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Exercise training prevented endothelium dysfunction from particulate matter instillation in Wistar rats

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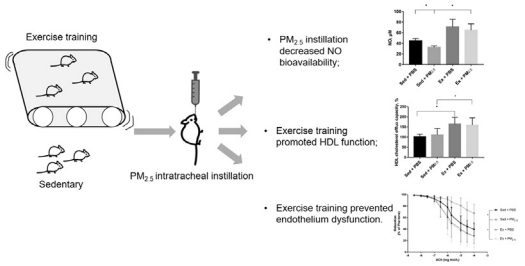
HIGHLIGHTS

- Exercise training significantly reduced the body weight of rats.
- $PM_{2.5}$ instillation decreased NO bioavailability.
- Exercise training promoted HDL function.
- Exercise training prevented endothelium dysfunction induced by $PM_{2.5}$ instillation.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to fine particulate matter ($PM_{2.5}$) can result in adverse cardiovascular responses including vascular endothelial dysfunction, whereas exercise training can promote cardiovascular health. However, whether exercise training can mitigate adverse vascular response to $PM_{2.5}$ has been less studied. In the present study, we aimed to investigate the preventive effect of exercise training on vascular endothelial dysfunction induced by $PM_{2.5}$ instillation. Six-week old male Wistar rats ($n = 32$) were divided into four groups (8 rats per group) by exercise status (sedentary vs. exercised) and $PM_{2.5}$ exposure (instilled vs. non-instilled). Rats received treadmill training with moderate-intensity intervals in week 1 to 6, followed by three repeated $PM_{2.5}$ instillation on every other day in week 7. Body weight and blood pressure were measured for each rat regularly during exercise training and before sacrifice. At sacrifice, thoracic aortas were isolated for functional response measurement to agonists. Nitric oxide bioavailability and high-density lipoprotein (HDL) function were also assessed. We observed that exercise training significantly reduced the body weight of rats, while $PM_{2.5}$ instillation had little effect. Neither exercise training nor $PM_{2.5}$ instillation had significant effects on blood pressure changes. However, exercise training effectively prevented endothelium-dependent vasorelaxation dysfunction and nitric oxide bioavailability reduction from subsequent $PM_{2.5}$ instillation. In addition, exercise training promoted HDL function which were characterized as increased HDL cholesterol level, cholesterol efflux capacity, and reduced oxidization index; whereas $PM_{2.5}$

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instillation showed limited adverse impact on HDL function. Collectively, our results indicated that exercise training could promote HDL function and protect against endothelium dysfunction from $PM_{2.5}$ instillation.

1. Introduction

Exposure to fine particulate matters (with aerodynamic diameter less than 2.5 μm, $PM_{2.5}$) in ambient air has been associated with increased risks of cardiovascular diseases, such as hypertension and atherosclerosis (Cohen et al., 2017). Although the underlying mechanisms are still not fully elucidated, it is believed that the abnormal vascular response characterized by reduction in vasorelaxation capacity or enhancement of vasocontraction might play important roles (Moller et al., 2011). Emerging epidemiological evidence showed negative associations between $PM_{2.5}$ exposure and nitric oxide (NO)-mediated vasorelaxation, implicating an involvement of NO in the $PM_{2.5}$-mediated vascular dysfunction (Krishnan et al., 2012; Tong et al., 2015). NO derived from endothelial nitric oxide synthase (eNOS) dilates all types of blood vessels through activating soluble guanylyl cyclase and increasing cyclic guanosine monophosphate (cGMP) in smooth muscle cells, playing an essential role in keeping normal vasorelaxation (Lundberg et al., 2015). In vivo studies further revealed that exposure to diesel exhaust particles, an important component of ambient $PM_{2.5}$, can decrease NO bioavailability by uncoupling eNOS and generating superoxide anion radicals, and result in the increase of vasocontraction (Rao et al., 2018).

To mitigate health risk of air pollution exposure, several intervention measures have been tailored and assessed in studies with the use of mask and/or air purifier, taking antioxidant supplement, as well as performing exercise among individuals or in laboratory animals (Shao et al., 2017; Zhong et al., 2017; Giles and Koehle, 2014; Li et al., 2017; Bolcas et al., 2019). Exercise training as a non-pharmacologic intervention measure has been particularly appealing, due to its pleiotropic beneficial effects that can promote cardiovascular health (Schuler et al., 2013). Exercise can be presumably beneficial on cardiovascular health by promoting high-density lipoprotein (HDL) functions (Sarzynski et al., 2015). Biologically, HDL plays a central role in reverse cholesterol transport (RCT), the major antiatherogenic process by which cholesterol in peripheral tissues including arterial wall can be transferred to liver for excretion through bile (Rader and Hovingh, 2014). Besides, HDL particles may also possess other potent biological activities, including antioxidative, anti-inflammatory and anti-contractile actions to initiate direct endothelial-protective effects (Ganjali et al., 2017). In a study conducted in patients with metabolic syndrome, three-month moderate intensity exercise training significantly increased the ability of HDL particles to receive free cholesterol and cholesterol ester, and to prevent $CuSO_4$-mediated low density lipoprotein (LDL) oxidation (Casella-Filho et al., 2011). Moreover, one in vitro study revealed that exercise training could modify HDL function via reducing malondialdehyde (MDA) bound to HDL, which can lead to a lower activation of PKC-BII, and result in higher activation of eNOS and synthesis of NO (Adams et al., 2013). However, it is still not clear whether these beneficial effects of HDL still exist when $PM_{2.5}$ exposure presents.

In the present study, we aimed to determine the effect of exercise training prior to $PM_{2.5}$ exposure on the endothelium-dependent vasorelaxation. To that extent, male Wistar rats first received moderate interval treadmill training in week 1 to 6, followed by three repeated $PM_{2.5}$ instillation on every other day in week 7. After sacrifice, thoracic aortas of rats were then isolated for functional response measurement to agonists, and NO bioavailability and high-density lipoprotein (HDL) function were further assessed.

2. Materials and methods

2.1. Experimental design

The experimental design that characterizes the exercise training and sequential $PM_{2.5}$ instillation protocol is summarized in Fig. 1. Experimental animals were divided into four groups (8 rats/group) by exercise status (sedentary vs. exercised) and $PM_{2.5}$ instillation (instilled vs. non-instilled) as follows: Sed + PBS (sedentary and non-$PM_{2.5}$-instilled), Sed + $PM_{2.5}$ (sedentary and $PM_{2.5}$-instilled), Ex + PBS (exercised and non-$PM_{2.5}$-instilled) and Ex + $PM_{2.5}$ (exercised and $PM_{2.5}$-instilled).

Six-week old male Wistar rats were purchased from Vital River Laboratory Technology Co. Ltd. (Beijing, China). Animals were raised under specific pathogen-free condition with a barrier system with a 12-hour light/dark cycle and free access to chow and tap water. Body weights were assessed at the end of week 0, 6 and 7 using an electronic balance (Youke, Shanghai, China). The protocols and the use of rats were approved by and in accordance with The Animal Care Committee of the Peking University. The rats were treated humanely and with regard for alleviation of suffering.

2.2. $PM_{2.5}$ particle sampling and solution preparation

$PM_{2.5}$ samples were collected in March 2017 in Beijing, China for use in the present study and the detailed procedures have been published elsewhere (Feng et al., 2019; Zhang et al., 2017). Briefly, $PM_{2.5}$ were collected on quartz fiber filters (Pall Life Science, Port Washington, NY, USA) using a high-volume air sampler (ThermoFisher Scientific, Waltham, MA, USA). The filters were cut into squares of 1–2 cm^2 and agitated in ultrapure water with an ultrasonic shaker (Hechuang, Suzhou, Jiangsu, China). The solution was then frozen and dried using a vacuum freeze dryer (ThermoFisher Scientific, Waltham, MA, USA). The dried $PM_{2.5}$ was weighed and kept at −20 °C before being diluted for the experiments.

To confirm the size distribution of achieved $PM_{2.5}$ samples, equivalent spherical diameter (ESD), a commonly used parameter for particle sizing, were measured using a field emission environmental scanning electron microscopy (FEI, Hillsboro, OR, USA). Particle area was determined by Image-J software and ESD was calculated using the formula: $ESD = 2 \times (area/\pi)^{1/2}$. Chemical compositions of $PM_{2.5}$ were analyzed

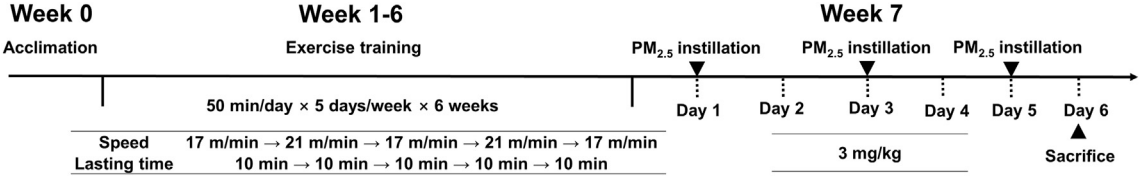

Fig. 1. Experimental protocol of exercise training and sequential $PM_{2.5}$ instillation in rats.

using inductively coupled plasma-atomic emission spectrometer (Shimadzu, Kyoto, Japan).

2.3. Exercise training and intratracheal instillation protocol

2.3.1. Exercise training

Experimental group of exercise Wistar rats were first accustomed to exercise in week 0 on a motor-driven treadmill (Zhenghua, Huaibei, Anhui, China), at a running speed of 10 m per minute at 0% inclination for 10 min per day. In the next six weeks (week 1 to 6), rats were then subjected to low intensity running (17 m/min, 10 min) at 0% inclination interspersed by moderate intensity running (21 m/min, 10 min) at 0% inclination for 50 min/day, 5 days/week. The animals were forced running in parallel tracks as groups on the same treadmill. Stimulations such as gentle physical handling and electric shock were used to secure low to moderate levels of exercise intensity, but were kept to a minimum. The groups of sedentary rats were also transported to the exercise training room, and stayed sedentary on the treadmill for the same length of time per day as those exercised on the treadmill.

2.3.2. Intratracheal instillation

Dried frozen $PM_{2.5}$ was diluted in phosphate buffer solution (PBS) at a concentration of 10 mg/mL and agitated in ultrapure water with an ultrasonic shaker for 10 min. The rats were anesthetized using intraperitoneal injection of 10% chloral hydrate (300 μL/100 g) and instilled with PBS or $PM_{2.5}$ at a volume of 300 μL/kg into the bronchus on day 1, 3 and 5 in week 7. Twenty-four hours after the last intratracheal instillation on day 6 of week 7, all rats were sacrificed. Arterial blood samples were collected in EDTA-vacuum tubes and centrifuged at 1200*g* for 15 min at 4 °C. The plasmas were stored at −80 °C before use.

2.4. Biological indicators measurement

2.4.1. Blood pressure

Blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured before sacrifice on day 6 in week 7 using a tail-cuff pressure analysis system (Softron, Tokyo, Japan) following the protocol described elsewhere (Chen et al., 2015). Before each measurement, the animals were kept within a net and sent into an insulation tube that is wrapped in a black bag to block light. The inside insulation tube was set at 37 °C and kept dark to create a comfortable and calming environment for the rats to eliminate the impact of agitation on blood pressure. Average blood pressure readings were obtained for each rat from three to five repeated measurements.

2.4.2. Vascular responses in isolated rat aortic rings

Vascular responses in isolated rat aortic rings were measured in thoracic aorta as described elsewhere (Munzel et al., 2017; Cuevas et al., 2015). To measure contractile and dilatory responses, the complete thoracic aorta was extracted after sacrifice and perivascular fat was removed. The aorta was cut into 5-mm segments in length and mounted on an 8-channel wire myograph (Danish Myo Technology, Aarhus N, Denmark). Each segment was placed in an individual organ chamber filled with 10 mL Krebs-Henseleit (K-H) solution. The chambers were kept at 37 °C and continuously aerated with 5% carbon dioxide balanced in 95% oxygen. Mounted vessels were allowed to equilibrate for 1 h to a resting distaining pressure equivalent to 1 g before being subjected to graded doses of agonists. After equilibration, vessel viability was assessed using a standard challenge of 60 mM KCl incubation. Then, the vessels were washed three times with K-H solution and allowed to equilibrate to their resting state before beginning procedures for graded dose responses.

Phenylephrine (PE) (Sigma-Aldrich, St. Louis, MO, USA) was added in graded doses (10 nM to 30 μM) to evaluate vasocontraction. PE responses were expressed as a percentage of the peak response to 60 mM KCl. Vessels were then washed three times with K-H solution and once equilibrated, vasorelaxation was evaluated by pre-contracting the segments with 3 μM PE and subsequently relaxing them with increasing concentrations (3 nM to 100 μM) of acetylcholine (ACh) (Sigma-Aldrich, St. Louis, MO, USA). Results yielded from the ACh were expressed as a percentage of pre-contraction by PE (3 μM). The maximum contraction and relaxation values were used to compare between groups.

2.4.3. Markers of vascular function

Thoracic aorta protein was extracted using a tissue homogenizer (Jingxin, Shanghai, China). The levels of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in thoracic aorta protein (150 μg) were assessed using commercial ELISA kits (Meimian, Yancheng, Jiangsu, China) according to the manufacturer's protocol. Total NO levels in thoracic aorta protein (400 μg) were assessed using a nitrate/nitrite assay kit (Beyotime, Haimen, Jiangsu, China). The levels of triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in plasma (2.5 μL) were assessed using commercial kits (Jiancheng, Nanjing, Jiangsu, China).

2.4.4. HDL function

HDL cholesterol efflux capacity (HDL-CEC) was measured by fluorescence-labeled cholesterol, which primarily evaluated ABCA1-specific CEC (Sankaranarayanan et al., 2011; Rohatgi et al., 2014). In brief, apoB was depleted in the plasma by polyethylene glycol (Sigma, St. Louis, MO, USA) (Mehta et al., 2012). RAW 264.7 macrophages were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO, Carlsbad, CA, USA) plus 10% fetal bovine serum (GIBCO, Carlsbad, CA, USA). Then, cells were plated on 96-well plates at a density of 10^4 cells/well over night. Cells were labeled by 5 μM BODIPY-cholesterol (Avanti, Miami, FL, USA) for 1 h, followed by washing with DMEM. Cells were then incubated with 2 μg/mL acyl-CoA cholesterol acyltransferase (ACAT) inhibitor (Sigma, St. Louis, MO, USA), 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP (Sigma, St. Louis, MO, USA) and DMEM containing 2.8% apoB-depleted plasma for 4 h. The resulting quantity of BODIPY cholesterol in the media or cells was individually determined with Multi-scan Spectrum (excitation 482 nm, emission 515 nm). The HDL-CEC data were expressed as % of Sed + PBS group.

HDL oxidization index (HDL-OI) was measured by the relative rates of dichlorofluorescein (DCF) in fluorescence that are proportional to the levels of lipid hydroperoxides in HDL (Navab et al., 2001; Patel et al., 2011). This cell free assay used $CuCl_2$ (Sigma, St. Louis, MO, USA) as the lipid hydroperoxides-inducing agent (Ou et al., 2005). The quantity of 10 μL dextran sulfate (10 mg/mL) (Sigma, St. Louis, MO, USA) solution was incubated with 100 μL of test plasma at room temperature for 5 min, and then centrifuged at 8000*g* for 10 min. The supernatant containing HDL-C was used in the experiments after cholesterol determination. DCFH-DA (Life Technologies, Carlsbad, CA, USA) was dissolved in fresh methanol at 0.5 mg/mL and was incubated at room temperature with protection from light for 30 min, resulting in the release of DCFH. Then, 100 μL HDL-C (10 μg/mL) was incubated with 10 μL $CuCl_2$ (60 mM) at 37 °C for 1 h using a black polystyrene 96-well plate. After incubation, 4 μL of DCFH solution was added to the HDL-Cu^{2+} mixture in each well. Rates of fluorescence (excitation, 485 nm; emission, 530 nm) were determined by Multi-scan Spectrum over the next hour at 15 min intervals. The HDL-OI data were expressed as % of Sed + PBS group.

2.5. Statistical analysis

Statistical comparisons among groups were evaluated by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) test using the SPSS 21 software package. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Chemical characterizations of $PM_{2.5}$

As shown in Fig. 2A, the PM achieved from the filters displayed differential size distributions and morphology. Over 85% of the collected PM showed an ESD less than 2.5 μm and the mean ESD of PM was 1.29 ± 0.86 μm (Fig. 2B). We observed that a small fraction of PM with an ESD over 2.5 μm which might be aggregated from small particles (i.e. $PM_{2.5}$). The chemical analysis of the $PM_{2.5}$ indicated that Ca, Fe, Al, K, and Mg were the major components of the collected $PM_{2.5}$ samples, with concentrations of 19.8, 11.1, 10.3, 6.7 and 5.2 μg/mg, respectively (Fig. 2C).

3.2. Effects of exercise training and $PM_{2.5}$ instillation on body weight and blood pressure

As shown in Fig. 3, the profiles showed increases in body weights in rats toward the end experiment in week 6 and 7 in all study rat groups. However, the body weights in exercised groups were about 10% less than that in sedentary groups at the end experiment in week 6 (Sed + PBS vs. Ex + PBS; Sed + $PM_{2.5}$ vs. Ex + $PM_{2.5}$), which persisted at the end experiment in week 7. By contrast, $PM_{2.5}$ did not show any effects on body weight between the groups of $PM_{2.5}$ instilled and non-instilled at the end experiment in week 7 (Sed + PBS vs. Sed + $PM_{2.5}$; Ex + PBS vs. Ex + $PM_{2.5}$).

Fig. 4 shows that no significant difference on the levels of blood pressure in all study rat groups remained at the end of experimental period (week 7), indicating no significant effects of exercise training or $PM_{2.5}$ instillation on blood pressure in the present study.

3.3. Exercise training prevented endothelium-dependent vasorelaxation dysfunction and NO bioavailability reduction by subsequent $PM_{2.5}$ instillation

No significant differences were observed in the percentage of PE-induced contraction across groups (Fig. 5A) and were therefore used to normalize Ach-mediated vasorelaxation. The results demonstrated that $PM_{2.5}$ instillation significantly reduced the maximum Ach-induced vasorelaxation in sedentary rats (Sed + PBS vs. Sed + $PM_{2.5}$) but not in exercised rats (Ex + PBS vs. Ex + $PM_{2.5}$) (Fig. 5B). Similarly, the levels of NO and eNOS in thoracic aorta were significantly reduced in sedentary rats but not in exercised rats after $PM_{2.5}$ instillation (Fig. 5C–D). By contrast, the levels of iNOS (Fig. 5E) remained unchanged both in sedentary and exercised rats after $PM_{2.5}$ instillation. These findings suggested that exercise training could increase NO production and inhibit endothelium-dependent vasorelaxation dysfunction induced by $PM_{2.5}$ exposure.

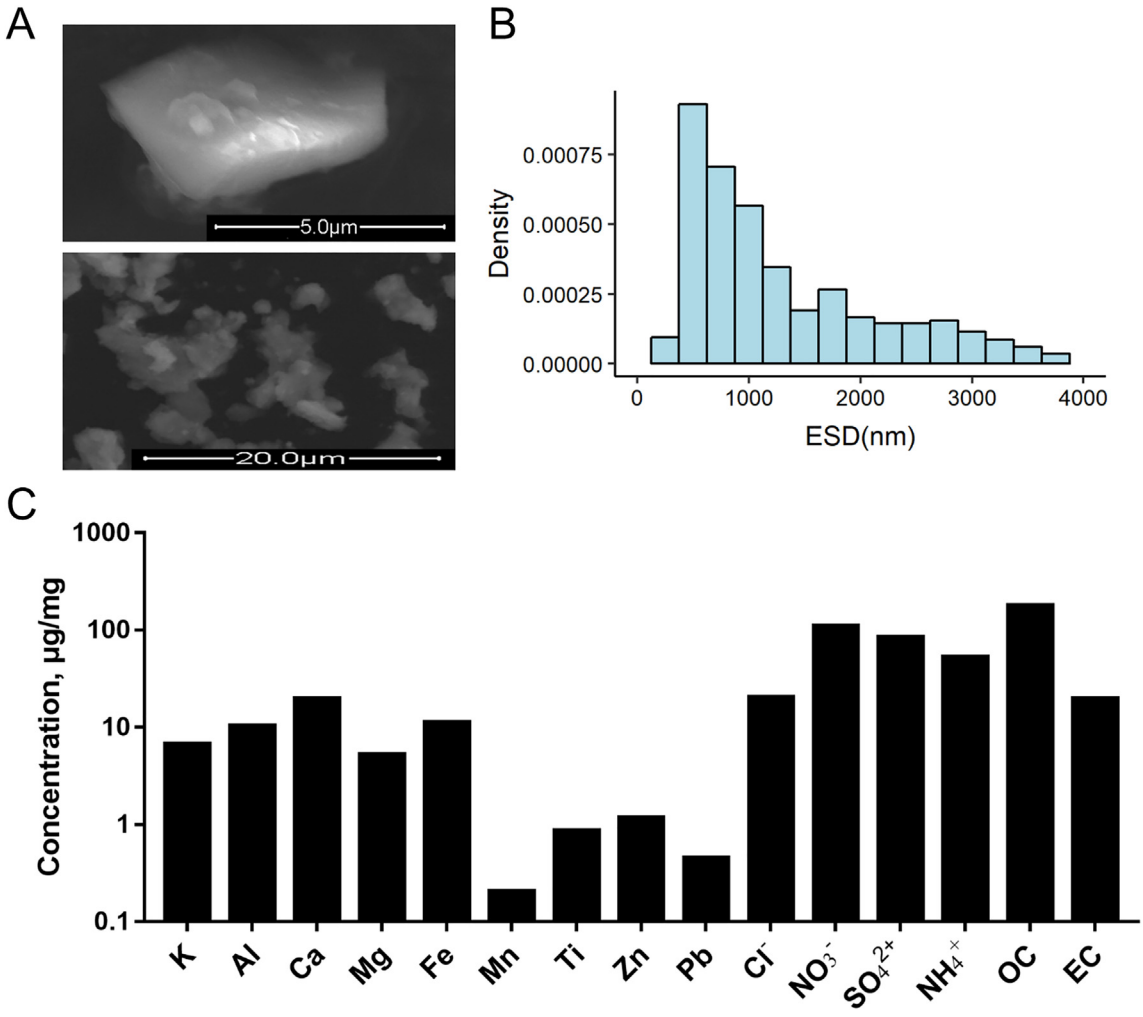

Fig. 2. Characteristics of ambient $PM_{2.5}$ collected for experimental instillation. (A) Scanning electron micrographs of collected $PM_{2.5}$ mass samples. (B) ESD distribution of collected $PM_{2.5}$. (C) Chemical compositions of collected $PM_{2.5}$ (μg/mg).

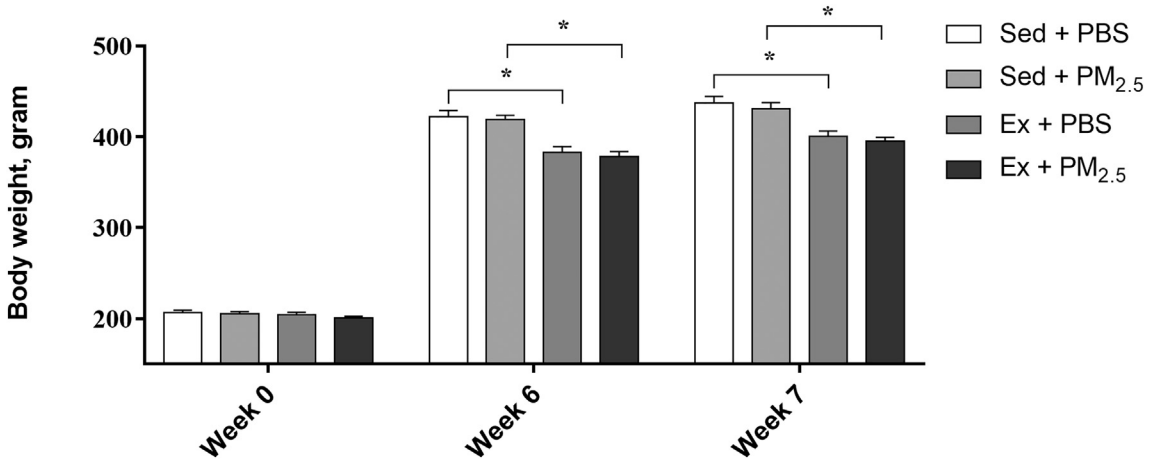

Fig. 3. Body weight of rats in groups received exercise training followed by $PM_{2.5}$ instillation.

3.4. Exercise training promoted HDL function

To explore the possible effect of exercise training on HDL, we measured HDL function including HDL-CEC and HDL-OI. As shown in Fig. 6, exercised rats had relatively higher levels of HDL-CEC and lower levels of HDL-OI compared with sedentary rats (Sed + PBS vs. Ex + PBS; Sed + $PM_{2.5}$ vs. Ex + $PM_{2.5}$), indicating that exercise training could significant promote HDL function. However, the levels of HDL-CEC and HDL-OI showed no significant changes between the groups of Sed + PBS and Sed + $PM_{2.5}$, Ex + PBS and Ex + $PM_{2.5}$, indicating that $PM_{2.5}$ instillation didn't significantly alter HDL function. Consistently, the levels of HDL-C showed significant changes with higher level in exercised rats (Sed + PBS vs. Ex + PBS; Sed + $PM_{2.5}$ vs. Ex + $PM_{2.5}$); while the levels of triglyceride, total cholesterol, and LDL-cholesterol mostly remained similar across groups with no significant effects by exercise training or $PM_{2.5}$ instillation (Fig. 7).

4. Discussion

In this study, we observed that exercise training could effectively promote HDL function, and prevent nitric oxide bioavailability reduction and endothelium-dependent vasorelaxation dysfunction from subsequent $PM_{2.5}$ instillation, which indicate potential beneficial effects of exercise training on endothelial function impaired by exposure to $PM_{2.5}$. These findings support the public health importance of regular exercise training that could induce adaptive responses through which increase cardiovascular capacity to cope with external stimulus such as air pollutants.

The major finding of the present study is that exercise training inhibited endothelium-dependent vasorelaxation dysfunction induced by subsequent $PM_{2.5}$ exposure. Exercise training has been recognized as an effective measure for preventing cardiovascular events in animals and human (Schuler et al., 2013; Ashton et al., 2018); however, few studies have been conducted under air pollution exposure. In a cigarette smoke-exposed mouse model, an eight-week prior exercise training was shown to alleviate respiratory inflammation induced by exposure to environmental cigarette smoke (Yu et al., 2012). Martinez-Campos et al. (2012) reported that a two-week exercise training significantly blocked ozone exposure induced increase in the levels of 8-isoprostane, malondialdehyde (MDA) and carbonyls in the plasma of Wistar rats. Epidemiological studies also showed that regular exercise training could be a protective factor against the effects that pollution have on cognition, oxygen transport, arterial stiffness and premature deaths (Chen et al., 2018; Wong et al., 2007; Molina-Sotomayor et al., 2019). However, one experimental study revealed that intratracheal instillation of residual oil fly ash in rats before an acute session of moderate intensity exercise promoted an increase in the level of MDA and a decrease in the activity of catalase in lung tissue (Heck et al., 2014). In

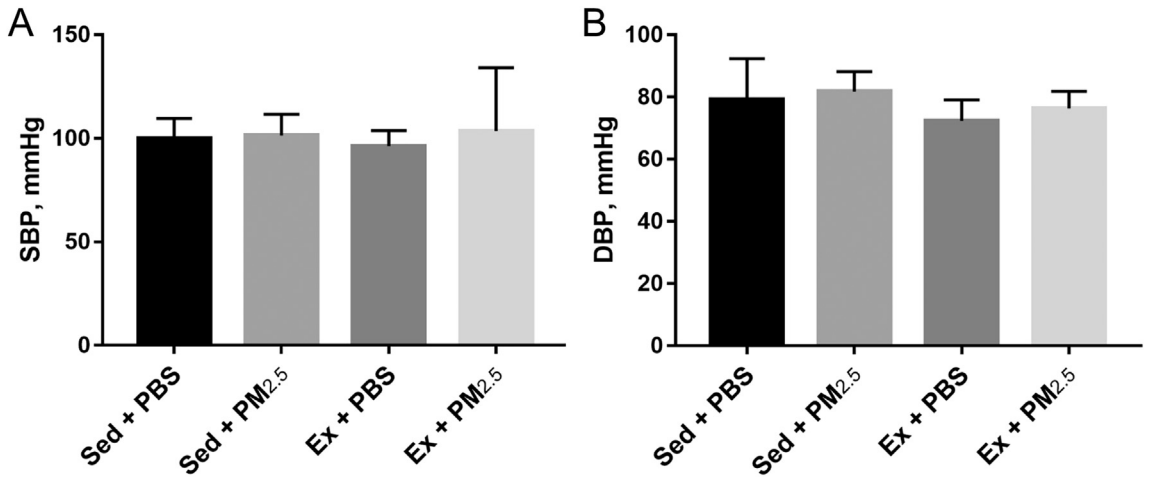

Fig. 4. Effect of exercise training and $PM_{2.5}$ instillation on (A) SBP, (B) DBP in groups received exercise training followed by $PM_{2.5}$ instillation.

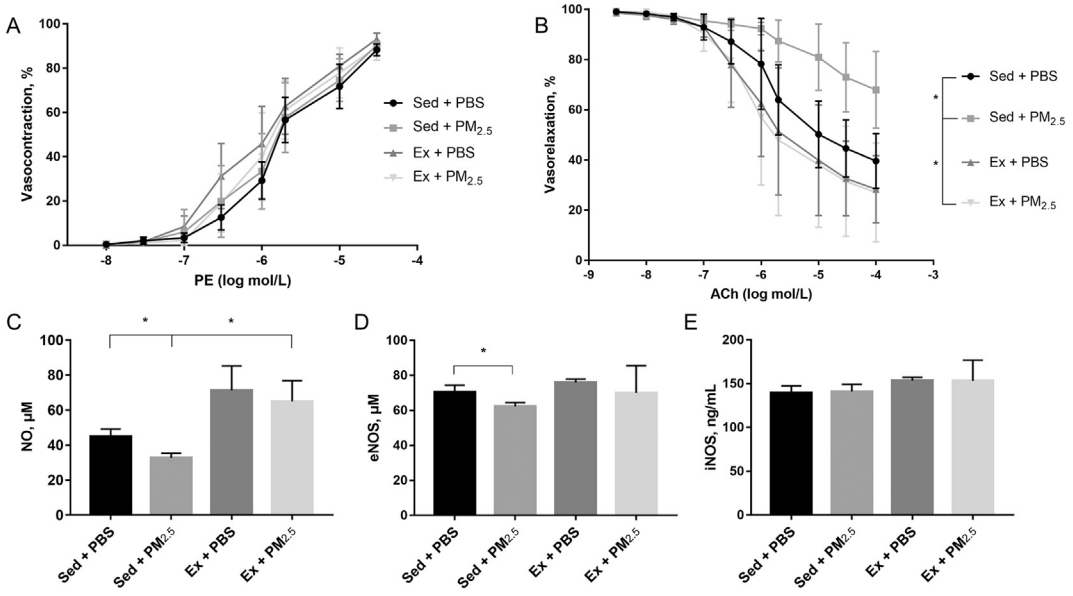

Fig. 5. Vascular responses of thoracic aorta. (A) Vasocontraction percent to KCl. (B) Vasorelaxation percent to phenylephrine. (C–E) Levels of NO, eNOS and iNOS in thoracic aorta.

a randomized crossover study, the extent of improvement in pulmonary function and pulse wave velocity observed in subjects with chronic obstructive pulmonary disease (COPD) and ischemic heart disease (IHD) were attenuated while walking in cleaner environment in comparison with walking in more polluted environment (Sinharay et al., 2018). Therefore, the question of whether exercise training performed under air pollution would introduce different consequence remains as an attractive hypothesis that needs further investigation.

Although it has been well documented that exercise training can bring health benefits, the exact protective mechanism by which it may protect against air pollution associated adverse cardiovascular effects remains unclear. It is proposed that exercise training may enable the body to gain certain adaptive responses to strengthen resistance against pathological processes promoted by air pollutants (Wong et al., 2007). Several studies have revealed that exercise training could enhance the activity of antioxidant enzymes and reduce the expression of inflammatory cytokines (Nemmar and Al-Salam, 2018; Abhijit et al., 2018). Additionally, exercise training was found to up-regulate the expression of genes related to energy pathways and lipid metabolism (Robinson et al., 2017; Ling and Rönn, 2014; Dantas et al., 2017). In the present study, we further observed that exercise training significantly promoted HDL function characterized as increased HDL cholesterol level, cholesterol efflux capacity, and reduced oxidization index, culminating in an increased NO production, a potent vasodilator. It has been well established that HDL could confer protection against vascular endothelial dysfunction through several eNOS/NO-dependent ways (Luscher et al., 2014). HDL could activate eNOS by regulating the cholesterol homeostasis of caveolae where eNOS locates and thus facilitating the interaction of caveolin 1 and eNOS (Garcia and Sessa, 2019). Furthermore, several components carried by HDL like sphingosine-1-phosphate (S1P) could induce eNOS phosphorylation by binding to corresponding receptors and thereby activating PI3K/Akt/eNOS signaling pathway (Keul et al., 2019). As a resultant vasoprotection of increased NO production, exercise training inhibited endothelium-dependent vasorelaxation dysfunction induced by $PM_{2.5}$ instillation as observed in the present study. However, in spite of an increased level of NO, we

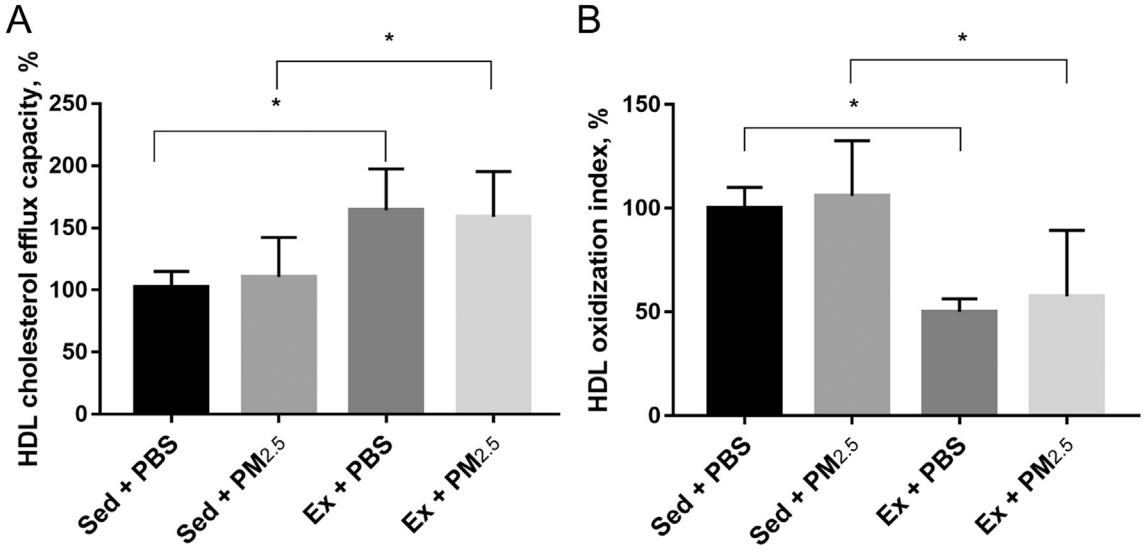

Fig. 6. Exercise promoted HDL function. (A) HDL cholesterol efflux capacity. (B) HDL oxidization index.

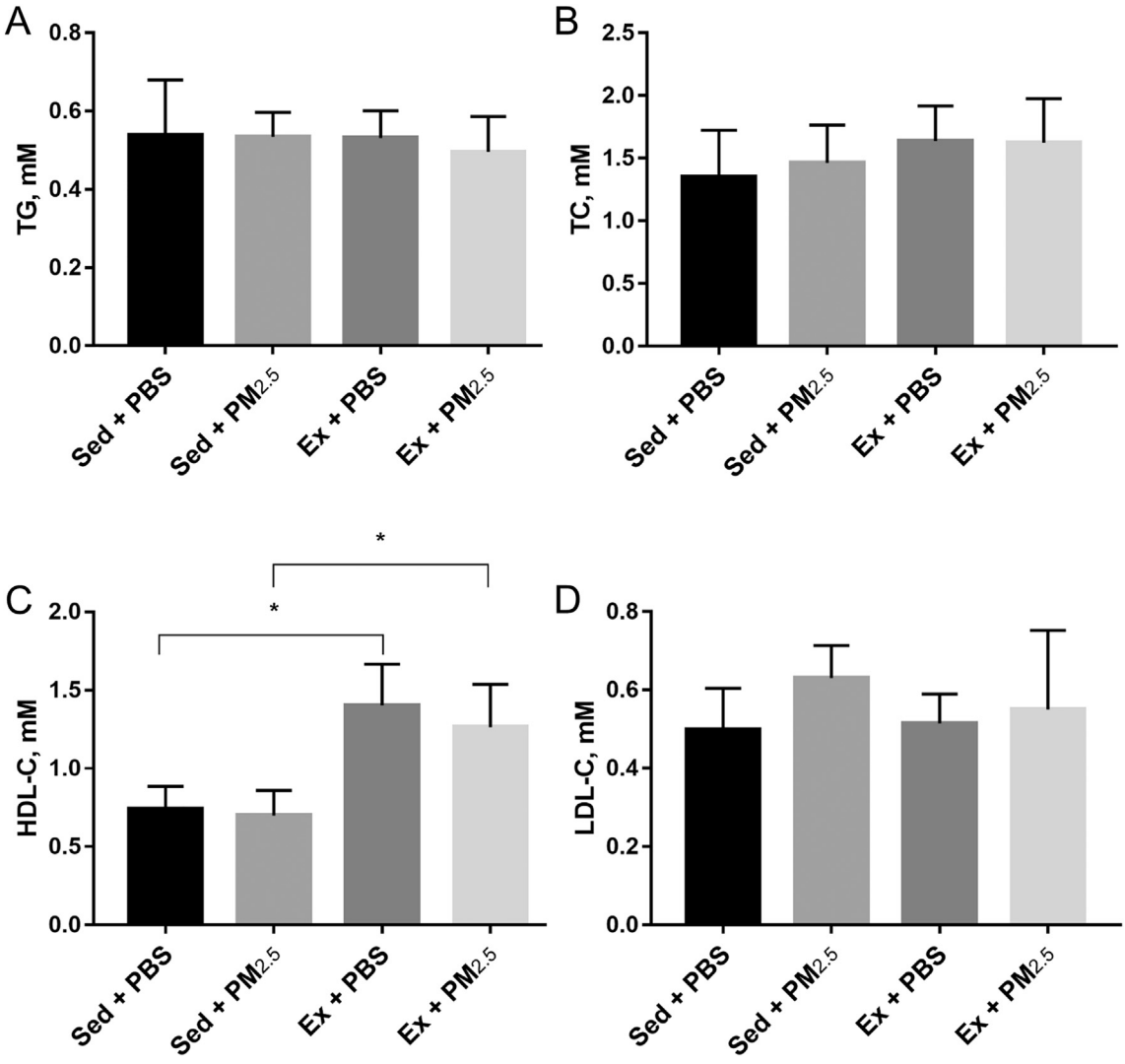

Fig. 7. Levels of (A) triglyceride, (B) total cholesterol, (C) HDL-cholesterol, and (D) LDL-cholesterol in plasma.

didn't observe significant effect of exercise training on total eNOS level. This may because exercise training only modifies the activity but not the expression of eNOS. The activity of eNOS is regulated by phosphorylation on several residues. The activity increases when phosphorylated at Ser-1177, Ser-635 and Ser-617, while decreases at Ser-116 and Thr-495 (Garcia and Sessa, 2019). Therefore, we speculated that the exercise-induced increase in NO bioavailability and finally the restoration of endothelial function impaired by subsequent $PM_{2.5}$ exposure may partly due to some altered HDL functions.

We observed that $PM_{2.5}$ instillation significantly reduced the levels of eNOS expression and NO bioavailability. Several studies have suggested that uncoupling of eNOS can act as a crucial step in vasomotor dysfunction and progression of atherosclerosis after PM exposure (Moller et al., 2016). Once uncoupled, the eNOS may produce superoxide, dramatically reducing the vasculature's ability to relax through this primary pathway (Yan et al., 2017). Using L-nitro-arginine-methyl-ester, an eNOS inhibitor, Knuckles et al. (2008) identified the uncoupling of eNOS as a mechanism for enhanced vasocontraction after diesel exhaust exposure. Simultaneously, the reduced NO bioavailability after $PM_{2.5}$ instillation may also attributed to a higher consumption by reactive oxygen species (Nurkiewicz et al., 2011). However, the reduced NO bioavailability observed in the present study may be independent of HDL function, as little effects on HDL-CEC and HDL-OI were observed after $PM_{2.5}$ instillation.

Several potential limitations of the present study should be taken into consideration. First, the study rats were under training for 6 weeks in a purified space, which limit generalizability of study results to exercise conducted in open environment with day-to-day exposure to air pollution. As aforementioned above, exercise training in polluted environment may curtail or even reverse the cardiorespiratory benefits of exercise (Sinharay et al., 2018; Heck et al., 2014). Second, the study rats only received moderate-intensity interval treadmill training, no other parallel exercise training groups like low-intensity or high-intensity were placed, which might limit our ability to identify the intensity of exercise training required for optimal benefits. Third, we didn't set up other dose groups of $PM_{2.5}$ instillation. Therefore, we were unable to assess the dose-response relationship between $PM_{2.5}$ instillation and endothelium-dependent vasorelaxation dysfunction. And lastly, we cannot determine if the alterations in HDL function have any causal relevance for NO bioavailability and endothelium-dependent vasorelaxation.

Considering the limitations of the present study, future study is warranted to examine the impact of exercise training on mitigating air pollution induced physiological responses, with respect to exercise type, duration, intensity and frequency. It may be important to use gene knockout rat models in future research to better understand the mechanisms that how exercise training might introduce potential improvements in endothelial function under air pollution exposure. In

addition, future study to leverage peripheral leukocytes from blood samples and next generation gene expression analysis may also provide additional surrogate markers to illustrate physiologic pathways involved in cardioprotective benefits.

5. Conclusions

In this study, we showed that exercise training promoted HDL function and protected against endothelium dysfunction from $PM_{2.5}$ instillation. These changes contribute to the normalization of the altered vascular function after $PM_{2.5}$ exposure by increasing endothelium dependent relaxation. Our findings provide strong evidence that regular exercise training is beneficial to the vascular system and could be considered as one of the intervention measures to reduce adverse cardiovascular responses of short-term air pollution exposure, which can be of potential public health importance.

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Declaration of competing interest

None.

References

Abhijit, S., Tripathi, S.J., Bhagya, V., Shankaranarayana Rao, B.S., Subramanyam, M.V., Asha Devi, S., 2018. Antioxidant action of grape seed polyphenols and aerobic exercise in improving neuronal number in the hippocampus is associated with decrease in lipid peroxidation and hydrogen peroxide in adult and middle-aged rats. Exp. Gerontol. 101, 101–112.

Adams, V., Besler, C., Fischer, T., Riwanto, M., Noack, F., Hollriegel, R., et al., 2013. Exercise training in patients with chronic heart failure promotes restoration of high-density lipoprotein functional properties. Circ. Res. 113 (12), 1345–1355.

Ashton, R.E., Tew, G.A., Aning, J.J., Gilbert, S.E., Lewis, L., Saxton, J.M., 2018. Effects of short-term, medium-term and long-term resistance exercise training on cardiometabolic health outcomes in adults: systematic review with meta-analysis. Br. J. Sports Med. 0, 1–9 (bjsports-2017-098970).

Bolcas, P.E., Brandt, E.B., Zhang, Z., Biagini Myers, J.M., Ruff, B.P., Khurana Hershey, G.K., 2019. Vitamin D supplementation attenuates asthma development following traffic-related particulate matter exposure. J. Allergy Clin. Immunol. 143 (1), 386–394.e3.

Casella-Filho, A., Chagas, A.C., Maranhao, R.C., Trombetta, I.C., Cesena, F.H., Silva, V.M., et al., 2011. Effect of exercise training on plasma levels and functional properties of high-density lipoprotein cholesterol in the metabolic syndrome. Am. J. Cardiol. 107 (8), 1168–1172.

Chen, Z., Wang, Y., Zhuo, L., Chen, S., Zhao, L., Luan, X., et al., 2015. Effect of titanium dioxide nanoparticles on the cardiovascular system after oral administration. Toxicol. Lett. 239 (2), 123–130.

Chen, X., Chen, W., Wang, Y., Han, Y., Zhu, T., 2018. Responses of healthy young males to fine-particle exposure are modified by exercise habits: a panel study. Environ. Health 17 (1), 88.

Cohen, A.J., Brauer, M., Burnett, R., Anderson, H.R., Frostad, J., Estep, K., et al., 2017. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet 389 (10082), 1907–1918.

Cuevas, A.K., Niu, J., Zhong, M., Liberda, E.N., Ghio, A., Qu, Q., et al., 2015. Metal rich particulate matter impairs acetylcholine-mediated vasorelaxation of microvessels in mice. Part Fibre Toxicol. 12, 14.

Dantas, W.S., Murai, I.H., Perandini, L.A., Azevedo, H., Moreira-Filho, C.A., Camara, N.O., et al., 2017. Acute exercise elicits differential expression of insulin resistance genes in the skeletal muscle of patients with polycystic ovary syndrome. Clin. Endocrinol. 86 (5), 688–697.

Feng, B., Li, L., Xu, H., Wang, T., Wu, R., Chen, J., et al., 2019. PM2.5-bound polycyclic aromatic hydrocarbons (PAHs) in Beijing: seasonal variations, sources, and risk assessment. J. Environ. Sci. (China) 77, 11–19.

Ganjali, S., Momtazi, A.A., Banach, M., Kovanen, P.T., Stein, E.A., Sahebkar, A., 2017. HDL abnormalities in familial hypercholesterolemia: focus on biological functions. Prog. Lipid Res. 67, 16–26.

Garcia, V., Sessa, W.C., 2019. Endothelial NOS: perspective and recent developments. Br. J. Pharmacol. 176 (2), 189–196.

Giles, L.V., Koehle, M.S., 2014. The health effects of exercising in air pollution. Sports Med. 44 (2), 223–249.

Heck, T.G., Nunes, R.B., Petry, M.R., Maslinkiewicz, A., Saldiva, P.H.N., Lago, P.D., et al., 2014. Residual oil fly ash (ROFA) inhalation promotes lung and heart oxidative stress without hemodynamic effects in exercising rats. J. Exercise Physiologyonline 17 (1), 78–89.

Keul, P., Polzin, A., Kaiser, K., Graler, M., Dannenberg, L., Daum, G., et al., 2019. Potent anti-inflammatory properties of HDL in vascular smooth muscle cells mediated by HDL-S1P and their impairment in coronary artery disease due to lower HDL-S1P: a new aspect of HDL dysfunction and its therapy. FASEB J. 33 (1), 1482–1495.

Knuckles, T.L., Lund, A.K., Lucas, S.N., Campen, M.J., 2008. Diesel exhaust exposure enhances venoconstriction via uncoupling of eNOS. Toxicol. Appl. Pharmacol. 230 (3), 346–351.

Krishnan, R.M., Adar, S.D., Szpiro, A.A., Jorgensen, N.W., Van Hee, V.C., Barr, R.G., et al., 2012. Vascular responses to long- and short-term exposure to fine particulate matter: MESA air (multi-ethnic study of atherosclerosis and air pollution). J. Am. Coll. Cardiol. 60 (21), 2158–2166.

Li, B., Hu, Y., Zhao, Y., Cheng, M., Qin, H., Cheng, T., et al., 2017. Curcumin attenuates titanium particle-induced inflammation by regulating macrophage polarization in vitro and in vivo. Front. Immunol. 8, 55.

Ling, C., Rönn, T., 2014. Epigenetic adaptation to regular exercise in humans. Drug Discov. Today 19 (7), 1015–1018.

Lundberg, J.O., Gladwin, M.T., Weitzberg, E., 2015. Strategies to increase nitric oxide signalling in cardiovascular disease. Nat. Rev. Drug Discov. 14 (9), 623–641.

Luscher, T.F., Landmesser, U., von Eckardstein, A., Fogelman, A.M., 2014. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. Circ. Res. 114 (1), 171–182.

Martinez-Campos, C., Lara-Padilla, E., Bobadilla-Lugo, R.A., Kross, R.D., Villanueva, C., 2012. Effects of exercise on oxidative stress in rats induced by ozone. ScientificWorldJournal 2012, 135921.

Mehta, N.N., Li, R., Krishnamoorthy, P., Yu, Y., Farver, W., Rodrigues, A., et al., 2012. Abnormal lipoprotein particles and cholesterol efflux capacity in patients with psoriasis. Atherosclerosis 224 (1), 218–221.

Molina-Sotomayor, E., Gonzalez Orb, M., Pradas de la Fuente, F., Carozzi Figueroa, G., Sanchez-Oliver, A.J., Gonzalez-Jurado, J.A., 2019. Effects of cardiorespiratory exercise on cognition in older women exposed to air pollution. Int. J. Environ. Res. Public Health 16 (2).

Moller, P., Mikkelsen, L., Vesterdal, L.K., Folkmann, J.K., Forchhammer, L., Roursgaard, M., et al., 2011. Hazard identification of particulate matter on vasomotor dysfunction and progression of atherosclerosis. Crit. Rev. Toxicol. 41 (4), 339–368.

Moller, P., Christophersen, D.V., Jacobsen, N.R., Skovmand, A., Gouveia, A.C., Andersen, M.H., et al., 2016. Atherosclerosis and vasomotor dysfunction in arteries of animals after exposure to combustion-derived particulate matter or nanomaterials. Crit. Rev. Toxicol. 46 (5), 437–476.

Munzel, T., Daiber, A., Steven, S., Tran, L.P., Ullmann, E., Kossmann, S., et al., 2017. Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. Eur. Heart J. 38 (37), 2838–2849.

Navab, M., Hama, S.Y., Hough, G.P., Subbanagounder, G., Reddy, S.T., Fogelman, A.M., 2001. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. J. Lipid Res. 42 (8), 1308–1317.

Nemmar, A., Al-Salam, S., 2018. Exercise Training Mitigates Water Pipe Smoke Exposure-Induced Pulmonary Impairment Via Inhibiting NF-kappaB and Activating Nrf2 Signalling Pathways (2018, 7459612).

Nurkiewicz, T.R., Porter, D.W., Hubbs, A.F., Stone, S., Moseley, A.M., Cumpston, J.L., et al., 2011. Pulmonary particulate matter and systemic microvascular dysfunction. Res. Rep. Health Eff. Inst. (164), 3–48.

Ou, J., Wang, J., Xu, H., Ou, Z., Sorci-Thomas, M.G., Jones, D.W., et al., 2005. Effects of D-4F on vasodilation and vessel wall thickness in hypercholesterolemic LDL receptor-null and LDL receptor/apolipoprotein A-I double-knockout mice on Western diet. Circ. Res. 97 (11), 1190–1197.

Patel, P.J., Khera, A.V., Jafri, K., Wilensky, R.L., Rader, D.J., 2011. The anti-oxidative capacity of high-density lipoprotein is reduced in acute coronary syndrome but not in stable coronary artery disease. J. Am. Coll. Cardiol. 58 (20), 2068–2075.

Rader, D.J., Hovingh, G.K., 2014. HDL and cardiovascular disease. Lancet 384 (9943), 618–625.

Rao, X., Zhong, J., Brook, R.D., Rajagopalan, S., 2018. Effect of particulate matter air pollution on cardiovascular oxidative stress pathways. Antioxid. Redox Signal. 28 (9), 797–818.

Robinson, M.M., Dasari, S., Konopka, A.R., Johnson, M.L., Manjunatha, S., Esponda, R.R., et al., 2017. Enhanced protein translation underlies improved metabolic and physical adaptations to different exercise training modes in young and old humans. Cell Metab. 25 (3), 581–592.

Rohatgi, A., Khera, A., Berry, J.D., Givens, E.G., Ayers, C.R., Wedin, K.E., et al., 2014. HDL cholesterol efflux capacity and incident cardiovascular events. N. Engl. J. Med. 371 (25), 2383–2393.

Sankaranarayanan, S., Kellner-Weibel, G., de la Llera-Moya, M., Phillips, M.C., Asztalos, B.F., Bittman, R., et al., 2011. A sensitive assay for ABCA1-mediated cholesterol efflux using BODIPY-cholesterol. J. Lipid Res. 52 (12), 2332–2340.

Sarzynski, M.A., Burton, J., Rankinen, T., Blair, S.N., Church, T.S., Després, J.-P., et al., 2015. The effects of exercise on the lipoprotein subclass profile: a meta-analysis of 10 interventions. Atherosclerosis 243 (2), 364–372.

Schuler, G., Adams, V., Goto, Y., 2013. Role of exercise in the prevention of cardiovascular disease: results, mechanisms, and new perspectives. Eur. Heart J. 34 (24), 1790–1799.

Shao, D., Du, Y., Liu, S., Brunekreef, B., Meliefste, K., Zhao, Q., et al., 2017. Cardiorespiratory responses of air filtration: a randomized crossover intervention trial in seniors living in Beijing: Beijing Indoor Air Purifier StudY, BIAPSY. Sci. Total Environ. 603-604, 541–549.

Sinharay, R., Gong, J., Barratt, B., Ohman-Strickland, P., Ernst, S., Kelly, F.J., et al., 2018. Respiratory and cardiovascular responses to walking down a traffic-polluted road compared with walking in a traffic-free area in participants aged 60 years and older with chronic lung or heart disease and age-matched healthy controls: a randomised, crossover study. Lancet 391 (10118), 339–349.

Tong, H., Rappold, A.G., Caughey, M., Hinderliter, A.L., Bassett, M., Montilla, T., et al., 2015. Dietary supplementation with olive oil or fish oil and vascular effects of concentrated ambient particulate matter exposure in human volunteers. Environ. Health Perspect. 123 (11), 1173–1179.

Wong, C.-M., Ou, C.-Q., Thach, T.-Q., Chau, Y.-K., Chan, K.-P., Ho, S.-Y., et al., 2007. Does regular exercise protect against air pollution-associated mortality? Prev. Med. 44 (5), 386–392.

Yan, F.X., Li, H.M., Li, S.X., He, S.H., Dai, W.P., Li, Y., et al., 2017. The oxidized phospholipid POVPC impairs endothelial function and vasodilation via uncoupling endothelial nitric oxide synthase. J. Mol. Cell. Cardiol. 112, 40–48.

Yu, Y.B., Liao, Y.W., Su, K.H., Chang, T.M., Shyue, S.K., Kou, Y.R., et al., 2012. Prior exercise training alleviates the lung inflammation induced by subsequent exposure to environmental cigarette smoke. Acta Physiol. (Oxf.) 205 (4), 532–540.

Zhang, S.Y., Shao, D., Liu, H., Feng, J., Feng, B., Song, X., et al., 2017. Metabolomics analysis reveals that benzo[a]pyrene, a component of PM2.5, promotes pulmonary injury by modifying lipid metabolism in a phospholipase A2-dependent manner in vivo and in vitro. Redox Biol. 13, 459–469.

Zhong, J., Karlsson, O., Wang, G., Li, J., Guo, Y., Lin, X., et al., 2017. B vitamins attenuate the epigenetic effects of ambient fine particles in a pilot human intervention trial. Proc. Natl. Acad. Sci. U. S. A. 114 (13), 3503–3508.