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Long-term Exposure to Ambient Fine Particulate Pollution Induces Insulin Resistance and Mitochondrial Alteration in Adipose Tissue

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We have previously shown that chronic exposure to ambient fine particulate matter (less than 2.5 μm in aerodynamic diameter, $PM_{2.5}$) pollution in conjunction with high-fat diet induces insulin resistance through alterations in inflammatory pathways. In this study, we evaluated the effects of $PM_{2.5}$ exposure over a substantive duration of a rodent's lifespan and focused on the impact of long-term exposure on adipose structure and function. C57BL/6 mice were exposed to $PM_{2.5}$ or filtered air (FA) (6 h/day, 5 days/week) for duration of 10 months in Columbus, OH. At the end of the exposure, $PM_{2.5}$-exposed mice demonstrated insulin resistance (IR) and a decrease in glucose tolerance compared with the FA-exposed group. Although there were no significant differences in circulating cytokines between $PM_{2.5}$- and FA-exposed groups, circulating adiponectin and leptin were significantly decreased in $PM_{2.5}$-exposed group. $PM_{2.5}$ exposure also led to inflammatory response and oxidative stress as evidenced by increase of Nrf2-regulated antioxidant genes. Additionally, $PM_{2.5}$ exposure decreased mitochondrial count in visceral adipose and mitochondrial size in interscapular adipose depots, which were associated with reduction of uncoupling protein 1 (UCP1) expression and downregulation of brown adipocyte-specific gene profiles. These findings suggest that long-term ambient $PM_{2.5}$ exposure induces impaired glucose tolerance, IR, inflammation, and mitochondrial alteration, and thus, it is a risk factor for the development of type 2 diabetes.

Key Words: **air pollution; inflammation; glucose tolerance; mitochondria.**

Accumulating evidence implicates fine particulate matter ($PM_{2.5}$) air pollution as a risk factor that leads to adverse human health effects in developed and developing countries (Kan *et al.*, 2009; Pope *et al.*, 1995; Samet *et al.*, 2000; Schwartz, 1999). Recent data from large population cohorts have provided compelling associations between $PM_{2.5}$ exposure and increased cardiovascular morbidity and mortality (Miller *et al.*, 2007; Pope *et al.*, 2004). Studying insulin resistance (IR) and type 2 diabetes mellitus development and its relation to air pollution may help clarify the causative relations between environmental factors and the development of cardiovascular risk (Franks *et al.*, 2010; Mattsson *et al.*, 2008). Inflammation and oxidative stress pathways in disease development may provoke maladaptive responses that, in turn, adversely affect organ function and appear to play critical roles in this process as demonstrated by numerous investigations (Dandona *et al.*, 2004; Hotamisligil, 2006; Houstis *et al.*, 2006; Shoelson *et al.*, 2006). The effects of $PM_{2.5}$ exposure on cardiovascular and pulmonary systems have been extensively studied with both short- and long-term exposures implicated in major adverse cardiovascular events (Brook *et al.*, 2010; Mills *et al.*, 2009). Although numerous studies have focused on the acute and short-term $PM_{2.5}$ exposure, the effect of long-term $PM_{2.5}$ exposure on mitochondrial alteration and IR has little been investigated, and underlying factors remain to be understood.

Adipose tissue exerts important endocrine and metabolic functions and plays a key role in the control of energy balance and lipid homeostasis (Park *et al.*, 2008). In mammals, adipocytes have been classified into two distinct types: white adipose tissue (WAT), the primary site of energy storage, and brown adipose tissue (BAT), specialized for energy expenditure (Spiegelman and Flier, 2001). The physiological role of BAT is to metabolize fatty acids and generate heat (Spiegelman and Flier, 2001). Due to these functional differences, the imbalance between WAT and BAT affects systemic energy balance and thus may contribute to the development of IR and adiposity. Recent studies suggest that BAT content and function may be an independent predictor of insulin sensitivity and potentially protecting against obesity (Cypess *et al.*, 2009). Although a number of studies suggest that air pollution may lead to inflammation, IR, and adiposity (Frampton, 2006;

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O'Neill *et al.*, 2007; Sun *et al.*, 2005, 2009; van Eeden *et al.*, 2001), few studies have documented the effect of long-term $PM_{2.5}$ air pollution on the alterations of adipose tissue depots.

Therefore, we hypothesized that long-term exposure to $PM_{2.5}$ air pollution would induce low grade, but prolonged, inflammatory response in adipose tissues, exert effects on function of BAT, and result in IR and mitochondrial dysfunction and thereby exacerbate the development of type 2 diabetes, obesity, and metabolic disorders. We employed an extended time of $PM_{2.5}$ exposure (10 months) to mimic $PM_{2.5}$ pollution exposure over a substantive duration of an individual's lifespan.

MATERIALS AND METHODS

Animals. Four-week-old male C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and were fed *ad libitum* standard rodent chow; filtered tap water was freely available. The protocols and the use of animals were approved by and in accordance with The Ohio State University (OSU) Animal Care and Use Committee.

$PM_{2.5}$ exposure. Mice were randomly assigned a group and were exposed to either ambient concentrated $PM_{2.5}$ or filtered air (FA) for 6 h/day, 5 days/week from April 2009 to January 2010 in Columbus, Ohio in a trailer-mounted exposure system, "Ohio Air Pollution Exposure System for Interrogation of Systemic Effects (OASIS) 1" (Laing *et al.*, 2010; Xu *et al.*, 2010; Ying *et al.*, 2009). The total duration of exposures was 1302 h.

Measurements of blood glucose and insulin. Before and after the exposure to FA or $PM_{2.5}$, an ip glucose tolerance test was performed. Briefly, the mice were fasted overnight and dextrose (2 mg/g body weight) was injected ip. Blood glucose measurements were conducted with an Elite Glucometer (Bayer) at baseline and 30, 60, 90, and 120 min after the dextrose injection. Insulin levels were determined using an Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem Inc., Downers Grove, IL). Homeostasis model assessment (HOMA) indices were calculated based on 1 mg of insulin as equivalent to 24 IU, using the formula HOMA = [fasting insulin concentration (ng/ml) × 24 × fasting glucose concentration (mg/dl)]/405 (Xu *et al.*, 2010).

Circulating inflammatory biomarkers, adiponectin, and leptin measurement. At the end of the study, blood samples were collected and spun, and serum was stored at −80°C for the analysis of cytokines, adiponectin, and leptin. Cytokine levels were determined by Cytometric Bead Array (BD Biosciences, San Jose, CA). Serum was incubated with beads specific for tumor necrosis factor (TNF), interferon γ (IFN-γ), monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), IL-10, and IL-12p70 according to the manufacturer's instructions. The total amount of cytokines was then determined using a BD LSR II instrument and analyzed by the BD CBA software (BD Biosciences). Adiponectin and leptin levels were determined by using an adiponectin and leptin quantification kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions.

Immunohistochemical staining. Immunohistochemical staining was performed as previously described (Xu *et al.*, 2010). Briefly, deparaffinized sections (5 μm) were subjected to heat-induced antigen retrieval. The slides were dipped in 0.3% H_2O_2 for 10 min to quench the endogenous peroxidase. The sections were then incubated in 1% BSA/PBS for 10 min, followed by overnight incubation with primary antibodies at 4°C. Afterward, the slides were incubated at room temperature for 2 h with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies. The stain was developed using Fast 3,3′-diaminobenzidine tablet sets (D4293; Sigma, St Louis, MO). The sections were counterstained with hematoxylin and examined by light microscopy. The primary antibodies were rat anti-mouse F4/80 (AbD Serotec, Raleigh, NC), rat anti-mouse UCP1 (Abcam), and rabbit anti-iNOS (Santa Cruz Biotechnology, Santa Cruz, CA). All measurements were conducted in a double-blinded manner by two independent investigators.

Quantification of superoxide production by dihydroethidium staining. The oxidative fluorescent probe dihydroethidium (DHE) was used to evaluate *in situ* O_2^- production on histological sections. DHE is a cell permeable dye that is oxidized by O_2^- to ethidium bromide, which subsequently intercalates with DNA and is trapped within cell nuclei. Cryosections (10 μm) were incubated with DHE (10 μM, Molecular Probes, Invitrogen) in a light-protected humidified chamber at room temperature for 30 min. Sections were then rinsed with phosphate buffered saline and analyzed with a fluorescent Nikon Eclipse FN1 microscope (Nikon, Tokyo, Japan). Acquisition settings of the camera were identical for all images. Automatic computer-based analysis was performed using MetaMorph software. Data are expressed as % fluorescence area.

Transmission electron microscopic imaging. To investigate the mitochondrial changes *in situ* between FA- and $PM_{2.5}$-exposed groups, we examined the ultrastructure of epididymal and interscapular adipose depots by transmission electron microscopic (TEM) as described previously (Xu *et al.*, 2011). There were five animals in each group for mitochondrial assessment. For the morphometric analysis, mitochondrial size and number were analyzed in 5 to 8 micrographs per group by NIH ImageJ software.

Real-time PCR. Epididymal and interscapular adipose tissues from the mice were excised, and RNA was isolated using Trizol reagent according to the manufacturer's instructions. Total RNA was then converted into cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The quantification of gene expression was determined by real-time PCR. All reactions were performed under the same condition: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. The primers for mouse uncoupling protein 1 (*Ucp1*), PRD1-BF1-RIZ1 homologous domain containing 16 (*Prdm16*), peroxisome proliferator-activated receptor-γ coactivator 1-α (*Pgc-1α*), CCAAT enhancing binding protein β (*C/ebpβ*), cell death-inducing DNA fragmentation factor–like effector A (*Cidea*), type 2 iodothyronine deiondinase (*Dio2*), peroxisome proliferator-activated receptor gamma 2 (*Pparg2*), elongation of very long-chain fatty acid 3 (*Elovl3*), NF-E2-related factor 2 (*Nrf2*), NAD(P)H quinone oxidoreductase 1 (*Nqo1*), glutamate-cysteine ligase modifier subunit (*Gclm*), and *β-actin* are showed in Table 1. *Beta-actin* was used as the control gene, and all data are presented as relative mRNA levels.

Western blotting. Liver, soleus muscle, epididymal, and interscapular fat depots were homogenized in lysis buffer (Thermo Scientific, Rockford, IL) with protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). The same amounts of protein (60 μg for epididymal fat depots and 20 μg for the others) were separated with SDS-PAGE in 10% polyacrylamide gel and then transferred to PVDF membranes (Bio-Rad, Hercules, CA). The membranes were immunoblotted with primary antibodies against UCP1 (1:1000, Abcam), phospho-Akt (Ser473, 1:1000, Cell Signaling, Danvers, MA), or Akt (1:1000, Cell Signaling), followed by treatment with HRP-conjugated anti-rabbit IgG (Santa Cruz Biotechnology) at a 1:5000 dilution. The membranes were detected with enhanced chemiluminescence (Super Signal West Pico; Thermo Scientific), followed by exposure to X-ray film. The protein bands on the X-ray film were scanned, and band density was calculated by Quantity One software (Bio-Rad).

Statistical analysis. Data are expressed as mean ± SE unless otherwise indicated. The results of the experiments were analyzed by unpaired *t* tests. All the analyses were performed using Graphpad Prism v5.0 (GraphPad Software, San Diego, CA). In all cases, a p value of <0.05 was considered as statistically significant.

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TABLE 1
Primers Used for Real-time PCR

Primer	Forward oligonucleotides	Reverse oligonucleotides
Ucp1	5′-ACTGCCACACCTCCAGTCATT-3′	5′-CTTTGCCTCACTCAGGATTGG-3′
Prdm16	5′-CAGCACGGTGAAGCCATTC-3′	5′-GCGTGCATCCGCTTGTG-3′
Pgc-1α	5′-CCCTGCCATTGTTAAGACC-3′	5′-TGCTGCTGTTCCTGTTTTC-3′
C/ebpβ	5′-ACGACTTCCTCTCCGACCTCT-3′	5′-CGAGGCTCACGTAACCGTAGT-3′
Cidea	5′-ATCACAACTGGCCTGGTTACG-3′	5′-TACTACCCGGTGTCCATTTCT-3′
Dio2	5′-AAGGCTGCCGAATGTCAACGAATG-3′	5′-TGCTGGTTCAGACTCACCTTGGAA-3′
Pparg2	5′-GCATGGTGCCTTCGCTGA-3′	5′-TGGCATCTCTGTGTCAACCATG-3′
Elovl3	5′-GATGGTTCTGGGCACCATCTT-3′	5′-CGTTGTTGTGTGGCATCCTT-3′
Nrf2	5′-TCACACGAGATGAGCTTAGGGCAA-3′	5′-TACAGTTCTGGGCGGCGACTTTAT-3′
NQO1	5′-ATGGCATCCAGTCCTCCATCAAGA-3′	5′-ACAAGTTAGTCCCTCGGCCATTGT-3′
Gclm	5′-CCACCAGATTTGACTGCCTTTGCT-3′	5′-AATCCTGGGCTTCAATGTCAGGGA-3′
β-actin	5′-TGTGATGGTGGGAATGGGTCAGAA-3′	5′-TGTGGTGCCAGATCTTCTCCATGT-3′

RESULTS

Exposure Characterization

The mean daily $PM_{2.5}$ concentration at the study site in Columbus, OH, was 12.7 (SD = 8.4) μg/m^3. The mean concentration of $PM_{2.5}$ in the exposure chamber during the 30 h/week of exposure was 94.4 μg/m^3 (~7-fold concentration from ambient $PM_{2.5}$ level).

Body and Organ Weight Measurements

Figure 1 illustrates the body and organ weights after 10-month exposure to $PM_{2.5}$ or FA. There were no significant differences in body weight (Fig. 1A). Interestingly, although the weight of visceral (epididymal) WAT in $PM_{2.5}$-exposed group was comparable to that in FA group (Fig. 1B), $PM_{2.5}$ exposure significantly decreased the interscapular BAT when compared with the FA-exposed group (Fig. 1C). Over time, we did not observe any statistically significant changes in body weight by long-term $PM_{2.5}$ exposure (Supplementary figure 1).

$PM_{2.5}$ Exposure Induced Glucose Intolerance and IR

To test whether long-term $PM_{2.5}$ exposure affects glucose metabolism, we performed an ip glucose tolerance test. As shown in Figure 1D, mice exposed to $PM_{2.5}$ showed significant elevations in glucose levels at each time point, indicating that long-term $PM_{2.5}$ exposure induced impaired glucose tolerance. The levels of fasting blood glucose and insulin were shown in Table 2. In addition, the HOMA-IR index, which is an indicator of insulin sensitivity (Katsuki *et al.*, 2001), was significantly higher in the $PM_{2.5}$-exposed mice than those in the FA-exposed mice (Table 2), suggesting that long-time $PM_{2.5}$ exposure induced IR.

Effect of $PM_{2.5}$ Exposure on Circulating Cytokines and Adipokines

We measured inflammatory biomarkers in the blood to see if long-term $PM_{2.5}$ exposure could induce systemic inflammation. As shown in Figure 2A, we did not find any significant differences in IL-12p70, TNF, IFN-γ, or MCP-1 between the 2 groups, whereas the levels of IL-6 and IL-10 were too low and nondetectable. As shown in Figures 2B and 2C, the levels of serum adiponectin and leptin were remarkably decreased by $PM_{2.5}$ exposure when compared with the FA control group ($p < 0.05$), suggesting that $PM_{2.5}$ exposure causes IR in adipose tissue (Sultan *et al.*, 2009).

$PM_{2.5}$ Exposure Increased Inflammation in Lung and Visceral Adipose Depot

We wanted to know whether long-term $PM_{2.5}$ exposure led to inflammation in the organs and tissues. As shown in Figures 3A and 3C, $PM_{2.5}$ exposure resulted in an increase in F4/80$^+$

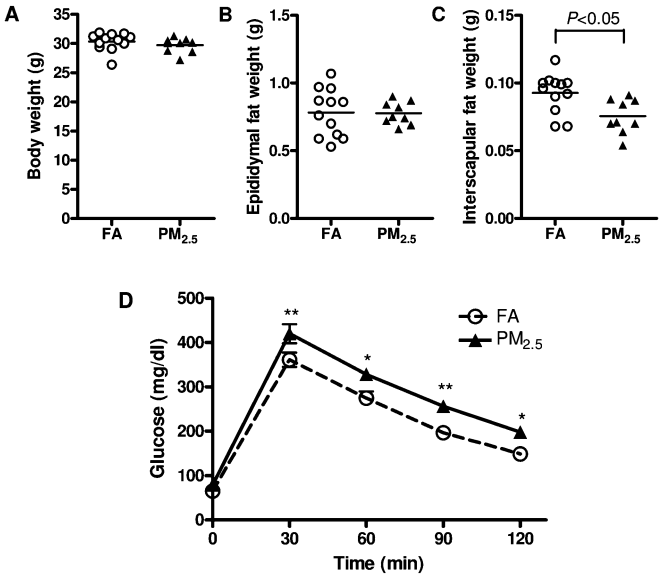

FIG. 1. Effect of $PM_{2.5}$ exposure on body weight (BW), organ weight, and glucose homeostasis. (A–C) Effect of $PM_{2.5}$ exposure on body weight (A), epididymal fat (B), and interscapular fat depots (C). (D) Intraperitoneal glucose tolerance test. $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^*p < 0.05$ versus FA group, $^{**}p < 0.01$ versus FA group.

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TABLE 2
Fasting Blood Glucose, Insulin, and HOMA-IR Index

	FA	$PM_{2.5}$	*p* Value
Glucose, mg/dl	65.13 ± 1.74	79.25 ± 5.39	0.026
Insulin (ng/ml)	0.68 ± 0.09	0.86 ± 0.10	0.215
HOMA-IR	2.29 ± 0.31	3.96 ± 0.51	0.015

Note. Values are mean ± SE. $N = 8$. HOMA-IR, homeostasis model assessment of IR.

macrophage recruitment in the lung. In addition, adipose tissue macrophages are thought to represent key cellular mediators of adipose tissue inflammatory response and IR development. We therefore investigated the inflammatory response to $PM_{2.5}$ exposure by immunohistochemistry in epididymal adipose tissue, which is regarded to play an important role in the development of IR, obesity, and type 2 diabetes mellitus (Dandona *et al*., 2004; Shoelson *et al*., 2006; Xu *et al*., 2003). $PM_{2.5}$ exposure induced an increase in F4/80$^+$ macrophage infiltrating into the WAT when compared with the FA group (Figs. 3B and 3D).

TEM Analysis of In Situ Mitochondria

We next tested the hypothesis that long-term $PM_{2.5}$ exposure accounted for mitochondrial alteration. Two quantitative parameters were used to assess mitochondria: mitochondrial number and size. Figure 4 shows representative TEM images of mitochondria in the interscapular (Fig. 4A) adipose depots in response to $PM_{2.5}$ exposure. Interestingly, $PM_{2.5}$ exposure did not lead to dramatic decline in mitochondrial number in interscapular brown adipose depots but did result in a significant decrease in average mitochondrial size compared with the FA group (Fig. 4B). On the other side, $PM_{2.5}$ exposure significantly reduced mitochondrial number in visceral adipose tissue, although there was no significant difference in mitochondrial size (Fig. 4C).

Brown Adipocyte-Specific Gene Profiles and UCP1 Expression in WAT and BAT

To determine brown adipocyte-specific gene profiles in response to long-term $PM_{2.5}$ exposure, we examined these gene expressions in WAT and BAT by real-time PCR analysis. As shown in Figure 5A, the mRNA levels of the brown adipocyte-specific gene *Ucp1*, *Prdm16*, *Pgc-1α*, and *Pparg2* were significantly decreased in the WAT in response to long-term $PM_{2.5}$ exposure, although we did not observe any significant difference in *C/ebpβ*, *Cidea*, *Dio2*, or *Elovl3* between these 2 groups. Consistent with *Ucp1* gene expression, the observation was further confirmed by Western blotting for UCP1 at protein level in the WAT, as shown in Figures 5B and 5C. Additionally, we wanted to know whether long-term $PM_{2.5}$ exposure caused any changes of the brown adipocyte-specific gene profiles in BAT. As shown in Figure 6A, the mRNA levels of *Ucp1* and *Pgc-1α* were significantly reduced in response to $PM_{2.5}$ exposure, although there were no significant

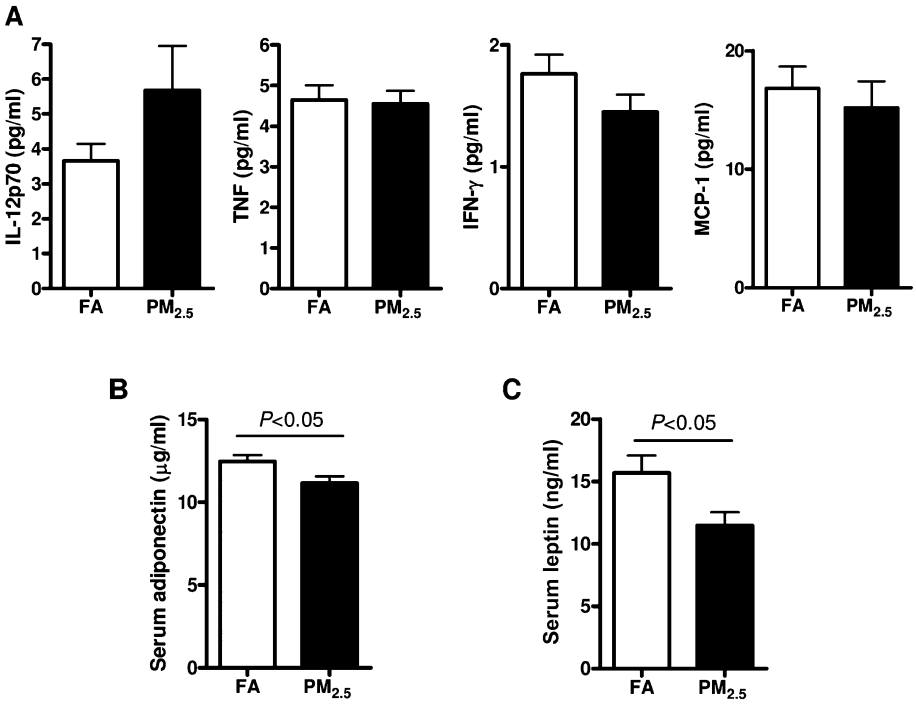

FIG. 2. The concentrations of circulating cytokines (IL-12p70, TNF, IFN-γ, and MCP-1) (A), adiponectin (B), leptin (C) in response to $PM_{2.5}$ exposure. $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group.

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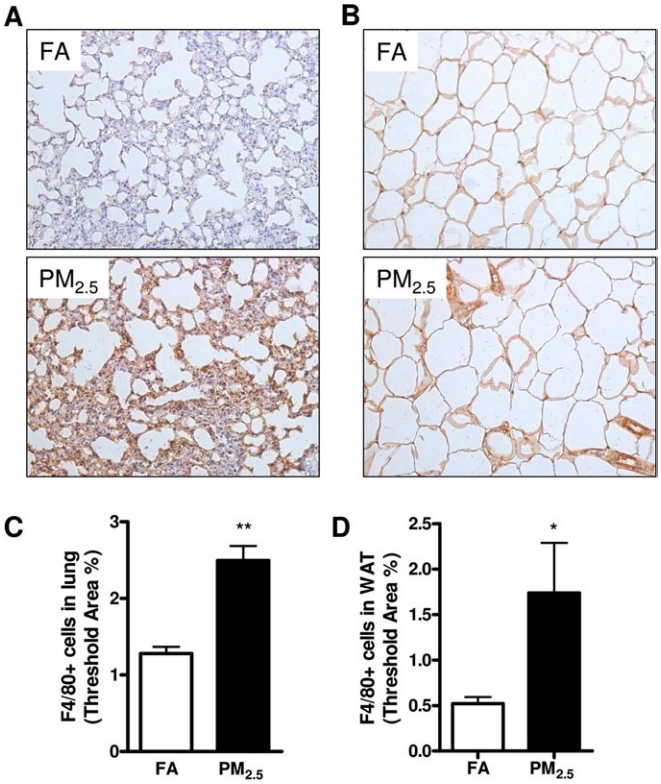

FIG. 3. Effect of $PM_{2.5}$ exposure on inflammatory response in the lung and WAT (epididymal) by immunohistochemistry. (A–D) Representative images (A–B) and statistical analysis (C–D) of immunohistochemistry for F4/80$^+$ macrophages in the lung (A, C) and WAT (B, D). Original magnification, ×200. $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^*p < 0.05$ versus FA group, $^{**}p < 0.001$ versus FA group.

changes in the other brown adipocyte-specific gene expressions. In line with the observations with regard to the alteration of *Ucp1* mRNA level, UCP1 expression at the protein level was significantly decreased by long-term $PM_{2.5}$ exposure in BAT, as demonstrated by immunohistochemistry (Figs. 6B and 6C) as well as Western blotting (Figs. 6D and 6E).

$PM_{2.5}$ Exposure Induced IR in Adipose Tissues, Liver, and Skeletal Muscle

Due to aforementioned observation that long-term $PM_{2.5}$ exposure led to systemic IR, we then further tested local IR in adipose tissue depots, liver, and skeletal muscle. As shown in Figure 7, we found that long-term $PM_{2.5}$ exposure decreased phosphorylation of Akt in the liver (Fig. 7A), skeletal muscle (Fig. 7B), WAT (Fig. 7C), and BAT (Fig. 7D) as compared with the FA-exposed control. There were no significant differences in the expression of total Akt between the groups in these tissues (data not shown).

$PM_{2.5}$ Exposure Induced Superoxide Production, Oxidative Stress, and Activated Nrf2 Signaling

As shown in Figure 8, long-term $PM_{2.5}$ exposure significantly induced superoxide production as determined by DHE staining

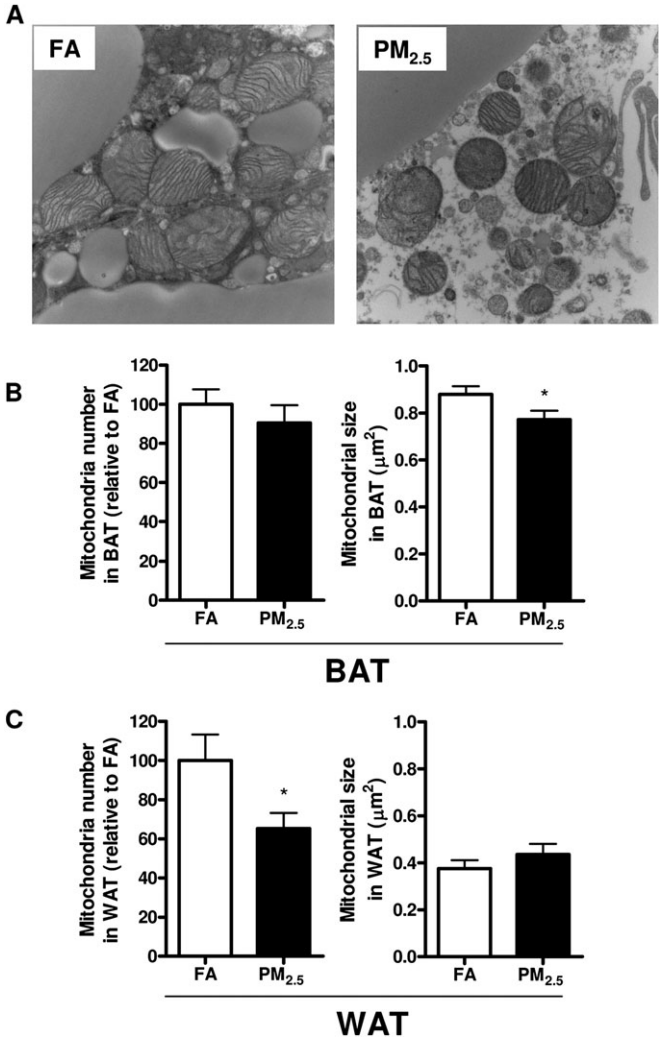

FIG. 4. Effect of $PM_{2.5}$ exposure on mitochondria in adipose tissues. (A–C) Representative TEM images in BAT (A) and quantification of mitochondrial number and size in BAT (B) and WAT (C). $N = 5$. $^*p < 0.05$ versus FA group. Original magnification, ×18,500. WAT (epididymal); BAT (interscapular).

(Figs. 8A and 8B) and resulted in higher expression of 3-nitrotyrosine in BAT depots, a marker of oxidative stress, by immunohistochemistry (Figs. 8C and 8D). Nevertheless, we did not observe any significant changes in iNOS expression in BAT depots by long-term $PM_{2.5}$ exposure (Supplementary figure. 2). We explored whether long-term $PM_{2.5}$ exposure led to phase II antioxidant responses that are mediated via Nrf2. After long-term $PM_{2.5}$ exposure, $PM_{2.5}$-exposed mice exhibited a significant increase in WAT (Fig. 8E) and BAT (Fig. 8F) depots in the expression of *Nrf2* as well as induction of *Nqo1* and *Gclm*, which are secondarily regulated by this transcription factor.

DISCUSSION

In this study, we evaluated the role of exposure to long-term $PM_{2.5}$ air pollution on inflammatory response, IR, oxidative

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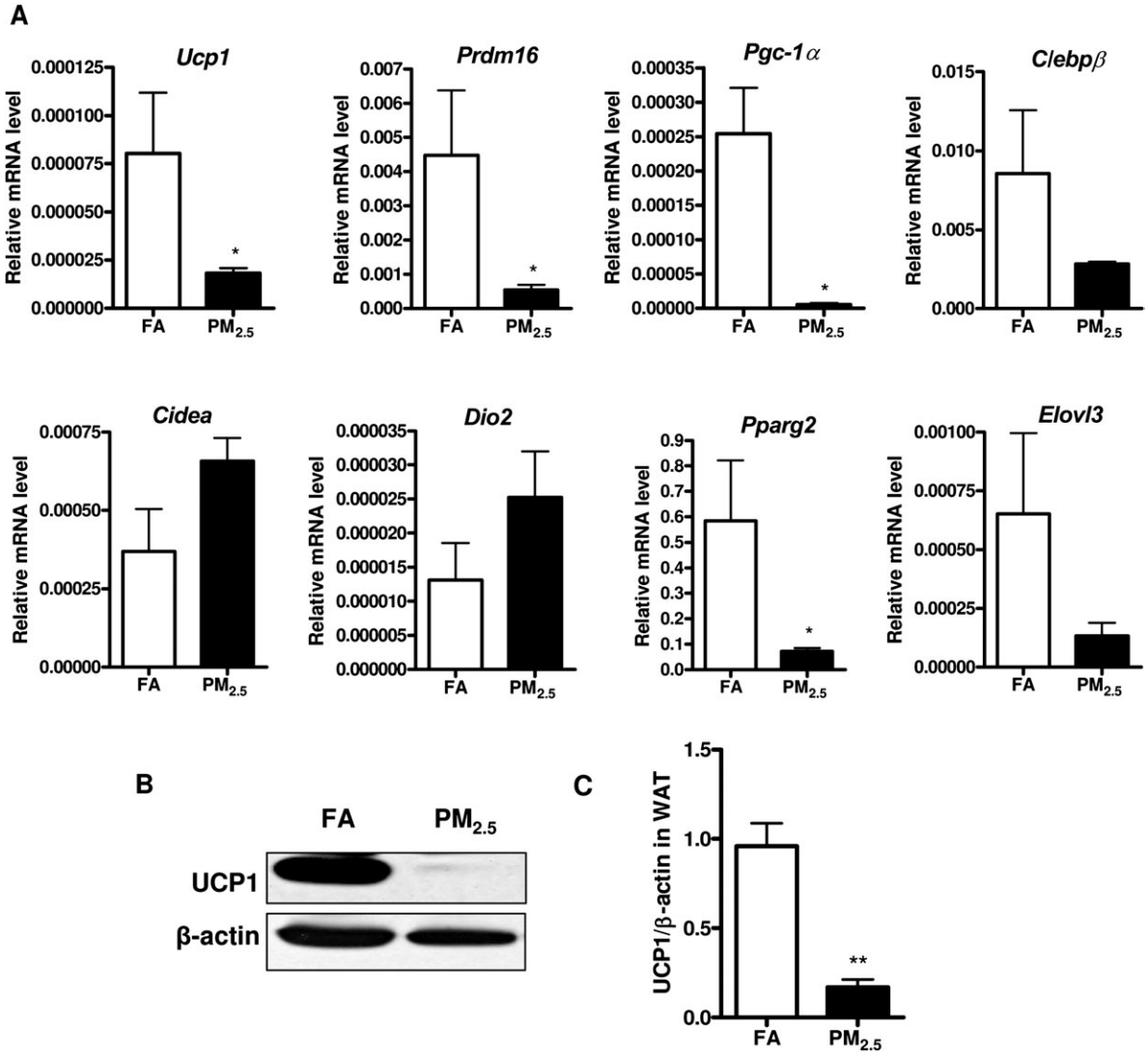

FIG. 5. Effect of $PM_{2.5}$ exposure on brown adipocyte-specific gene profiles and UCP1 expression in visceral WAT. (A) Brown adipocyte-specific gene expressions in WAT. (B and C) Representative bands (B) and statistical analysis (C) of Western blotting for UCP1 expression in WAT in response to $PM_{2.5}$ exposure. $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^{*}p < 0.05$ versus FA group, $^{**}p < 0.001$ versus FA group. Uncoupling protein 1 (*Ucp1*), PRD1-BF1-RIZ1 homologous domain containing 16 (*Prdm16*), peroxisome proliferator-activated receptor-γ coactivator 1-α (*Pgc-1α*), CCAAT enhancing binding protein β (*C/ebpβ*), cell death-inducing DNA fragmentation factor–like effector A (*Cidea*), type 2 iodothyronine deiondinase (*Dio2*), peroxisome proliferator-activated receptor gamma 2 (*Pparg2*), elongation of very long-chain fatty acid 3 (*Elovl3*).

stress, and mitochondrial alteration, as well as changes in brown adipocyte-specific gene profiles in both WAT and BAT in mice. To our knowledge, this is the first to study the changes in mitochondria, UCP1 expression, and brown adipocyte-specific gene profiles in response to long-term $PM_{2.5}$ exposure. There are some important findings in this study. First, long-term $PM_{2.5}$ exposure induces systemic and local IR, impaired glucose tolerance, and inflammatory responses in the lung and visceral adipose depots. Secondly, long-term $PM_{2.5}$ exposure results in oxidative stress as evidenced by elevated levels of 3-nitrotyrosine and a significant increase of Nrf2-regulated antioxidant genes in the $PM_{2.5}$-exposed mice. In addition, long-term $PM_{2.5}$ exposure decreases mitochondrial number in WAT, reduces mitochondrial size in BAT, and lowers UCP1 expression and several brown adipocyte-specific gene expressions in adipose depots, and thereby leading to mitochondrial dysfunction in adipose tissue.

Because human beings are constantly exposed to ambient $PM_{2.5}$, long-term $PM_{2.5}$ exposure in laboratory studies has been pursued in the past decade and is regarded as a sound approach to mimic real-world human exposures. Our previous studies have investigated different exposure time periods associated with different diseases, which were from a relatively short-term period of 10 weeks to relatively long-term exposures of up to 6 months (Sun *et al.*, 2005, 2008, 2009; Xu *et al.*, 2010). Different from the studies by intratracheal or nasal exposure

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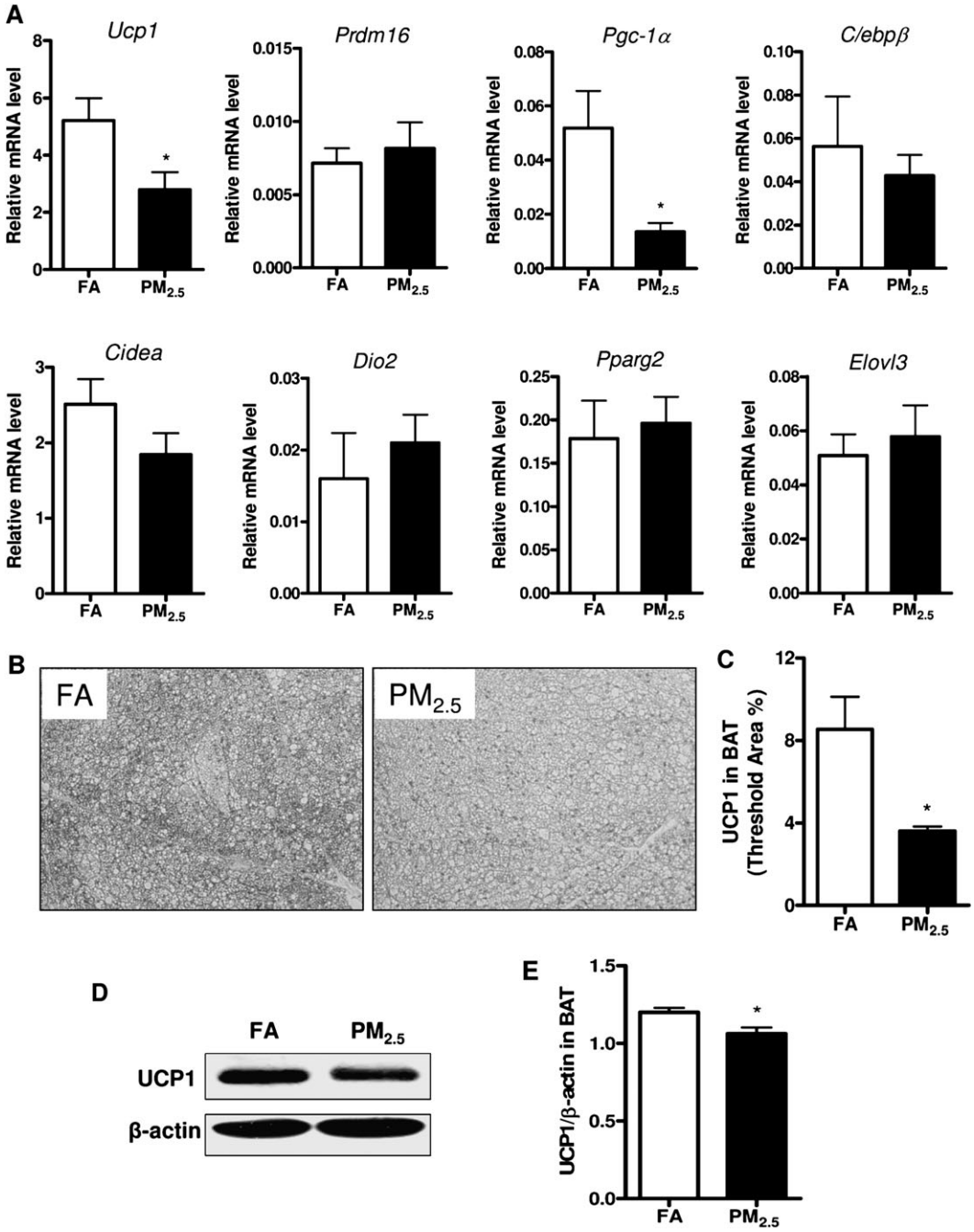

FIG. 6. Effect of $PM_{2.5}$ exposure on brown adipocyte-specific gene profiles and UCP1 expression in interscapular BAT. (A) Brown adipocyte-specific gene expressions in BAT. (B and C) Representative images (B) and statistical analysis (C) of immunohistochemistry for UCP1 expression in BAT in response to $PM_{2.5}$ exposure. (D and E) Representative bands (D) and statistical analysis (E) of Western blotting for UCP1 expression in BAT in response to $PM_{2.5}$ exposure. $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^{*}p < 0.05$ versus FA group. Uncoupling protein 1 (*Ucp1*), PRD1-BF1-RIZ1 homologous domain containing 16 (*Prdm16*), peroxisome proliferator-activated receptor-γ coactivator 1-α (*Pgc-1α*), CCAAT enhancing binding protein β (*C/ebpβ*), cell death-inducing DNA fragmentation factor–like effector A (*Cidea*), type 2 iodothyronine deiondinase (*Dio2*), peroxisome proliferator-activated receptor gamma 2 (*Pparg2*), elongation of very long-chain fatty acid 3 (*Elovl3*).

in vivo and others by cell culture *in vitro* (Kongerud *et al.*, 2006; Mutlu *et al.*, 2007; Pozzi *et al.*, 2003), the mice in the current study were exposed to the $PM_{2.5}$ particles over a duration of 10 months, which is roughly equivalent to a human exposure period of ~40 years (Flurkey *et al.*, 2007), which might be sufficient to cause IR and increases in the prevalence of type 2 diabetes and susceptibility to cardiovascular diseases.

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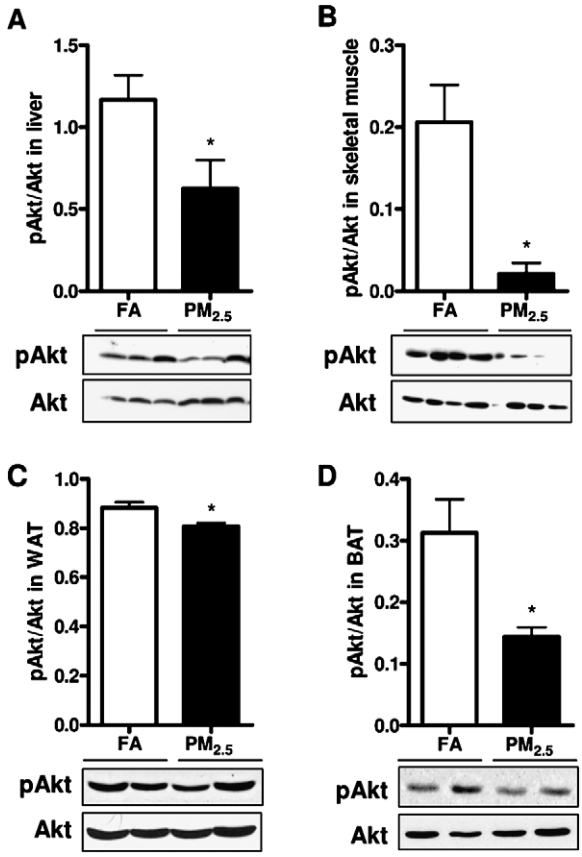

FIG. 7. IR in the liver, skeletal muscle, and adipose tissues in response to $PM_{2.5}$ exposure. (A–D) Western blotting for phosphorylated (pAkt) and total Akt in the liver (A), soleus skeletal muscle (B), visceral WAT (C), and interscapular BAT (D). $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^{*}p < 0.05$ versus FA group.

A number of studies have shown that exposure to $PM_{2.5}$ is associated with a systemic proinflammatory response in humans and animals (Calderon-Garciduenas *et al.*, 2008; Mutlu *et al.*, 2007; Thompson *et al.*, 2010; Xu *et al.*, 2010). Inflammation and oxidative stress pathways play critical roles in this process of IR, adiposity, and the development of cardiovascular risk in response to $PM_{2.5}$ air pollution, as demonstrated by numerous investigations (Dandona *et al.*, 2004; Hotamisligil, 2006; Houstis *et al.*, 2006; Shoelson *et al.*, 2006; Xu *et al.*, 2010). A number of studies have highlighted the innate immune mechanisms as the critical role that is responsible for the pathophysiological abnormalities (Dandona *et al.*, 2004; Zhou *et al.*, 2009). In addition, accumulating studies have revealed that exposure to concentrated ambient particulate matter air pollution is related to acute and chronic effects on cardiac function, increased amounts of more invasive aortic plaque, inflammation, and IR as well as adiposity (Chen and Hwang, 2005; Hwang *et al.*, 2005; Sun *et al.*, 2005, 2008, 2009; Ying *et al.*, 2009). In line with these findings, our results demonstrate that although long-term exposure to $PM_{2.5}$ did not induce systemic inflammation, it did result in inflammatory responses in the lung and visceral adipose tissue and systemic IR as well as local IR, as indicated by diminished insulin-mediated phosphorylation of Akt at Ser^{473} in the liver, skeletal muscle, and adipose tissues. Additionally, our findings suggest that long-term $PM_{2.5}$ exposure induces oxidative stress, which is consistent with some other investigations (Araujo *et al.*, 2008).

BAT serves a very important function in protection from diet-induced obesity, diabetes, and IR (Hamann *et al.*, 1996). Therefore, promoting functional BAT and/or brown-like adipocytes within WAT depots is associated with improved metabolic phenotypes and could be a potential target for the treatment of obesity via the modulation of metabolic rate. UCP1, which is specifically expressed in BAT mitochondria, is largely responsible for the uncoupling of respiration from ATP synthesis resulting in dissipation of energy as heat and thereby playing a pivotal role in thermogenesis and protecting against reactive oxygen species (Ricquier, 2005). Accordingly, BAT is of importance in the maintenance of body temperature and energy balance. Recently, "brite" (*br*own-*i*n-whi*te*) adipocytes have been discovered in WAT and display several classical brown adipocyte characteristics (Nedergaard and Cannon, 2010; Petrovic *et al.*, 2010). Besides UCP1, proteins such as Dio2, Cidea, and Elovl3 have been shown to be highly enriched in BAT (Silva and Larsen, 1983). PGC-1α, a coactivator of multiple transcription factors such as NRF-1/2 and PPAR (Evans and Scarpulla, 1990; Ferre, 2004), is also highly expressed in BAT but relatively low in WAT (Lin *et al.*, 2005). It is likely that brite adipose has unique physiological/thermogenic function associated with the regulation of body adiposity (Enerback, 2010). To evaluate further the hypothesis that mitochondria could be affected by long-term $PM_{2.5}$ exposure, we measured the expression of UCP1 and several key genes related to mitochondrial function and regulatory networks that govern expression of genes during BAT differentiation and mitochondrial biogenesis by real-time PCR in the BAT and WAT based on the previous studies (Lagouge *et al.*, 2006; Petrovic *et al.*, 2010). In this study, we demonstrate that long-term exposure to $PM_{2.5}$ decreased UCP1 expression and several brown adipocyte-specific gene expressions in both WAT and BAT. Furthermore, long-term $PM_{2.5}$ exposure reduced mitochondrial number in WAT and mitochondrial size in BAT. These findings suggest that long-term $PM_{2.5}$ exposure induces alterations of adipose tissue and leads to mitochondrial dysfunction. The underlying mechanisms responsible for this adverse effect in response to ambient $PM_{2.5}$ air pollution need to be further investigated.

In summary, our data suggest that long-term $PM_{2.5}$ exposure induces inflammatory responses in the lung and visceral adipose, IR, and mitochondrial alteration. These findings suggest an important public health impact on human populations.

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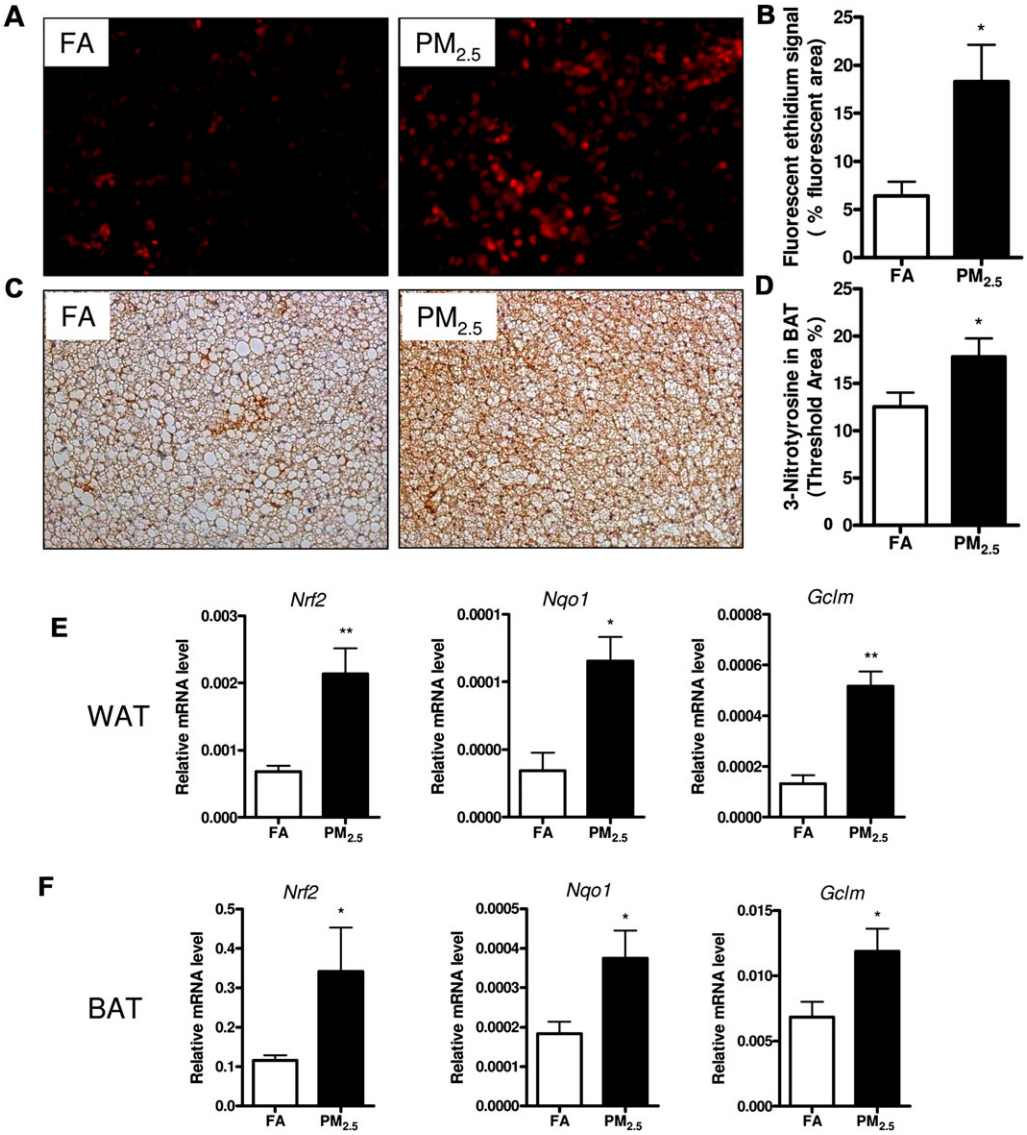

FIG. 8. Effect of $PM_{2.5}$ exposure on superoxide production and oxidative stress in interscapular BAT and Nrf2 signaling in WAT and BAT. (A and B) Representative images (A) and quantification (B) for superoxide production, as determined by dihydroethidium (DHE) staining. (C and D) Representative images (C) and quantification (D) of immunohistochemistry for 3-nitrotyrosine as a marker of oxidative stress. Original magnification, ×200. (E and F) Real-time PCR data for Nrf2 signaling in WAT (E) and BAT (F). $N = 11$ in FA group; $N = 9$ in $PM_{2.5}$ group. $^{*}p < 0.05$ versus FA group, $^{**}p < 0.001$ versus FA group. NF-E2-related factor 2 (*Nrf2*), NAD(P)H quinone oxidoreductase 1 (*Nqo1*), glutamate-cysteine ligase modifier subunit (*Gclm*).

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