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

BAOYU ZHANG[*], RUILI YIN[*], JIANAN LANG, LONGYAN YANG, DONG ZHAO and YAN MA

Beijing Key Laboratory of Diabetes Prevention and Research, Centre for Endocrine Metabolic and
Immune Disease, Luhe Hospital, Capital Medical University, Beijing 101149, P.R. China

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 acetic 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 I 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.

Correspondence to: Professor Dong Zhao or Dr Yan Ma, Beijing Key Laboratory of Diabetes Prevention and Research, Centre for Endocrine Metabolic and Immune Disease, Luhe Hospital, Capital Medical University, 82 Xinhua South Road, Tongzhou, Beijing 101149, P.R. China
E-mail: zhaodong@ccmu.edu.cn
E-mail: my0320@163.com

[*]Contributed equally

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/m3) 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 intraperitoneally 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 ultra-pure 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
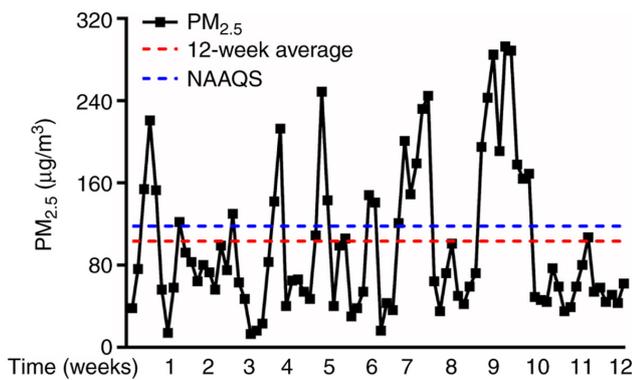
Figure 1. Concentration of ambient $PM_{2.5}$ during the exposure time. The mean daily concentration of ambient $PM_{2.5}$ prior to streptozotocin injection is present. The red dotted line represents the average level of $PM_{2.5}$ (103.27 $\mu g/m^3$) during the exposure time and the blue dotted line represents the average concentration of $PM_{2.5}$ based on the NAAQS of Beijing (118.08 $\mu g/m^3$). $PM_{2.5}$, particulate matters, diameter <2.5 μm; NAAQS, National Ambient Air Quality Standard.

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
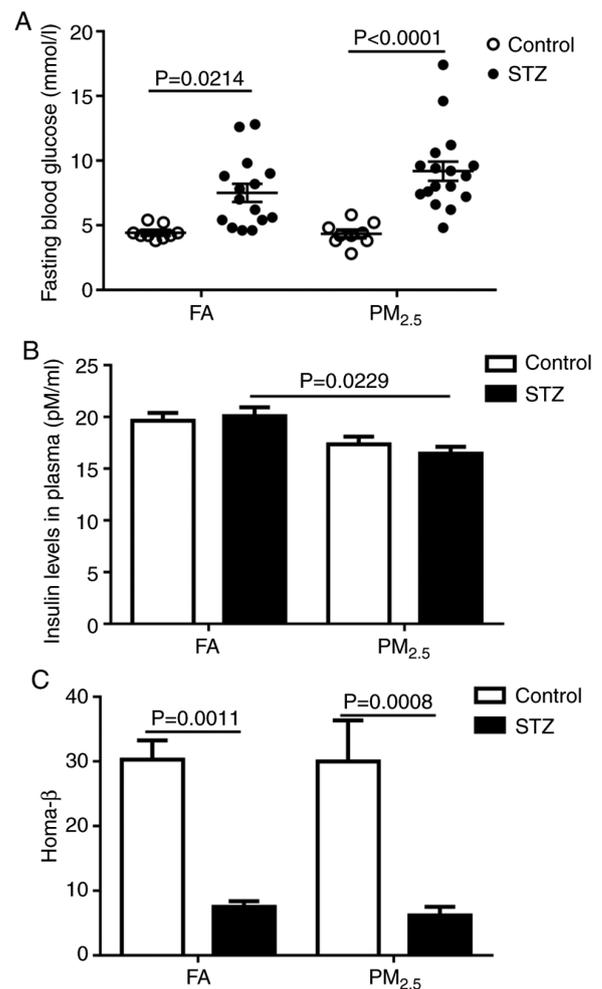
Figure 2. Effects of $PM_{2.5}$ exposure on glucose metabolism. After 12 weeks of FA or $PM_{2.5}$ exposure, mice were injected with STZ (40 mg/kg) or an equivalent dose of acetic buffer for 5 consecutive days. (A) At 4 weeks after the last injection, mice were fasted overnight for the fasting blood 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.

concentration of ambient $PM_{2.5}$ prior to STZ injection was presented in Fig. 1. Across the study, the average $PM_{2.5}$ levels of the Beijing National Ambient Air Quality Standard and in the exposure system were 118.08 $\mu g/m^3$ and 103.27 $\mu g/m^3$, respectively. This result showed that it is ~1.1-fold lower than the average $PM_{2.5}$ National Ambient Air Quality Standard in China (29).

Effects of $PM_{2.5}$ pre-exposure on FBG and insulin levels. $PM_{2.5}$ exposure did not increase FBG levels compared with the FA group. Additionally, at 4 weeks after STZ injection, there was no significant difference in FBG levels between mice pre-exposed to $PM_{2.5}$ and those in the FA group (Fig. 2A). Using FBG levels ≥7 mmol/l as the criteria for impaired glucose level, the incidence of impaired glucose level upon STZ injection was 53.3% (8/15) in the FA group and 77.8% (14/18) in the $PM_{2.5}$ group, although this was not significant (data not shown). The data suggested that mice pre-exposed to $PM_{2.5}$ may be prone to developing impaired glucose metabolism; however,
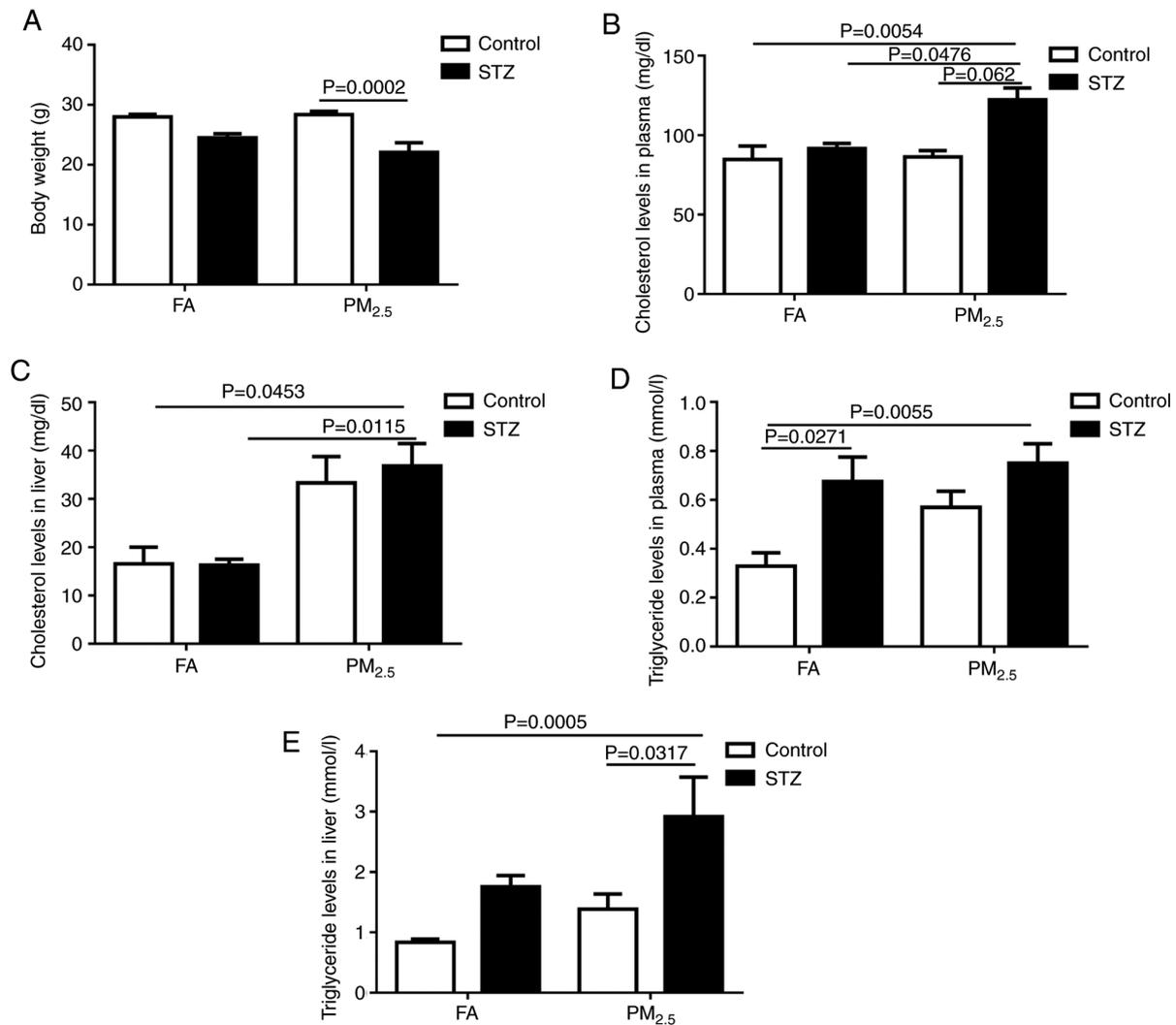
Figure 3. Effects of PM$_{2.5}$ on body weight and lipid profile. After 12 weeks of FA or PM$_{2.5}$ exposure, mice were injected with STZ (40 mg/kg) or an equivalent dose of acetic buffer for 5 consecutive days. After 4 weeks, after overnight fasting, (A) body weights and cholesterol levels in the (B) plasma and (C) liver were obtained. Triglyceride levels in the (D) plasma and (E) liver were also measured. Data are presented as the mean ± SEM (n=8-14). FA, filtered air; PM$_{2.5}$, particulate matters, diameter <2.5 μm; STZ, streptozotocin.

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 ≥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-β revealed that STZ injection similarly reduced β 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-1β and TNFα are involved in the incidence and progression of diabetes (30). ELISA revealed that PM$_{2.5}$ exposure may increase IL-1β levels compared with FA exposure in the plasma of STZ-treated mice, whilst STZ exposure also elevated IL-1β levels compared with the
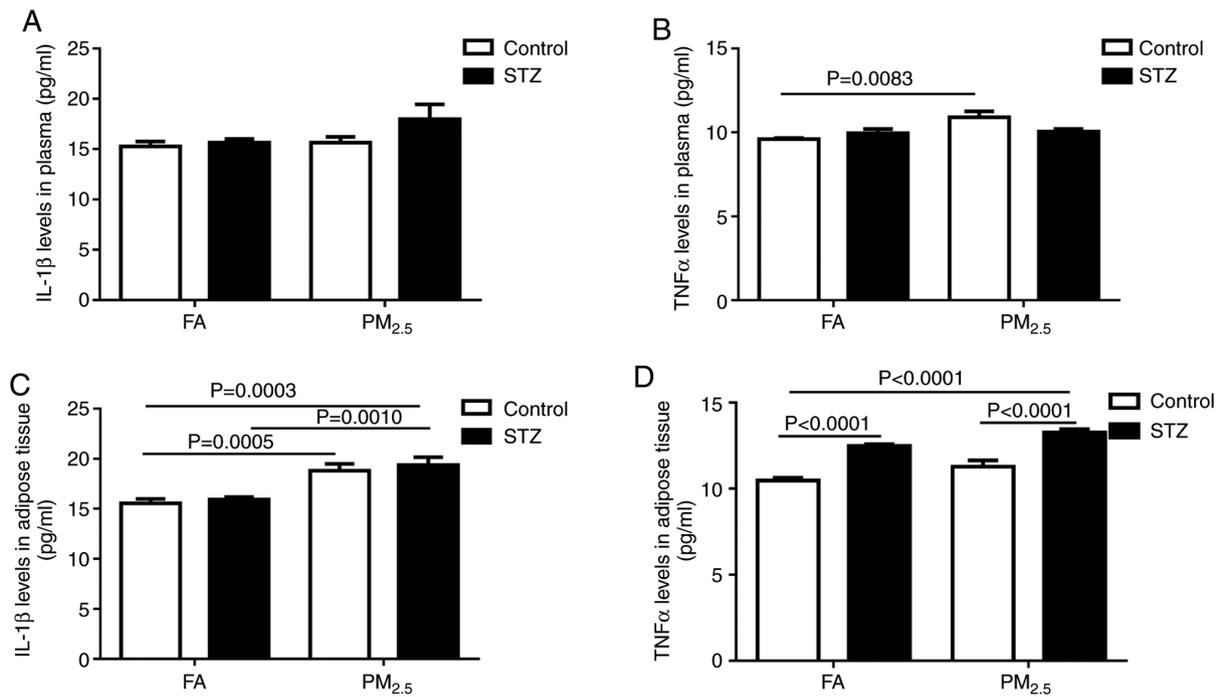
Figure 4. PM$_{2.5}$ exposure affects inflammatory responses in plasma and adipose tissues. After 12 weeks of FA or PM$_{2.5}$ exposure, mice were injected with STZ (40 mg/kg) or an equivalent dose of acetic buffer for 5 consecutive days. Plasma levels of (A) IL-1β and (B) TNFα in mice after STZ injection. Expression of (C) IL-1β and (D) TNFα in adipose tissue protein homogenates after PM$_{2.5}$ exposure and STZ injection. 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; IL-1β, interleukin-1β; TNFα, tumor necrosis factor-α.

control; however, there was no statistically significant difference (Fig. 4A). TNFα levels in plasma were elevated in mice in the PM$_{2.5}$ 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, PM$_{2.5}$ 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 PM$_{2.5}$ exposure (Fig. 4D). These data suggested that PM$_{2.5}$ pre-exposure increased inflammation in mice treated with STZ in adipose tissue.

PM$_{2.5}$ 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 PM$_{2.5}$ 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 PM$_{2.5}$ 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 PM$_{2.5}$ exposure in a dose-dependent manner (Fig. 5A-D). Furthermore, PM$_{2.5}$ exposure

significantly decreased insulin secretion in response to 2.5 and 20 mM glucose (Fig. 5E). These findings suggested that PM$_{2.5}$ exposure may aggravate β cell damage by increasing inflammation and inhibiting insulin secretion.

Discussion

In the present study, the effect of pre-exposure to PM$_{2.5}$ 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 PM$_{2.5}$, 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 PM$_{2.5}$ decreased the level of insulin, and elevated cholesterol levels in the blood, and cholesterol and triglyceride contents in the liver; iii) exposure to PM$_{2.5}$ stimulated inflammation in the present study by increasing TNFα and IL-1β levels in macrophages and pancreatic β cells; and iv) exposure to PM$_{2.5}$ decreased glucose-induced insulin secretory function. Collectively, the present study indicated that pre-exposure to PM$_{2.5}$ 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 PM$_{2.5}$ exposure and cardiovascular injury (35), atherosclerosis (36) and ischemic stroke (37). Consistent with these findings, exposure to PM$_{2.5}$ increased diabetic prevalence in pregnant women (38), general populations (12,39) or elderly individuals (40). Aside from increased diabetic prevalence, long-term exposure to PM$_{10}$ is associated with hyperglycemia, as reflected by FBG and hemoglobin A1c levels, and insulin
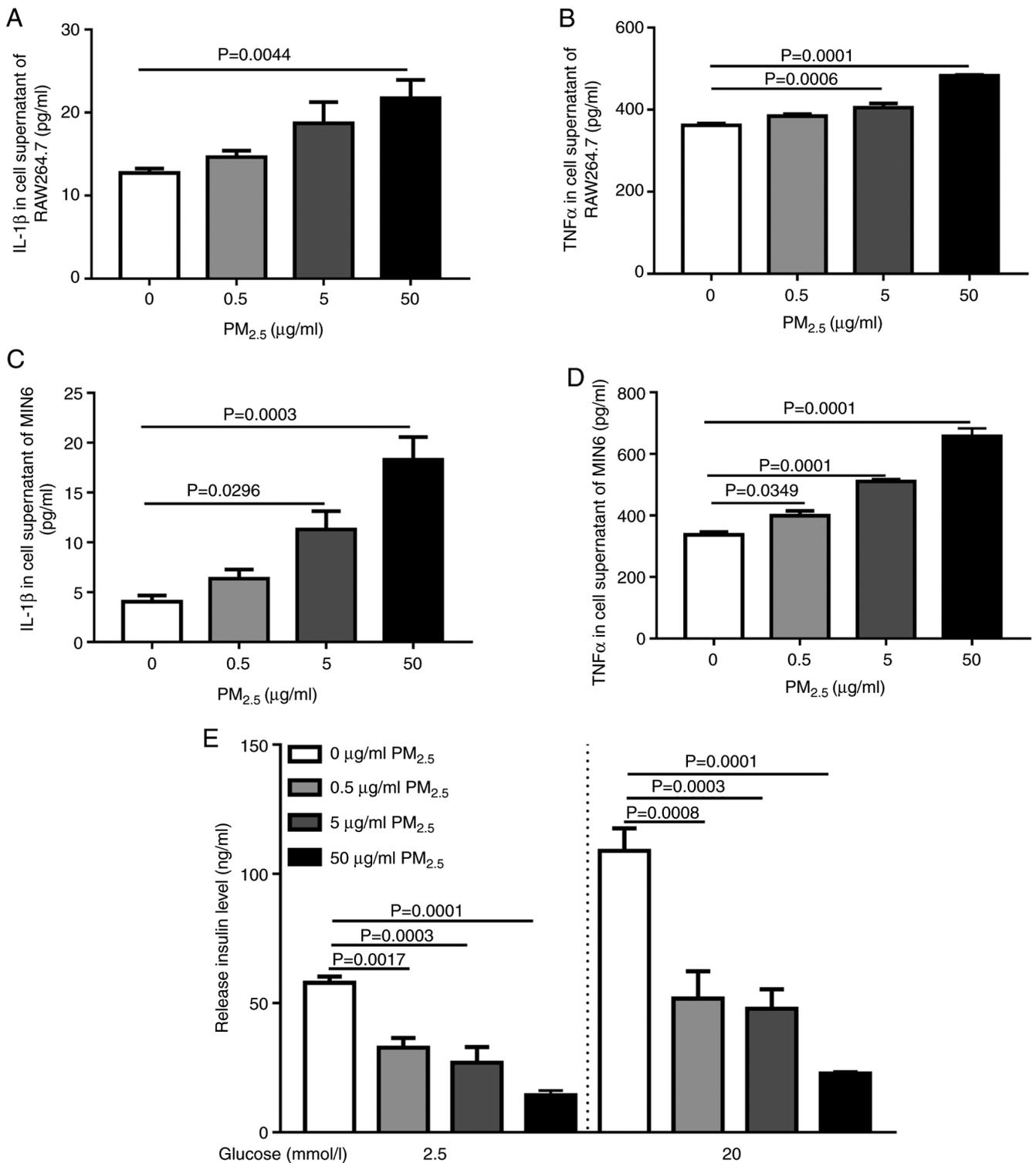
Figure 5. PM$_{2.5}$ treatment stimulates inflammation and decreases glucose-induced insulin secretion. Mouse RAW264.7 macrophages and MIN6 β cells were treated with PM$_{2.5}$ (0-50 µg/ml). (A) IL-1β and (B) TNFα levels in the supernatant of RAW264.7 cells were quantified via ELISA. (C) IL-1β and (D) 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.

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

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 monocyte 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.

Acknowledgements

Funding

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.

References

1. International Diabetes Federation: IDF Diabetes Atlas. 6th edition, 2013.
2. Hu C and Jia W: Diabetes in China: Epidemiology and genetic risk factors and their clinical utility in personalized medication. Diabetes 67: 3-11, 2018.
3. Ramos-Rodríguez M, Raurell-Vila H, Colli ML, Alvelos MI, Subirana-Granés M, Juan-Mateu J, Norris R, Turatsinze JV, Nakayasu ES, Webb-Robertson BM, *et al*: The impact of proinflammatory cytokines on the β-cell regulatory landscape provides insights into the genetics of type 1 diabetes. Nat Genet 51: 1588-1595, 2019.
4. Rorsman P and Ashcroft FM: Pancreatic β-cell electrical activity and insulin secretion: Of mice and men. Physiol Rev 98: 117-214, 2018.
5. Yang BY, Fan S, Thiering E, Seissler J, Nowak D, Dong GH and Heinrich J: Ambient air pollution and diabetes: A systematic review and meta-analysis. Environ Res 180: 108817, 2020.
6. Zhang H, Dong H, Ren M, Liang Q, Shen X, Wang Q, Yu L, Lin H, Luo Q, Chen W, *et al*: Ambient air pollution exposure and gestational diabetes mellitus in Guangzhou, China: A prospective cohort study. Sci Total Environ 699: 134390, 2020.
7. Renzi M, Cerza F, Gariazzo C, Agabiti N, Cascini S, Di Domenicantonio R, Davoli M, Forastiere F and Cesaroni G: Air pollution and occurrence of type 2 diabetes in a large cohort study. Environ Int 112: 68-76, 2018.
8. Nogueira JB: Air pollution and cardiovascular disease. Rev Port Cardiol 28: 715-733, 2009.
9. Kodros JK, Volckens J, Jathar SH and Pierce JR: Ambient particulate matter size distributions drive regional and global variability in particle deposition in the respiratory tract. Geohealth 2: 298-312, 2018.
10. Li Y, Sun B, Shi Y, Jiang J, Du Z, Chen R, Duan J and Sun Z: Subacute exposure of PM2.5 induces airway inflammation through inflammatory cell infiltration and cytokine expression in rats. Chemosphere 251: 126423, 2020.
11. Abramson MJ, Wigmann C, Altug H and Schikowski T: Ambient air pollution is associated with airway inflammation in older women: A nested cross-sectional analysis. BMJ Open Respir Res 7: 7, 2020.
12. Coogan PF, White LF, Yu J, Burnett RT, Seto E, Brook RD, Palmer JR, Rosenberg L and Jerrett M: PM2.5 and diabetes and hypertension incidence in the Black Women's Health Study. Epidemiology 27: 202-210, 2016.
13. Liu C, Yang C, Zhao Y, Ma Z, Bi J, Liu Y, Meng X, Wang Y, Cai J, Chen R, *et al*: Associations between long-term exposure to ambient particulate air pollution and type 2 diabetes prevalence, blood glucose and glycosylated hemoglobin levels in China. Environ Int 92-93: 416-421, 2016.
14. Weinmayr G, Hennig F, Fuks K, Nonnemacher M, Jakobs H, Möhlenkamp S, Erbel R, Jöckel KH, Hoffmann B and Moebus S; Heinz Nixdorf Recall Investigator Group: Long-term exposure to fine particulate matter and incidence of type 2 diabetes mellitus in a cohort study: Effects of total and traffic-specific air pollution. Environ Health 14: 53, 2015.
15. Bekki K, Ito T, Yoshida Y, He C, Arashidani K, He M, Sun G, Zeng Y, Sone H, Kunugita N, *et al*: PM2.5 collected in China causes inflammatory and oxidative stress responses in macrophages through the multiple pathways. Environ Toxicol Pharmacol 45: 362-369, 2016.
16. Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, Cai Y, Ostrowski MC, Lu B, Parthasarathy S, *et al*: Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 119: 538-546, 2009.
17. Liu C, Xu X, Bai Y, Zhong J, Wang A, Sun L, Kong L, Ying Z, Sun Q and Rajagopalan S: Particulate Air pollution mediated effects on insulin resistance in mice are independent of CCR2. Part Fibre Toxicol 14: 6, 2017.
18. Tomita T: Apoptosis of pancreatic β-cells in Type 1 diabetes. Bosn J Basic Med Sci 17: 183-193, 2017.
19. Fantuzzi G: Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 115: 911-919, quiz 920, 2005.
20. Jager J, Grémeaux T, Cormont M, Le Marchand-Brustel Y and Tanti JF: Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 148: 241-251, 2007.
21. Wasserman DH, Wang TJ and Brown NJ: The vasculature in prediabetes. Circ Res 122: 1135-1150, 2018.
22. National Research Council (US) Institute for Laboratory Animal Research: Guide for the Care and Use of Laboratory Animals. National Academies Press, Washington, DC, USA, 1996.
23. Sioutas C, Koutrakis P and Burton RM: A technique to expose animals to concentrated fine ambient aerosols. Environ Health Perspect 103: 172-177, 1995.
24. Chen LC and Nadziejko C: Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. V. CAPs exacerbate aortic plaque development in hyperlipidemic mice. Inhal Toxicol 17: 217-224, 2005.
25. Imrich A, Ning Y and Kobzik L: Insoluble components of concentrated air particles mediate alveolar macrophage responses in vitro. Toxicol Appl Pharmacol 167: 140-150, 2000.
26. Arhi CS, Bottle A, Burns EM, Clarke JM, Aylin P, Ziprin P and Darzi A: Comparison of cancer diagnosis recording between the Clinical Practice Research Datalink, Cancer Registry and Hospital Episodes Statistics. Cancer Epidemiol 57: 148-157, 2018.
27. Wallace TM, Levy JC and Matthews DR: Use and abuse of HOMA modeling. Diabetes Care 27: 1487-1495, 2004.
28. Yip WCY, Sequeira IR, Plank LD and Poppitt SD: Prevalence of Pre-Diabetes across Ethnicities: A review of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) for classification of dysglycaemia. Nutrients 9: 9, 2017.
29. Ma X, Jia H, Sha T, An J and Tian R: Spatial and seasonal characteristics of particulate matter and gaseous pollution in China: Implications for control policy. Environ Pollut 248: 421-428, 2019.
30. Carrero JA, McCarthy DP, Ferris ST, Wan X, Hu H, Zinselmeyer BH, Vomund AN and Unanue ER: Resident macrophages of pancreatic islets have a seminal role in the initiation of autoimmune diabetes of NOD mice. Proc Natl Acad Sci USA 114: E10418-E10427, 2017.
31. Rakotoarivelo V, Lacraz G, Mayhue M, Brown C, Rottembourg D, Fradette J, Ilangumaran S, Menendez A, Langlois MF and Ramanathan S: Inflammatory cytokine profiles in visceral and subcutaneous adipose tissues of obese patients undergoing bariatric surgery reveal lack of correlation with obesity or diabetes. EBioMedicine 30: 237-247, 2018.
32. Coppack SW: Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 60: 349-356, 2001.
33. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, Wabitsch M, O'Brien PE and Harrison LC: Pro-inflammatory CD11c$^+$CD206$^+$ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes 59: 1648-1656, 2010.
34. Biden TJ, Boslem E, Chu KY and Sue N: Lipotoxic endoplasmic reticulum stress, β cell failure, and type 2 diabetes mellitus. Trends Endocrinol Metab 25: 389-398, 2014.
35. Pope CA III, Bhatnagar A, McCracken JP, Abplanalp W, Conklin DJ and O'Toole T: Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Circ Res 119: 1204-1214, 2016.
36. Bai Y and Sun Q: Fine particulate matter air pollution and atherosclerosis: Mechanistic insights. Biochim Biophys Acta 1860: 2863-2868, 2016.
37. Tian Y, Xiang X, Wu Y, Cao Y, Song J, Sun K, Liu H and Hu Y: Fine particulate air pollution and first hospital admissions for ischemic stroke in Beijing, China. Sci Rep 7: 3897, 2017.
38. Fleisch AF, Gold DR, Rifas-Shiman SL, Koutrakis P, Schwartz JD, Kloog I, Melly S, Coull BA, Zanobetti A, Gillman MW, *et al*: Air pollution exposure and abnormal glucose tolerance during pregnancy: The project Viva cohort. Environ Health Perspect 122: 378-383, 2014.
39. Pearson JF, Bachireddy C, Shyamprasad S, Goldfine AB and Brownstein JS: Association between fine particulate matter and diabetes prevalence in the U.S. Diabetes Care 33: 2196-2201, 2010.
40. Chuang KJ, Yan YH, Chiu SY and Cheng TJ: Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68: 64-68, 2011.

41. Khafaie MA, Salvi SS, Ojha A, Khafaie B, Gore SD and Yajnik CS: Particulate matter and markers of glycemic control and insulin resistance in type 2 diabetic patients: Result from Wellcome Trust Genetic study. J Expo Sci Environ Epidemiol 28: 328-336, 2018.

42. Ding S, Yuan C, Si B, Wang M, Da S, Bai L and Wu W: Combined effects of ambient particulate matter exposure and a high-fat diet on oxidative stress and steatohepatitis in mice. PLoS One 14: e0214680, 2019.

43. Rajagopalan S, Park B, Palanivel R, Vinayachandran V, Deiuliis JA, Gangwar RS, Das L, Yin J, Choi Y, Al-Kindi S, et al: Metabolic effects of air pollution exposure and reversibility. J Clin Invest 130: 6034-6040, 2020.

44. Shoenfelt J, Mitkus RJ, Zeisler R, Spatz RO, Powell J, Fenton MJ, Squibb KA and Medvedev AE: Involvement of TLR2 and TLR4 in inflammatory immune responses induced by fine and coarse ambient air particulate matter. J Leukoc Biol 86: 303-312, 2009.

45. Zhao C, Liao J, Chu W, Wang S, Yang T, Tao Y and Wang G: Involvement of TLR2 and TLR4 and Th1/Th2 shift in inflammatory responses induced by fine ambient particulate matter in mice. Inhal Toxicol 24: 918-927, 2012.

46. Jin Y, Wu W, Zhang W, Zhao Y, Wu Y, Ge G, Ba Y, Guo Q, Gao T, Chi X, et al: Involvement of EGF receptor signaling and NLRP12 inflammasome in fine particulate matter-induced lung inflammation in mice. Environ Toxicol 32: 1121-1134, 2017.

47. Li Z, Wu Y, Chen HP, Zhu C, Dong L, Wang Y, Liu H, Xu X, Zhou J, Wu Y, et al: MTOR suppresses environmental particle-induced inflammatory response in macrophages. J Immunol 200: 2826-2834, 2018.

48. Schulthess FT, Paroni F, Sauter NS, Shu L, Ribaux P, Haataja L, Strieter RM, Oberholzer J, King CC and Maedler K: CXCL10 impairs beta cell function and viability in diabetes through TLR4 signaling. Cell Metab 9: 125-139, 2009.

49. Lee T, Yun S, Jeong JH and Jung TW: Asprosin impairs insulin secretion in response to glucose and viability through TLR4/JNK-mediated inflammation. Mol Cell Endocrinol 486: 96-104, 2019.

50. Liu C, Ying Z, Harkema J, Sun Q and Rajagopalan S: Epidemiological and experimental links between air pollution and type 2 diabetes. Toxicol Pathol 41: 361-373, 2013.

51. Ma QY, Huang DY, Zhang HJ, Wang S and Chen XF: Exposure to particulate matter 2.5 (PM2.5) induced macrophage-dependent inflammation, characterized by increased Th1/Th17 cytokine secretion and cytotoxicity. Int Immunopharmacol 50: 139-145, 2017.

52. Mancini SJ, White AD, Bijland S, Rutherford C, Graham D, Richter EA, Viollet B, Touyz RM, Palmer TM and Salt IP: Activation of AMP-activated protein kinase rapidly suppresses multiple pro-inflammatory pathways in adipocytes including IL-1 receptor-associated kinase-4 phosphorylation. Mol Cell Endocrinol 440: 44-56, 2017.

53. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE and White MF: Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 277: 1531-1537, 2002.

54. Dou L, Wang S, Sun L, Huang X, Zhang Y, Shen T, Guo J, Man Y, Tang W and Li J: Mir-338-3p mediates Tnf-A-induced hepatic insulin resistance by targeting PP4r1 to regulate PP4 expression. Cell Physiol Biochem 41: 2419-2431, 2017.

55. Löfgren P, van Harmelen V, Reynisdottir S, Näslund E, Rydén M, Rössner S and Arner P: Secretion of tumor necrosis factor-alpha shows a strong relationship to insulin-stimulated glucose transport in human adipose tissue. Diabetes 49: 688-692, 2000.

56. Russo L and Lumeng CN: Properties and functions of adipose tissue macrophages in obesity. Immunology 155: 407-417, 2018.