PM$_{2.5}$ promotes β cell damage by increasing inflammatory factors in mice with streptozotocin

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Abstract. Emerging evidence indicates that exposure to fine particulate matter contributes to the onset of diabetes. The present study aimed to investigate the mechanism of particulate matters (PM)$_{2.5}$ affecting glucose homeostasis in mice with type 1 diabetes mellitus. Male C57BL/6 mice were housed under filtered air (FA) or PM$_{2.5}$ for 12 weeks and then received intraperitoneal injection of streptozotocin (STZ; 40 mg/kg) or acetate buffer daily for 5 days. At 4 weeks after the last injection, fasting glucose was tested. In the plasma and liver, cholesterol levels were determined by cholesterol oxidase-peroxidase and triglyceride levels were determined by triglycerophosphate oxidase-peroxidase. Homeostasis model assessment of β cell function (Homa-β) was computed based on fasting insulin and glucose levels. Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) levels in plasma, visceral adipose tissues, RAW264.7 macrophages and MIN6 pancreatic β cells treated with PM$_{2.5}$ (0-50 µg/ml) were quantified via ELISA. Before STZ injection, fasting blood glucose (FBG) levels were similar between FA and PM$_{2.5}$ groups. After STZ injection, FBG levels were higher in mice pre-exposed to PM$_{2.5}$ compared with those pre-exposed to FA. When taking FBG levels ≥7 mmol/l as the criteria for impaired glucose level, its incidence was 53.3% and 77.8% in FA and PM$_{2.5}$ groups, respectively. Independent of STZ injection, IL-1β levels in the adipose tissue were upregulated in mice pre-exposed to PM$_{2.5}$ compared with FA. The addition of PM$_{2.5}$ stimulated IL-1β and TNFα production in macrophages and pancreatic β cells, and inhibited the secretion of insulin from MIN6 cells in a dose-dependent manner. In conclusion, pre-exposure of PM$_{2.5}$ impaired pancreatic β cells in mice upon STZ injection, partially via enhanced inflammation, and suppressed the secretion of insulin.

Introduction

Diabetes mellitus prevalence is increasing and becoming a common health problem worldwide; according to the report from The International Diabetes Federation, there were an estimated 382 million individuals with diabetes in 2013 and the number may rise to 592 million by 2035 worldwide (1). Similarly, the prevalence of diabetes mellitus in China was 5.5% in 2001 and increased to 10.9% in 2013 (2). The early stages of type 1 diabetes mellitus (T1DM) are characterized by local autoimmune inflammation and progressive loss of insulin-producing pancreatic β cells, and β cells can respond to a pro-inflammatory environment and remodeling of the regulatory landscape in T1DM (3). As β cells fail to produce adequate amounts of insulin for glucose homeostasis, patients develop hyperglycemia (4). Thus, there is a need to determine how to repair impaired β cells in order to prevent diabetes progression.

Among all etiological factors that contribute to the onset of diabetes, air pollution has received considerable attention (5-7). Airborne particulate matters (PM) consist of airborne solid particles and liquid droplets (8). PM with a diameter <10 µm can deposit in the tracheobronchial tree (9), PM with a diameter <2.5 µm, termed PM$_{2.5}$, easily move down into the alveoli and enter the circulatory system (8). Epidemiological evidence has illustrated the positive association between ambient PM$_{2.5}$ and the extent of inflammation (10,11), as well as the incidence of metabolic syndrome (12) and type 2 diabetes (13), in cross-sectional studies. In separate cohort studies, long-term exposure of PM$_{2.5}$ increased the risk of diabetes in cohorts after 5.1 or 16 years of follow-up (12,14), indicating a link between PM$_{2.5}$ and incidence of diabetes.
When PM$_{2.5}$ particles processed from the atmosphere are added to cultivated macrophages, they activate Toll-like receptor (TLR)2 and TLR4 signaling pathways, stimulating NF-κB transcription, and leading to interleukin-1β (IL-1β) and cyclooxygenase-2 production (15). In vivo, PM$_{2.5}$ exposure in combination with a sustained high-fat diet enhances the infiltration of macrophages into adipose tissues and promotes tumor necrosis factor-α (TNFα) production in wild-type mice (16). Exposure to PM$_{2.5}$ alone does not alter fasting blood glucose (FBG) levels despite increased macrophage infiltration into adipose tissues in wild-type mice (17). Environmental factors are involved in the development of T1DM, providing an opportunity to detect and prevent further autoimmune destruction of β cells via therapeutic intervention (18). As the role of inflammation in glucose homeostasis and β cell destruction has been established (19-21), it was hypothesized that mice with pre-exposure to PM$_{2.5}$ may be prone to greater impairments in glucose tolerance and β cell function when challenged with diabetic triggers. Therefore, in the present study, wild-type mice were pre-exposed to an ambient PM$_{2.5}$ environment for 12 weeks and then administered an intraperitoneal streptozotocin (STZ) injection.

Materials and methods

Mice and treatment. All the animal experiments were approved by the Animal Care and Use Committee of Luhe Hospital, Capital Medical University (2020 LH-KS-020; Beijing, China) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (22). In the present study, 8-week-old male C57BL/6 mice (n=51; weight, 25-30 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.

Mice were randomly exposed to ambient PM$_{2.5}$ (n=27) or filtered air (FA; n=24) from November 2016 to February 2017. PM$_{2.5}$ exposure (14-289 µg/m$^3$) was performed using a ‘real-world’ versatile aerosol concentration enrichment system in Tongzhou District, Beijing, China as described by Sioutas et al (23) and further modified by Chen et al (24). During the exposure time, the dynamic daily concentration of ambient PM$_{2.5}$ was monitored using an individual particle monitor (pDR1500; Thermo Fisher Scientific, Inc.). The FA mice were exposed to an identical environment with the exception of a highly efficient particulate air filter positioned in the inlet valve to remove PM$_{2.5}$ in the air stream. The mice in the exposure chamber were fed regular chow and distilled water ad libitum, and raised under suitable temperature (22±2°C) and relative humidity (40-60%) conditions with a 12:12-h light/dark cycle.

At 12 weeks after exposure, 18 mice in the PM$_{2.5}$ group and 15 mice in the FA group were intraintraperitoneally injected with STZ (40 mg/kg) in acetic buffer daily for 5 consecutive days. The rest of mice received an equivalent volume of the acetic buffer. At 4th week after the last injection, mice were weighed and plasma samples were collected from the tail and reading by Accu-check Performa glucometer (Roche Diagnostics) followed fasting overnight. To further dissect the impact of PM$_{2.5}$ on the features of diabetic mice, STZ-injected mice with FBG levels ≥7 mmol/l were investigated in the following analysis (STZ-treated with FA, n=8; STZ-treated with PM$_{2.5}$, n=14; acetic-treated with FA, n=9; and acetic-treated with PM$_{2.5}$, n=9). All mice were euthanized via an intraperitoneal injection of 150 mg/kg pentobarbital, blood samples (1 ml) were collected from the inferior vena cava after euthanasia, and then fat and liver tissues were dissected and stored at -80°C. Death was confirmed based on the absence of heart beat, breathing and reflexes. Blood samples were centrifuged at 1,000 x g at 4°C for 10 min, and the plasma was collected for further analysis.

PM$_{2.5}$ particle preparation. PM$_{2.5}$ samples were collected regularly in Tongzhou (Beijing, China) between November 2016 and February 2017. As described previously by Imrich et al (25), PM$_{2.5}$ samples were collected on Teflon filters (diameter, 47 mm; Whatman plc; Cytiva), then particles were isolated by putting the Teflon filters into 50-ml centrifuge tubes and probe-sonicating for 2 min at 40 kHz at room temperature in ultrapure water before drying the filters in a drying oven. The final concentration of the PM$_{2.5}$ particle extracts was 5 mg/ml. PM$_{2.5}$ particles were stored at -80°C for cell experiments.

Lipid measurement. Liver samples were homogenized using lysis buffer (cat. no. C1053; Applygen Technologies, Inc.) and quantified by BCA (Thermo Fisher Scientific, Inc.). Liver and plasma levels of triglyceride (cat. no. 0220) and cholesterol (cat. no. 0180) were measured using reagent kits (Biosino Bio-Technology & Science Inc.) by triglycerophosphate oxidase-peroxidase and cholesterol oxidase-peroxidase according to the manufacturer's instructions.

ELISA. The levels of IL-1β (cat. no. 432604) and TNFα (cat. no. 430904) in plasma, protein extracts from adipose tissues and cell media were determined via ELISA according to the manufacturer's instructions (both BioLegend, Inc.) and calculated as previously described (26). The levels of insulin in plasma and cell supernatant were evaluated via ELISA using a kit (cat. no. EZRMI-13K; EMD Millipore) according to the manufacturer's instructions.

Assessment of β cell function. Homeostasis model assessment of β cell function (Homa-β) was performed using fasting insulin and glucose levels according to the formula: Homa-β = [(360 x insulin level)(glucose (mg/dl - 63)] (27).

Cell culture and treatment. The mouse macrophage cell line RAW264.7 was purchased from American Type Culture Collection and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS; HyClone; Cytiva) and 1% penicillin-streptomycin. The mouse pancreatic β cell line MIN6 was a gift from Professor Yang (Peking University Health Science Center, Beijing, China) and cultured in DMEM (cat. no. C11995500BT; Gibco; Thermo Fisher Scientific, Inc.), supplemented with 15% FBS, 1% penicillin-streptomycin and 5 µl/l β-mercaptoethanol. Cells were maintained in an incubator at 37°C with 5% CO$_2$.

After reaching 100% confluence, cells were treated with PM$_{2.5}$ particles (0, 0.5, 5 or 50 µg/ml) for 24 h. After harvesting, the cell and the supernatant were collected at 10,000 x g at 4°C for 3 min. For insulin secretion, the MIN6 cell were
treated with PM$_{2.5}$ for 24 h at 37˚C with 5% CO$_2$, followed by one wash with prewarmed Krebs-Ringer bicarbonate buffer (KRB; Coolaber) without glucose, and then incubation in KRB without glucose at 37˚C with 5% CO$_2$ for 1 h. Cells were then incubated in KRB with 0, 2.5 or 20 mmol/l glucose for 1 h at 37˚C. After incubation, the supernatant was collected for ELISA.

Protein extraction. Proteins were extracted from adipose tissues using lysis buffer (cat. no. C1053; Applygen Technologies, Inc.) in a ratio of 1:1:100 (protein inhibitor: PMSF: RIPA lysis buffer, respectively) and then calibrated to a consistent concentration using a BCA protein assay kit (Thermo Fisher Scientific, Inc.) before being subjected to ELISA.

Statistical analysis. Data were expressed as the mean ± SEM. All cell experiments were repeated three times. In PM$_{2.5}$-treated cell experiments, one-way ANOVA followed by Dunnett’s post hoc test was used for data with normal distribution. When there were more than two experimental treatments, two-way ANOVA followed by Sidak’s was used by comparing treated groups with the control. Both WHO$_{1999}$ and ADA$_{2003}$ recommend cut points for IGT as 7.8-11.0 mmol/l measured at the 2 h time point of an OGTT (28). Since the T1DM model was used in the present study, FBG levels ≥7 mmol/l were used as the criteria for impaired glucose level. The incidence of mice with impaired glucose level was analyzed by Fisher’s exact test, using FBG levels ≥7 mmol/l as the criteria for impaired glucose level. P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using GraphPad Prism version 7.00 (GraphPad Software, Inc.).

Results

Ambient PM$_{2.5}$ levels in the exposure period. Throughout the study, mice were exposed to either FA or PM$_{2.5}$ in a ‘real-world’ ambient PM$_{2.5}$ exposure system. During the exposure time, the dynamic daily concentration of ambient PM$_{2.5}$ was monitored using an individual particle monitor. The average daily concentration of ambient PM$_{2.5}$ prior to STZ injection was presented in Fig. 1. The mean daily concentration of ambient PM$_{2.5}$, prior to streptozotocin injection is present. The red dotted line represents the average level of PM$_{2.5}$ (103.27 µg/m$^3$) during the exposure time and the blue dotted line represents the average concentration of PM$_{2.5}$ based on the NAAQS of Beijing (118.08 µg/m$^3$). PM$_{2.5}$, particulate matters, diameter <2.5 µm; NAAQS, National Ambient Air Quality Standard.

Figure 1. Concentration of ambient PM$_{2.5}$ during the exposure time. The mean daily concentration of ambient PM$_{2.5}$ prior to streptozotocin injection is present. The red dotted line represents the average level of PM$_{2.5}$ (103.27 µg/m$^3$) during the exposure time and the blue dotted line represents the average concentration of PM$_{2.5}$ based on the NAAQS of Beijing (118.08 µg/m$^3$). PM$_{2.5}$, particulate matters, diameter <2.5 µm; NAAQS, National Ambient Air Quality Standard.

Effects of PM$_{2.5}$ exposure on glucose metabolism. After 12 weeks of FA or PM$_{2.5}$ exposure, mice were injected with STZ (40 mg/kg) or an equivalent dose of acetic buffer for 5 consecutive days. (A) At 4 weeks after the last injection, mice were fasted overnight for the fasting blood glucose test. (B) Insulin levels in the plasma were quantified via ELISA. (C) Homa-β. Data are presented as the mean ± SEM (control with FA, n=8; control with PM$_{2.5}$, n=8; STZ-treated with FA, n=8; STZ-treated with PM$_{2.5}$, n=14). FA, filtered air; PM$_{2.5}$, particulate matters, diameter <2.5 µm; STZ, streptozotocin; Homa-β, homeostasis model assessment of β cell function.

Figure 2. Effects of PM$_{2.5}$ exposure on glucose metabolism. After 12 weeks of FA or PM$_{2.5}$ exposure, mice were injected with STZ (40 mg/kg) or an equivalent dose of acetic buffer for 5 consecutive days. (A) At 4 weeks after the last injection, mice were fasted overnight for the fasting blood glucose test. (B) Insulin levels in the plasma were quantified via ELISA. (C) Homa-β. Data are presented as the mean ± SEM (control with FA, n=8; control with PM$_{2.5}$, n=8; STZ-treated with FA, n=8; STZ-treated with PM$_{2.5}$, n=14). FA, filtered air; PM$_{2.5}$, particulate matters, diameter <2.5 µm; STZ, streptozotocin; Homa-β, homeostasis model assessment of β cell function.
no significant difference was observed. To further dissect the impact of PM$_{2.5}$ on the features of diabetic mice, STZ-injected mice with FBG levels $\geq 7$ mmol/l were investigated in the following analysis (STZ-treated with FA, $n=8$; STZ-treated with PM$_{2.5}$, $n=14$). ELISA revealed that pre-exposure to PM$_{2.5}$ significantly reduced fasting insulin levels in mice after STZ injection; meanwhile, pre-exposure to PM$_{2.5}$ also down-regulated the insulin level; however, there was no significant difference in acetic buffer groups (Fig. 2B). Homa-$\beta$ revealed that STZ injection similarly reduced $\beta$ cell function in FA and PM$_{2.5}$ mice (Fig. 2C). These data suggested that PM$_{2.5}$ exposure may contribute to the impairments in glucose metabolism and insulin levels after STZ injection.

**Effects of PM$_{2.5}$ pre-exposure on body weight and lipid profile.** Compared with mice injected with acetic buffer, mice receiving STZ treatment exhibited significantly reduced body weights in the PM$_{2.5}$ group (Fig. 3A). Compared with the FA group injected with acetic buffer, mice exposed to PM$_{2.5}$ and STZ injection exhibited significantly elevated cholesterol levels in the plasma and liver (Fig. 3B and C). In the plasma, STZ injection significantly increased cholesterol levels in mice when exposed to PM$_{2.5}$ (Fig. 3B). Furthermore, pre-exposure to PM$_{2.5}$ increased both the plasma and liver cholesterol levels compared with FA and STZ injection (Fig. 3B and C).

For triglyceride profiles, compared with the FA injected with acetic buffer group, mice exposed to PM$_{2.5}$ and STZ injection exhibited significantly elevated triglyceride levels in the plasma and liver (Fig. 3D and E). In plasma, STZ injection increased triglyceride levels in the FA group (Fig. 3D); and in the liver, STZ injection increased triglyceride levels in the PM$_{2.5}$ group (Fig. 3E). These findings indicated that PM$_{2.5}$ exposure combined with STZ may affect lipid metabolism.

**Effects of PM$_{2.5}$ pre-exposure on inflammation.** Accumulating evidence suggests that IL-1$\beta$ and TNF$\alpha$ are involved in the incidence and progression of diabetes (30). ELISA revealed that PM$_{2.5}$ exposure may increase IL-1$\beta$ levels compared with FA exposure in the plasma of STZ-treated mice, whilst STZ exposure also elevated IL-1$\beta$ levels compared with the
control; however, there was no statistically significant difference (Fig. 4A). TNFα levels in plasma were elevated in mice in the PM₂.₅ group compared with the FA group (Fig. 4B). As adipose tissues are the main source of IL-1β and TNFα (31), the adipose tissues of mice were dissected for protein extraction. Compared with the FA group, PM₂.₅ exposure significantly increased IL-1β production in both acetic buffer- and STZ-treated adipose tissues (Fig. 4C); however, STZ significantly upregulated TNFα production in adipose tissues compared with the control independent of FA or PM₂.₅ exposure (Fig. 4D). These data suggested that PM₂.₅ pre-exposure increased inflammation in mice treated with STZ in adipose tissue.

**PM₂.₅ increases inflammation and decreases glucose-induced insulin secretion.** Cytokines appear to be major regulators of adipose tissue metabolism, especially TNFα and IL-1β (32). The density of adipose tissue macrophages is associated with adipose tissue inflammatory markers and insulin resistance, and inflammation has been described as causally related to decreased insulin secretion from β-cells (33,34). Pancreatic β cells are the main source of insulin. Therefore, the effects of PM₂.₅ on IL-1β and TNFα production in macrophages and pancreatic β cells, as well as pancreatic β cell insulin secretion, were evaluated. Mouse RAW264.7 macrophage cells and mouse MIN6 pancreatic β cells were treated with PM₂.₅ at different concentrations (0-50 µg/ml) for 24 h, and the supernatant was collected. ELISA revealed that both RAW264.7 and MIN6 cells significantly increased release of TNFα and IL-1β levels following PM₂.₅ exposure in a dose-dependent manner (Fig. 5A-D). Furthermore, PM₂.₅ exposure significantly decreased insulin secretion in response to 2.5 and 20 mM glucose (Fig. 5E). These findings suggested that PM₂.₅ exposure may aggravate β cell damage by increasing inflammation and inhibiting insulin secretion.

**Discussion**

In the present study, the effect of pre-exposure to PM₂.₅ on β cell function in mice challenged with a diabetic trigger was investigated. The major findings are summarized as follows: i) When pre-exposed to PM₂.₅, there was a non-significant trend towards mice developing diabetes following STZ injection based on increased numbers of animals with IGT; ii) in STZ-injected mice, exposure to PM₂.₅ decreased the level of insulin, and elevated cholesterol levels in the blood, and cholesterol and triglyceride contents in the liver; iii) exposure to PM₂.₅ stimulated inflammation in the present study by increasing TNFα and IL-1β levels in macrophages and pancreatic β cells; and iv) exposure to PM₂.₅ decreased glucose-induced insulin secretory function. Collectively, the present study indicated that pre-exposure to PM₂.₅ may accelerate impairments to β cells upon STZ injection, which may be partially mediated via increased inflammation.

Studies have reported positive associations between PM10 or PM₂.₅ exposure and cardiovascular injury (35), atherosclerosis (36) and ischemic stroke (37). Consistent with these findings, exposure to PM₂.₅ increased diabetic prevalence in pregnant women (38), general populations (12,39) or elderly individuals (40). Aside from increased diabetic prevalence, long-term exposure to PM₁₀ is associated with hyperglycemia, as reflected by FBG and hemoglobin A1c levels, and insulin...
resistance (IR), as determined by the Homa-IR index, in patients with diabetes (41).

To explore the mechanism underlying the effects of PM$_{2.5}$ exposure on diabetes prevalence, mouse and rat models have been frequently used; in these models, animals are either exposed to PM$_{2.5}$ alone or simultaneously in combination with a high-fat diet (42,43). Studies have found that PM$_{2.5}$ is taken up by macrophages via TLR2 and TLR4 (44,45). Downstream of TLR2 and TLR4, adaptor protein myeloid differentiation primary response 88 mediates inflammatory responses and

Figure 5. PM$_{2.5}$ treatment stimulates inflammation and decreases glucose-induced insulin secretion. Mouse RAW264.7 macrophages and MIN6 β cells were treated with PM$_{2.5}$ (0-50 µg/ml). (A) IL-1β and (B) TNFα levels in the supernatant of RAW264.7 cells were quantified via ELISA. (C) IL-1β and (D) TNFα levels in the supernatant of MIN6 cells were quantified via ELISA. (E) Insulin levels in the supernatant of MIN6 cells following glucose treatment were analyzed via ELISA. Data are presented as the mean ± SEM (n=3). PM$_{2.5}$, particulate matters, diameter <2.5 µm; IL-1β, interleukin-1β; TNFα, tumor necrosis factor-α.
activation of NF-κB transcription, both of which contribute to inflammatory cytokine production in alveolar and peripheral blood (46,47). Consistently, activation of TLR4/JNK-induced inflammation has been reported to result in impaired insulin secretion and apoptosis in MIN6 cells (48,49). Following circulation, these inflammatory cytokines induce endoplasmic reticulum stress and deteriorate brown adipocyte activity by reducing uncoupling protein 1 expression in brown adipose tissues (50), exaggerate endothelial cell dysfunction (47) and promote macrophage infiltration into fat tissues (16). Additionally, PM$_{2.5}$ exposure stimulates the transition of M2 macrophages into M1 macrophages and increases Th1/Th17 cell number in peripheral tissues (45,51), which further reinforces inflammation and IR. However, whether long-term PM$_{2.5}$ exposure prior to a diabetic trigger such as STZ injection could enhance IGT and IR has not been established. Therefore, the present study was performed to investigate this, and the findings validated the hypothesis that pre-exposure to PM$_{2.5}$ promoted the onset of impaired glucose level in mice challenged with STZ. However, the observed reduction in insulin levels was comparable between STZ-injected mice pre-exposed to FA or PM$_{2.5}$, whereas Homa-β was comparable between acetic buffer and STZ groups. Therefore, it appears that the adverse effect of PM$_{2.5}$ was primarily in relation to insulin levels rather than insulin sufficiency.

The present study subsequently investigated how PM$_{2.5}$ may participate in the progression of impaired β cell function. Despite functioning via different mechanisms, IL-1β and TNFα signaling interferes with insulin signaling pathways, thus serving as fundamental pathogenic factors underlying IR (52). IL-1β downregulates insulin substrate receptor-1 (IRS-1) expression in adipocytes and hepatocytes (19,20), whereas TNFα induces JNK phosphorylation that phosphorylates IRS-1 at Ser307 (53,54). Decreased IRS-1 protein expression and phosphorylation abrogate insulin signal transduction in peripheral cells (55). In addition, TNFα inhibits glucose transport in adipocytes (55). In the present study, increased IL-1β and TNFα production was observed in adipose tissues of mice exposed to PM$_{2.5}$ and STZ injection. Macrophages and β cells were hypothesized to be the sources of increased IL-1β and TNFα production, as release of both factors was increased in both cell types when treated with PM$_{2.5}$ particles. Following STZ injection, insulin levels were significantly decreased in PM$_{2.5}$-exposed mice, but the levels of IL-1β in the peripheral blood did not increase further in PM$_{2.5}$-exposed mice compared with FA-exposed ones. By contrast, IL-1β and TNFα expression were increased in adipose tissues from PM$_{2.5}$-exposed mice compared with mice in the FA group following STZ injection, indicating enhanced inflammation by PM$_{2.5}$ exposure and STZ in vivo. Subsequently, RAW264.7 and MIN6 cells were treated with PM$_{2.5}$ particles, and the levels of IL-1β and TNFα were significantly upregulated in a dose-dependent manner. The secretion of insulin from MIN6 cells was similarly decreased following PM$_{2.5}$-exposure.

The present study must be interpreted in the context of some potential limitations. First, macrophages are critical mediators in adipose tissues (56). The kinetics of IL-1β and TNFα production in macrophages residing in adipose tissues could not be evaluated in vivo. Instead, the release of inflammatory cytokines was studied at only one timepoint (24 h) in RAW264.7 and MIN6 mouse cell lines. Second, when compared with FA injected with acetic buffer, liver cholesterol and triglyceride levels demonstrated no significant change in FA injected with STZ. However, whether and how increased cholesterol and triglyceride in mice could facilitate impairment of glucose levels was not studied, as it was not the main goal of the present study. Further experimental verification and in-depth exploration will be conducted in our subsequent research, including investigation of the TLR4/JNK signaling pathway in PM$_{2.5}$-induced β cell injury.

In conclusion, the present study indicated that pre-exposure of PM$_{2.5}$ impaired pancreatic β cells in mice upon STZ injection, which may be partially mediated via increased IL-1β and TNFα production in macrophages and adipose tissues.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YM conceived the study. BZ analyzed the data. RY and JL acquired and interpreted the data. LY was a major contributor in writing the manuscript, revised it critically for important intellectual content and analyzed the data from cell experiments. DZ acquired the cell data and gave approval of the final version to be published. LY, RY and DZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the animal experiments were approved by the Animal Care and Use Committee of Luhe Hospital, Capital Medical University (Beijing, China).

Patient consent for publication

Not applicable.
Competing interests
The authors declare that they have no competing interests.

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