the liver, PPARα is a key regulator of fatty acid oxidation and anti-inflammatory response, while PPARγ is required to prevent inflammation and to maintain lipid and glucose homeostasis in Kupffer cells and hepatocytes [20]. Downregulation of PPARα may be partially responsible for hepatic steatosis in PM_{2.5}-exposed animals due to reduction of fatty acid oxidation. Additionally, glycogen synthase, the rate-limiting enzymes for hepatic glycogen production, is a target of PPARα and PPARγ [31]. The decreased expression of hepatic PPARα and PPARγ may also contribute to the reduction of hepatic glycogen storage in PM_{2.5}-exposed mice (Fig. 2A and B). Together, downregulation of PPARα and PPARγ, upregulation of inflammatory responses, and impairment of IRS1 signaling in the liver are critical to the progression of hepatic steatosis, inflammation, as well as impaired glucose and insulin homeostasis in mice exposed to PM_{2.5} for 10 weeks.

Our findings need to be placed in the context of levels of air pollution observed in the US. Recent epidemiologic studies have linked air pollution to the development of type-2 diabetes [2–4]. Diabetes prevalence in the US increased with increasing PM_{2.5} concentrations, as evidenced by a 1% increase in diabetes prevalence seen with a 10 μg/m³ increase in PM_{2.5} exposure [4]. The relationship remained consistent even for counties within guidelines for EPA PM_{2.5} exposure limits. Interestingly, those with the highest exposure showed a >20% increase in dia-

betes prevalence compared with those with the lowest levels of PM_{2.5}, an association that persisted after controlling for diabetes risk factors [4]. In the US, 6 of the top 25 cities considered most polluted as reported by the American Lung Association from 2007 to 2011 (www.stateoftheair.org/2011/city-rankings) are from the Midwestern region (Pittsburgh, PA; Cincinnati, OH; Cleveland, OH; Detroit, MI; Indianapolis, IN; Chicago, IL). Columbus, where our animals were exposed to PM_{2.5}, is a regional representative of Midwest source of air pollution in the US (Supplementary Fig. 1). The results from our study are consistent with maladaptive responses caused by air pollutants that may interact with other risk factors in facilitating whole body metabolic impairment. These findings significantly contribute to our understanding of air pollution-associated systemic disease, especially in the context of the burgeoning national and international epidemic of metabolic disease.

Financial support

Portions of this work were supported by National Institutes of Health (NIH) Grants DK090313 and ES017829 to KZ, American Heart Association Grants 0635423Z and 09GRNT2280479 to KZ, NIH Grants ES016588, ES017412, and ES018900 to QS, and NIH grants R01ES019616, R01ES017290, and R01ES015146 to SR.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

The underlying research reported in the study was funded by the NIH Institutes of Health.

Acknowledgements

We thank Dr. Scott Friedman for providing LX-2 cells and Dr. Michael Tainky for providing AP-1 reporter plasmids.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhep.2012.08.009>.

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