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Moderate-intensity physical activity reduces systemic inflammation and maintains cardiorespiratory function following chronic particulate matter$_{2.5}$ exposure in rats

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ABSTRACT

Aims: The purpose of the current study is to 1) examine the beneficial effects of moderate levels of physical activity (PA) on functional and biochemical markers of the cardiorespiratory system, 2) establish the detrimental effects of a single, daily particulate matter (PM) exposure event on cardiorespiratory function and 3) determine if exercising during daily PM exposure increases the deleterious effects caused by PM exposure due to increased inhalation of particulates on cardiorespiratory function.

Methods: Four groups of 16 rats were used: control (CON), PA, $PM_{2.5}$ exposed and PA combined with $PM_{2.5}$ exposure (PA + PM). Animals were purchased at 4 weeks old. However, both PA and PM exposure was initiated when the animals reached 8 weeks of age, for 8 weeks.

Results: PA alone did not alter body weight or blood pressure (BP) compared to control animals. However, there was a significant decrease in epididymal fat pad mass in the PA group. The PM exposed rats were hypertensive, showed increased systemic inflammation and oxidative stress, and had decreased spleen mass without pathological changes in the cardiac action potential or impaired vascular function. PA was able to decrease systemic inflammation in PM exposed animals, including a reduction in IL-6 serum levels, however, this did not translate to an improvement in BP or vascular reactivity. Smooth muscle relaxation in the trachea from the combination PA + PM group was not significantly different to CON and PA groups but was significantly higher than the PM group.

Conclusions: The current study showed that while there is an increased cardiovascular disease (CVD) risk associated with PM exposure, engaging in PA during exposure events imposes no increased risk with exercise providing a protective mechanism against some of the biochemical signaling changes caused by inhaled PM.

1. Introduction

A physically inactive lifestyle has been shown to be detrimental to long-term health as evidenced by the associated increased risk of obesity and many chronic diseases including cardiovascular disease (CVD), metabolic syndrome and diabetes [1–3]. Physical activity (PA) is associated with improved metabolic function and reduced systemic inflammation [4]. Regular PA engagement is also linked to a reduction in the risk of developing specific CVDs such as endothelial dysfunction, atherosclerosis, hypertension and cardiovascular events irrespective of age, body mass index and ethnicity [4]. Weight-loss that is often associated with PA has been shown to have many health benefits including improvement of endothelial dysfunction and decreased systemic inflammation [5]. Weight-loss induced by both dietary and PA changes also results in similar health benefits [6,7]. Rodent studies using treadmill running at 1 h/day, 5 days/weeks for 13 weeks were able to diminish diet-induced obesity and mild hypertension [8]. Similarly, swim training of rats 2 h/day, 5days/week for 6 weeks showed that PA reduced insulin resistance induced via a high-fat diet [9]. Therefore, moderate intensity PA can prevent key changes induced by metabolic syndrome and obesity. It has also been shown that strenuous and extreme physical activity can also increase levels of inflammatory cytokines [10–12]. Marathon runners have been found to have increased TNF-α, IL-6 and IL-1β following a race [10,11]. This makes duration and intensity an important consideration when considering physical activity to reduce risk of CVD development or treatment.

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Particulate matter (PM) is a component of air pollution and its composition and size vary from location to location [13]. Most common sources of PM are traffic exhaust and the manufacturing industry. Studies show that elemental analysis of exhaust samples contained metals hazardous to organisms such as Al, Cd, Cr, Cu, Fe, Mg, Ni, Pb, and Zn [14]. Animal and epidemiological studies demonstrate that both acute and chronic PM exposure increases risk of cardiovascular events and can accelerate the progression of the disease [15–17]. Biochemically, PM exposure has been associated with increases in systemic tumour necrosis factor-α and interleukin-6 levels [18]. These findings are consistent with short-term studies of *in vitro* and *in vivo* inhalational exposure to PM, producing both marked pulmonary and also systemic inflammatory responses [19]. Oxidative stress in the microvascular wall has also been shown to be increased after PM exposure [20] which together with the heightened inflammatory response may provide a mechanism for the initiation of CVD however the precise mechanisms are yet to be established. The majority of people have increased exposure to PM and pollution when engaged in outdoor leisure or exercise activities in high population dense/close to the roadside/city areas.

PA conducted close to the roadside increases the risk of PM exposure [21,22]. Given this evidence and widespread promotion of active travel as a means to meet PA guidelines [23] which commonly occurs near roads, the role of PA in PM increasing the risk of CVD should be explored further. Individuals who engage in walking as their form of PA have been shown to have a higher level of exposure to PM ($495\,\mu g/m^{-3}$) than other modes of transport [24]. Another study showed that volunteers who completed three 8-h trips during which they alternated using a car, the subway and walking all while traveling the same route encountered similar high levels of exposure in New York City [25]. This increase in active travel also accompanies rising inhalation rates [22] and increased airflow velocity, leading to an increase in the quantity of pollutants and PM inhaled deeper into the respiratory tract [21].

The purpose of the current study is to 1) examine the beneficial effects of moderate levels of PA on functional and biochemical markers of the cardiorespiratory system, 2) establish the detrimental effects of moderate, daily but chronic PM exposure on cardiorespiratory function and 3) determine if engaging in PA during PM exposure increases the deleterious effects caused by PM exposure due to increased inhalation of particulates on cardiorespiratory function.

2. Methods

2.1. Animals and animal care

Male Wistar rats were randomized into one of four experimental groups: control (CON), physical activity (PA), particulate matter exposed (PM) and PA combined with particulate matter exposure (PA + PM). Animals were purchased at 4 weeks old. PA and PM exposure was initiated (PM, PA, and PA + PM) when the animals reached 8 weeks of age for a period of 8 weeks (Fig. 1) with 16 animals in each group. There were 16 animals in each group. All animals were euthanized at 16 weeks of age. Control animals were fed standardized rat and mouse nuts (Norco Stockfeeds; South Lismore, NSW, Australia) and were exposed to room air. All experimental procedures were approved by the CQUniversity Animal Ethics Research Committee (approval A10/11-265) and were conducted in accordance with the National Health and Medical Research Council (NHMRC) guidelines.

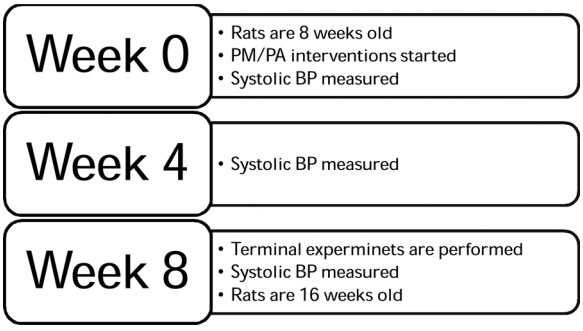

Fig. 1. Timeline of study design.

2.2. Physical activity protocol

The physical activity protocol used in the groups subjected to physical activity (PA and PA + PM), was that described in van Waveren et al. [26]. Being 30 min long to match the time frame of PM exposure described below.

2.3. Exposure to $PM_{2.5}$

PM and PA + PM groups were subjected to whole-body inhalation 400 μg/m3 of $PM_{2.5}$ for 30 min per day, 5 days per week in an enclosed chamber. PA + PM groups were exposed during engagement in PA. The CON and PA groups were exposed to room air under the same environmental conditions. PM was obtained from Powder Technology Inc. (Burnsville, MN, USA). Constituents of the PM used included silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), iron (III) oxide (Fe_2O_3), sodium oxide (Na_2O), calcium oxide (CaO), magnesium oxide (MgO), titanium oxide (TiO_2) and potassium oxide (K_2O). Typical chemical composition was SiO_2 68–76 %, Al_2O_3 10–15 %, Fe_2O_3 2–5 %, Na_2O 2–4 %, CaO 2–5 %, MgO 1–2 %, TiO_2 0.5–1 % and K_2O 2-5 % of the total weight. Studies have previously used commercially purchased PM to produce adverse health effects in animal models [15]. The concentration of PM was based upon epidemiological studies, including, current dosages used in human and animal models of pollution [27] and current National Environment Protection Measures for Ambient Air Quality of exposure to PM, set by the National Environment Protection Council. Product was chosen to simulate a combination of natural sources (including top-soil) of PM and man-made sources (industrial, commercial, automotive, etc.). The PM exposure concentration was $400\,\mu g/m^3$ over the 30 minute exposure period. This was measured using TSI incorporated (Shoreview, MN, USA), SIDEPAK personal aerosol monitor, model AM510. The PM was aerosolized using a vibrating platform, which was adjusted manually to control dispersion.

2.4. Body mass and systolic blood pressure

Using similar methods to Fenning et al., 2005 [28], systolic BP readings were taken at treatment weeks 0, 4, 8 and 12, via tail-cuff plethysmography and body mass collected weekly for all groups.

2.5. Terminal assessments

Rats were euthanized using a 0.2 ml/kg i.p. Injection of sodium pentobarbitone (375 mg/ml), following the end of the treatment period. A 5 ml blood sample was collected after euthanasia from the abdominal vena cava and internal organs were removed and weighed including the heart, kidneys, liver, spleen and fat pads.

2.6. 4-HNE, NO, IL-6 and IL-1B

Biochemical assessments were performed using ELISA kits- Cell Biolabs' Oxiselect ™ HNE adduct ELISA kit (Catalog Number STA-338) (Biolabs, San Diego, CA, USA), NO (total) Detection Kit (Catalog Number ADI-917-020) (Enzo Life Sciences Int'l, INC. Butler Pike Plymouth Meeting, PA, USA), R&D Systems Quantikine Rat IL-1β/IL-1F2 Immunoassay (Catalog Number RLB00) (R&D Systems, Minneapolis, MN, USA) and R&D Systems Quantikine Rat IL-6 Immunoassay (Catalog Number R6000B) (R&D Systems, Minneapolis,

MN, USA).

2.7. Vascular reactivity in isolated vascular tissues

Using protocol obtained from Fenning et al. [28], vascular reactivity was assessed in sections of the thoracic aorta. Cumulative concentration-response curves (CRC) to noradrenaline (NA), acetylcholine (ACh) (NA pre-contraction) and sodium nitroprusside (NaNO) (NA pre-contraction using 70 % submaximal dose) were completed. Vascular function in isolated pulmonary arteries was assessed using a method adapted from van Waveren et al. [26].

2.8. Assessment of cardiac electrophysiological changes

Using methods detailed in Fenning et al., 2005 [28], cardiac electrophysiological function was examined. Action potential duration (APD) at 20 %, 50 %, and 90 % of repolarization and action potential amplitude, were measured.

2.9. Airway reactivity in isolated sections of Trachea and bronchioles

Tracheal rings were isolated and cleaned, then were stabilized at 37 °C modified KHB, bubbled with carbogen gas. After equilibration CRC to carbachol (CAR), 5-hydroxytryptamine (5-HT) and isoprenaline (ISO) were completed. Pulmonary function in isolated bronchioles was assessed via a 4-bath wire-myograph system (Danish Myograph Technologies, Denmark). Following normalization and equilibration procedures consistent with previously used, CRC was performed using 5-HT, ACh and ISO (ACh pre-contraction using 70 % submaximal dose).

2.10. Drugs and chemicals

The drugs that were used in this study (NA, ACh, CAR, 5-HT, ISO and NaNO) were purchased from the Sigma Chemical Company, St Louis MO, USA. Serial dilutions of the drugs needed to perform the study were produced using distilled water.

2.11. Statistical analysis

Data is expressed as mean ± standard error mean (SEM). Statistical analysis was performed using either one-way or two-way analysis of variance (ANOVA) and students' *t*-test where appropriate, with Bonferroni post-tests. Data between groups were analyzed using one-way ANOVA with Bonferroni post-test. Data between groups over multiple time points were analyzed using two-way ANOVA with Bonferroni post-test. Results were considered significant when $P < 0.05$ with analysis carried out using GraphPad Prism v5 (GraphPad Software La Jolla, CA 92037 USA).

3. Results

3.1. Responses following PA

There were no significant changes noted in body mass, organ mass or in the PA group compared to the CON and PM groups (Table 1). Epididymal fat pad mass was significantly decreased in the PA group compared to the CON group, all other fat pads remained unaltered (Table 1). In the physical activity (PA and PA + PM) rats there was a significant increase ($P < 0.05$) in lipid peroxidation, however a significant decrease ($P < 0.05$) in serum IL-6 concentrations was observed compared to both the CON and PM groups (Table 1).

There was no significant change in systolic BP noted between the groups following PA (Fig. 2). Similarly, there were no significant changes in contractile aortic responses or endothelial independent or dependent relaxation responses following exercise training (Fig. 3A and B). ISO mediated relaxation of the trachea, however, was significantly ($P < 0.05$) increased in the PM group when compared to the CON and PA + PM treated groups (Fig. 4E).

3.2. Responses following PM exposure

No significant changes were noted in body mass, fat pads, organ mass (with exception of the spleen), oxidative stress markers in the PM group compared to the CON and PA groups (Table 1). Terminal spleen mass was significantly decreased in the PM group when compared to CON animals (Table 1). Markers of systemic inflammation, serum IL-6 and IL-1β were significantly increased following PM exposure. Serum IL-6 was significantly increased versus the CON animals and serum IL-1β compared to all other treatment groups (Table 1).

Hypertension was induced by PM exposure with a significant increase in systolic BP of 16 % at 4 weeks and 14 % after 8 weeks of exposure compared to the age-matched CON animals (Fig. 2). There was no significant change in contractile aortic responses to NA or endothelial independent or dependent relaxation responses following PM exposure (Fig. 3A and B).

PM exposure significantly altered bronchiole contractile responses to ACh in comparison to the CON and PA groups (Fig. 4A). Tracheal relaxation to ISO was also reduced in comparison in the CON and PA + PM groups (Fig. 4E).

Exposure to PM did not alter any of the assessed cardiac electrophysiological parameters (Table 2).

3.3. Responses following PA + PM exposure

Body mass, fat pad mass and organ mass following 8 weeks of treatment were not significantly altered by exercise during PM exposure ($P > 0.05$) (Table 1). Serum IL-6 concentrations were decreased by PA (PA and PA + PM) compared to the CON and PM exposed animals (Table 1). As a marker of lipid peroxidation and oxidative stress, 4-HNE concentrations in the PA groups (PA and PA + PM) were increased in comparison to the CON and PM exposed groups (Table 1). The combination of exercise training and PM exposure increased lipid peroxidation beyond the levels observed in the PM and PA animals (Table 1).

Rats subjected to both PM and the PA + PM developed hypertension to a similar extent as the PM group with an increase of 13 % and 19 % at 4 and 8 weeks of treatment respectively (Fig. 2). Exercise training failed to significantly reduce the increased BP induced by PM exposure (Fig. 2).

There was no significant change in contractile aortic responses to NA or endothelial independent or dependent relaxation responses in the PA + PM animals compared to the other treatment groups (Fig. 3A and B). However, ISO mediated relaxation of the trachea was significantly ($P < 0.05$) altered in the PA + PM group compared to the CON, PM and PA groups (Fig. 4E).

There was no alteration in any of the cardiac electrophysiological parameters (Table 2).

4. Discussion

The current study investigated the cardiopulmonary changes caused by the exposure of PM during PA engagement in a rat model. This study showed that while there is an increased risk of oxidative stress and inflammation-promoting CVD following PM exposure, engaging in PA in dusty environments imposes minimal additional risk. Additionally, it appears that exercising while exposed to PM provides a protective mechanism against some of the damaging signaling processes triggered by PM exposure. The PM exposed rats were hypertensive with no changes in organ hypertrophy except in the spleen, which was decreased. The reduction in terminal spleen weight is hypothesized to be part of the changing immune response seen in both the PM exposed groups; the downstream effects of this are unknown.

The addition of PA to PM exposure (PA + PM) resulted in a

Table 1
Biochemical, organ mass and fat pad parameters.

	CON	PA	PM	PA + PM
Body mass (g)	444.8 ± 7.6	465.5 ± 8.9	457.9 ± 10.7	470.0 ± 12.0
Retroperitoneal fat pads (g)	7.8 ± 1.1	7.9 ± 0.6	7.9 ± 0.6	8.1 ± 0.8
Subcutaneous fat pads (g)	8.3 ± 1.0	8.5 ± 0.7	9.0 ± 0.6	9.8 ± 0.7
Epididymal fat pads (g)	8.5 ± 1.0	6.2 ± 0.3[a]	7.1 ± 0.3	6.8 ± 0.3
Mesenteric fat pads (g)	7.6 ± 0.9	7.4 ± 0.4	6.1 ± 0.4	6.6 ± 0.4
LV and septum (g)	2.2 ± 0.08	2.0 ± 0.08	2.0 ± 0.08	1.9 ± 0.15
RV (g)	0.5 ± 0.05	0.4 ± 0.03	0.4 ± 0.03	0.5 ± 0.05
Liver (g)	36.9 ± 0.7	36.8 ± 0.6	34.5 ± 0.1	37.2 ± 0.1
Spleen (g)	2.8 ± 0.1	2.7 ± 0.1	2.2 ± 0.1[b]	2.4 ± 0.1
4-HNE (mmol/L)	0.4 ± 0.2	10.4 ± 0.9[c]	2.5 ± 0.6	16.0 ± 2.0[d]
IL-6 (pg/mL)	770.0 ± 91.9	194.3 ± 31.5[e]	1340.0 ± 52.4[f]	404.3 ± 55.2[g]
IL-1β (pg/mL)	105.4 ± 13.6	49.4 ± 8.8[h]	199.8 ± 20.9[i]	66.1 ± 12.1
Nitrate/Nitrite (μmol/L)	22.6 ± 1.2	33.6 ± 2.7	26.7 ± 3.3	32.5 ± 4.4

Data expressed as Mean ± SEM; n = 16 for all groups. [a] P < 0.05 PA vs. CON for epididymal fat pad mass, [b] P < 0.05 PM vs. CON for spleen mass, [c] P < 0.05 PA vs. CON and PM for 4-HNE, [d] P < 0.05 PA + PM vs. CON, PA and PM for 4-HNE, [e] P < 0.05 PA vs. CON and PM for IL-6, [f] P < 0.05 PM vs CON, [g] P < 0.05 PA + PM vs. CON and PM for IL-6, [h] P < 0.05 PA vs. CON for IL-1β, [i] P < 0.05 PM vs. CON, PA and PA + PM for IL-1β.

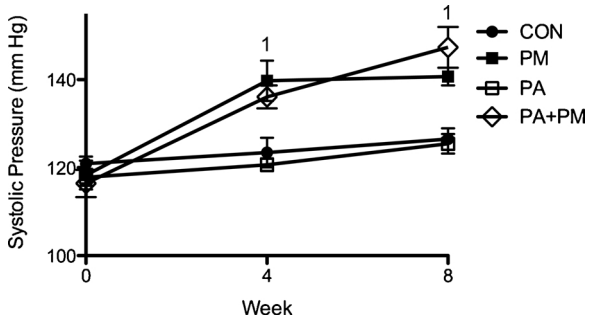

Fig. 2. Systolic blood pressure.
BP = blood pressure. Data expressed as Mean ± SEM; n = 16 for all groups. 1 P < 0.05 PA + PM vs. CON and PM vs. CON.

significantly lower trachea relaxation compared to PM, however, it was elevated compared to the control group, but this difference was not significant. PA in combination with PM normalized bronchiole and trachea responses. PA during chronic PM exposure increased spleen weights back towards control levels. Rises in serum IL-6 and IL-1b were used as biomarkers of systemic inflammation. Alterations is spleen weight has been shown to be a marker of toxic effects on the immune system (Dayan et al., 1998). The reduction in splenic weights that accompanied these changes are consistent with observations from other rodent studies that PM exposure causes immunotoxicity through induction of spleen autophagy and oxidative stress (Su et al., 2019).

PM size plays a significant role in the damage it can cause, as the smaller the particles are the deeper they can be inhaled into the airway, causing an increased risk for morbidities from PM inhalation [29]. Most particles exceeding 10 μm in nasal-breathing will be trapped by cilia and mucous in the nasal cavity [30]. With coarse PM (< 10 μm) deposited in the trachea or bronchi [31–35], however they are usually effectively removed through processes such as sneezing or coughing [30]. Particles between approximately 5 and 10 μm are most likely deposited in the tracheobronchial tree [32,33]. With the majority of particles less than 2.5 μm in diameter ($PM_{2.5}$), this size particle is able to enter and deposit in the respiratory tract and into the alveoli [21,32,33,36]. With smaller particles, less than 0.1 μm postulated to be able to translocate into the systemic circulation through the pulmonary capillary bed [37]. This may result in tissue injury at the site of PM delivery, leading to inflammatory and oxidative responses being upregulated. This could trigger smooth muscle contraction and initiate a response at the site of exposure leading to functional complications.

PM significantly increased systolic BP, with PA unable to attenuate the increase. Studies have found that exposure to PM increases BP, mean arterial pressure and heart rate leading to enhanced CVD complications [16]. PA is hypothesised to lower BP by multiple mechanisms including a reduction in sympathetic tone [38]. As such, the elevated BP in the PM + PA group compared to the CON animals may be due to constant activation of the sympathetic nervous system triggered by PM exposure and increased activity [39]. Elevated sympathetic and renin-angiotensin-aldosterone system drive following severe essential hypertension was compounded by high-intensity exercise which failed to improve haemodynamic parameters [39]. Although the current study was at a moderate intensity of PA, we can speculate similar circumstances were a factor in the BP results observed in our study with a chronically activated sympathetic drive masking the depressor benefits of exercise training [39]. PM exposure increases BP via baroreceptor reflex, which is dependent on the sympathetic tone [38,39]. Thus, the increased BP observed in the PA + PM groups compared to the CON group may be related to PM disrupting the effect of PA on sympathetic tone. However, inflammatory and oxidative pathways and the 'dose' of PA in the PA + PM animals may be insufficient to overcome the disruption caused by the exposure to PM and lower BP.

Reduced systemic inflammation is one of the mechanisms responsible for PA lowering overall CVD pulmonary disorder risk [18,40]. Our results indicate that PA was able to significantly reduce serum IL-6 and IL-1β levels compared to the PM exposed group. With increased oxidative stress and inflammation present, secondary cardiovascular complications such as vascular dysfunction would typically be expected to occur following exposure to PM. However, the younger age of rat model used in this study potentially required a longer duration of PM to induce a more significant cardiorespiratory functional change, similar to those seen in recent studies [18,27]. This could have been achieved by either a further two months of exposure or a more extended daily duration. However, as the goal of the study was to try to replicate exposure to PM whilst engaging in PA we feel that the 30-minute daily exposure was acceptable. The results in this study also support current literature stating long-term PA interventions are able to decrease significant systemic inflammation and lower overall CVD risk [41,42].

Human-based research has shown that PA is independently associated with a lower C-reactive protein (CRP) level [43]. Animal studies have also shown that regular exercise reduces inflammation by providing an anti-inflammatory environment, by increasing interleukin-10 and decreasing CRP [41]. Another study showed expression of inflammatory markers was increased in adipose tissue from high fat fed mice with PA reversing the increased expression of these inflammatory cytokines [42]. This chronic increase in systemic inflammation can impact endothelial function, with Tamagawa, *et* al, finding repeated exposure to PM_{10} caused both lung and systemic inflammation which was associated with endothelial dysfunction [44]. Tamagawa, et al.,

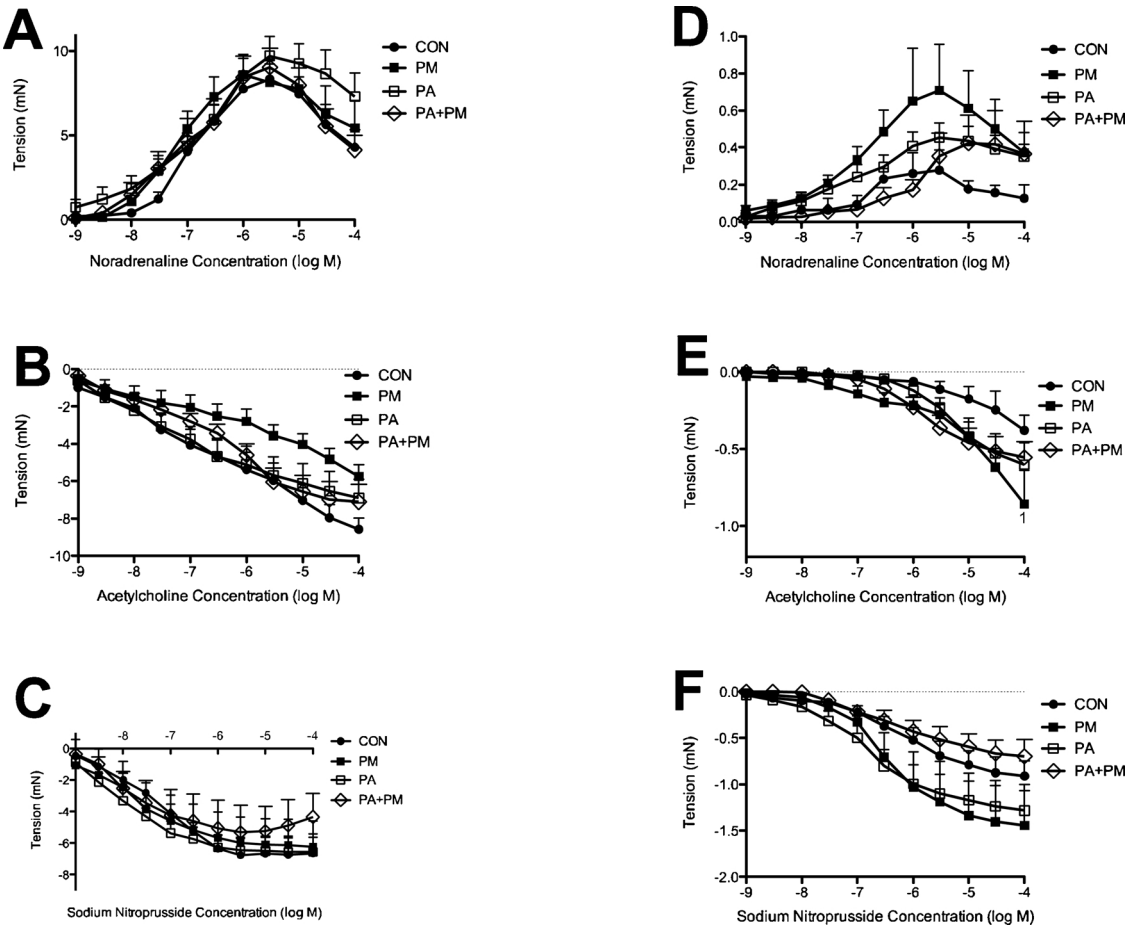

Fig. 3. Vascular reactivity.
A. Noradrenaline mediated contraction of the thoracic aorta. **B.** Endothelium-dependent relaxation by acetylcholine of noradrenaline pre-contracted thoracic aorta. **C.** Endothelium-independent relaxation by acetylcholine of noradrenaline pre-contracted thoracic aorta. **D.** Noradrenaline mediated contraction of the pulmonary artery. **E.** Endothelium-dependent relaxation by acetylcholine on noradrenaline pre-contracted pulmonary artery. **F.** Endothelium-independent relaxation by sodium nitroprusside on noradrenaline pre-contracted pulmonary artery. Data expressed as Mean ± SEM; n = 16 for all groups. 1 $P < 0.05$ vs. CON.

hypothesised that exposure to PM has a prolonged impact on blood vessels, and speculated that this contributes to the vascular events associated with exposure to air pollution [44]. Even with exposure to PM, which is believed to increase during PA due to an increase in respiration rate [21,22], the PA + PM group saw a decrease in systemic inflammation compared to the PM group.

Our results indicate normal vascular function regardless of PM exposure. With the young age of the animal model, relatively short daily duration of exposure and the moderate pressor response of PM, normal vessel reactivity was maintained. Increased systemic inflammation increased ROS and decreased NO bioavailability play a role in endothelial dysfunction and the pathogenesis of CVD [45]. Reduced NO bioavailability is an essential feature of oxidative stress [45]. Exposure studies in healthy residents who live in city/industrial seaport areas showed to finely dispersed particles in atmospheric air causes the oxidative modification of proteins and DNA [46], this highlights even in healthy individuals there can be a change. Cardiovascular risk factors including hypercholesterolemia, hypertension, diabetes, and smoking are associated with impairment of the various constitutive NO systems [47]. Surprisingly, we found that although markers of oxidative stress were increased in the PA and PA + PM exposed groups, serum NO metabolites were relatively unchanged. It can be hypothesised that NO availability remained the same across the treatment groups due in part to the young age of the rats and the moderate degree of PM exposure and helped to protect normal cardiovascular function.

4-HNE concentrations were used as a measure for oxidative stress in this study. 4-HNE protein adduct concentration was significantly increased in the PM exposed group compared to the CON animals. One of the proposed mechanisms by which ambient PM is believed to exert its pro-inflammatory effects is the generation of oxidative stress by its chemical compounds and metals [48]. PM is believed to cause a pro-inflammatory state through the increase in ROS [48]. The chemical compounds and metals found in PM are absorbed into the systemic circulation after being inspired [48], triggering cellular responses and damaging endothelial cells. Oxidative stress has also been shown to be increased in the microvascular wall after PM exposure, regardless of which oxygen radicals are elevated after PM exposure [20]. Coupled with changes in circulating inflammatory cytokines, the synergistic effect together with ROS plays a role in developing hypertension following short to moderate PM exposure, which may translate, into overt functional changes in more extreme exposure scenarios.

Oxidative stress was also significantly increased following PA. PA has been shown to generate increased ROS but at the same time can up-regulate defenses and constitutive NO release [49,50]. Research has indicated a reduction in oxidative stress in PA models, this is believed to be due to the increase in the anti-inflammatory environment that exercise provides, which is linked to inducing an increase in the anti-oxidant defense systems [41]. In this study, an increase in our marker of oxidative stress was significantly increased in the PA and PA + PM group compared to our CON and PM exposed animals. A trend showed an exaggeration between the PA and PA + PM exposed groups.

PM exposure is also known to cause pulmonary injury, the proposed

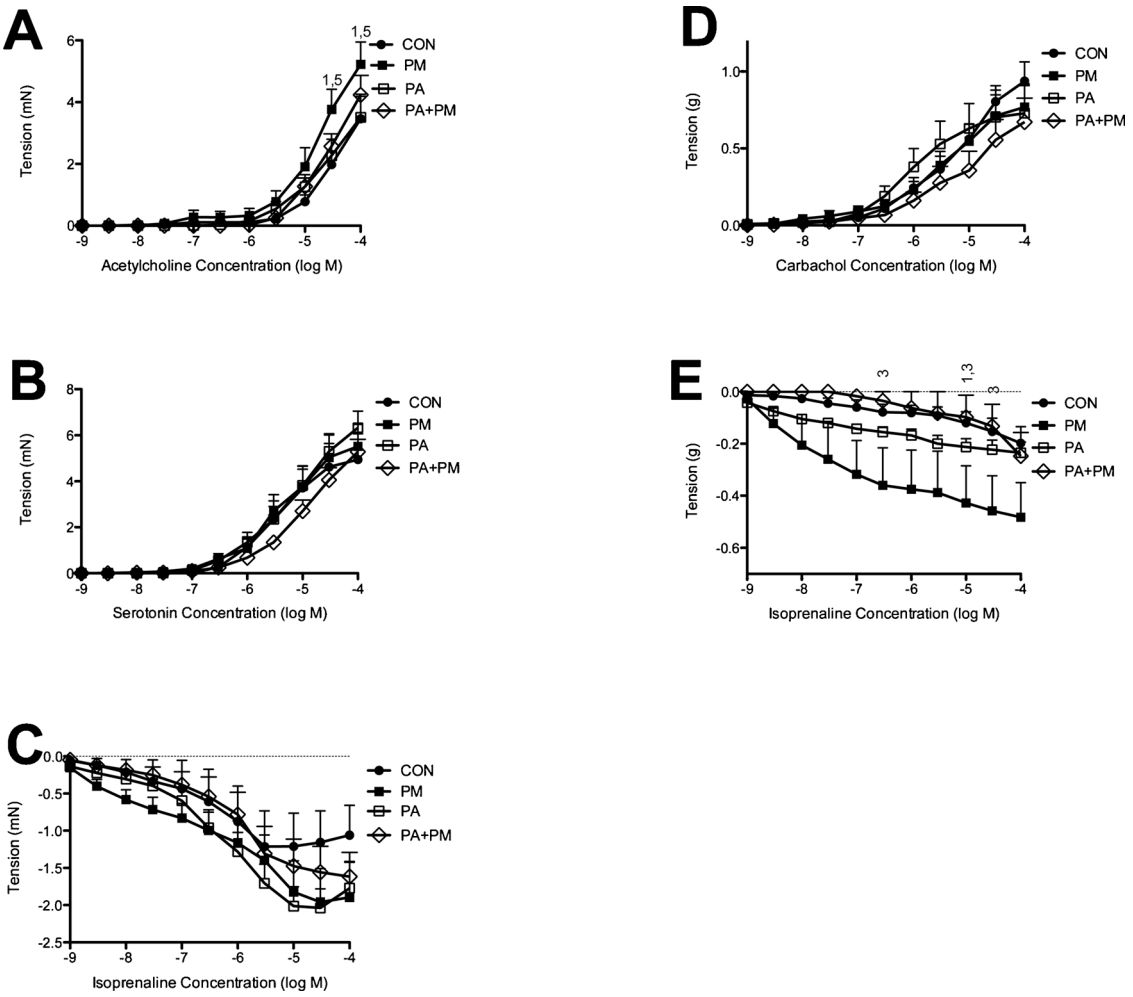

Fig. 4. Airway reactivity.
A. Acetylcholine-mediated contraction of the bronchioles. **B.** Serotonin-mediated contraction of the bronchioles. **C.** Isoprenaline mediated relaxation of the bronchioles. **D.** Carbachol mediated contraction of the trachea. **E.** Isoprenaline mediated relaxation on carbachol pre-contracted trachea. Data expressed as Mean ± SEM; n = 16 for all groups. 1 P < 0.05 CON vs. PM, 2 P < 0.05 CON vs. PA + PM, 3 P < 0.05 PM vs. PA + PM, 4 P < 0.05 PA vs. PA + PM, 5 P < 0.05 PM vs. PA.

mechanisms include direct lung damage and neutrophilic inflammation [51]. PM sizes ranging from 10-0.2 μm has been shown to induced mass-dependent antioxidant, proinflammatory, and cytotoxic responses to different degrees [52]. It was shown that the ranging sizes also decreased cell viability [52]. It has been hypothesised that this connection with inflammation and even oxidative stress may be a possible mechanistic action [51]. Airway responsiveness is also increased in these animals with 50 % in the study having an increased reaction to ACh [51]. Similar results were seen in our study, where PM exposed animals had an increased contraction to ACh in isolated bronchiole tissue compared to the control group leading to enhanced airways hypersensitivity. Interestingly, animals exposed to both PA and PM showed a decrease in airways reactivity compared to the PM group, which may indicate a reduced sensitivity to PM irritants despite the increased delivery of PM with physical activity.

Repeated exposure to PM_{10} has been shown to cause both lung and systemic inflammation [44]. The inflammatory responses seen in the lung were characterized predominantly by activation and infiltration of alveolar macrophages, and the systemic inflammatory response was characterized by an increase in circulating leukocytes and IL-6 levels [44]. These inflammatory responses were also associated with endothelial dysfunction [44]. Whereas, exercise training has been shown to reduce lung oedema formation after pulmonary ischemia-reperfusion in the rat [50]. PA prior to lung ischemia-perfusion injury attenuated inflammatory oedema and induced significant reductions in serum levels of TNF-a and IL-1β, without altering IL-10 levels [50]. Without the

Table 2
Assessment of cardiac electrophysiological changes.

	CON	PA	PM	PA + PM
RMP	−69.47 ± 1.67	−64.13 ± 1.72†	−72.10 ± 1.90	−60.99 ± 1.54*†
APA	66.16 ± 2.76	65.60 ± 2.70	69.47 ± 2.83	61.56 ± 2.41
APD20	23.58 ± 0.53	24.50 ± 0.34	25.00 ± 0.67	25.06 ± 0.69
APD50	32.17 ± 1.43	34.01 ± 1.64	32.94 ± 1.72	33.33 ± 1.27
APD90	70.60 ± 5.80	77.22 ± 4.20	71.92 ± 3.90	80.73 ± 3.45

Data expressed as Mean ± SEM; n = 16 for all groups. * P < 0.05 vs. CON and † P < 0.05 vs. PM.

protective benefits of PA, the increase in pulmonary inflammation escapes the lungs and leads to systemic and secondary pathological responses [53,54]. Even though it is expected that there would be increased inhalation [21,22] of PM particles in the PA + PM group in comparison to the PM group there was improvements in systemic inflammation and normalized bronchiole and trachea concentration response curves. Clearly, exercise provides a protective response to PM exposure.

PM exposure in young rats failed to modulate the cardiac action potential despite increases in systemic inflammation, oxidative stress, and BP. It has been shown that PM can induce cardiac arrhythmias following differing concentrations and durations of exposures [17–19,27]. Our results would indicate that the 8 weeks duration of exposure and the young age of the rats together with the maintained serum NO metabolite levels helped to prevent secondary cardiovascular complications observed in these previous studies. Exercising during PM exposure did not alter any of these parameters.

In conclusion, the current study's results were consistent with the literature, showing PA's ability to reduce overall systemic inflammation. PA was able to prevent some of the damage to the cardiopulmonary system that was caused by PM. In part, these results are consistent with the hypothesis, that PA induced improvements in airway responsiveness, are mediated by a reduction in systemic inflammation. These results suggest that PA participation while exposed to moderate concentrations of $PM_{2.5}$ does not exaggerate the risk of CVD. Also, that engaging in PA during PM exposure in young individuals is no worse than being exposed to PM alone and that exercise may help to offset any pathological processes. Furthermore, individuals should limit their exposure to PM during daily life and while participating in PA to avoid the ill health effects associated with PM exposure.

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Disclosures

None.

CRediT authorship contribution statement

Alannah van Waveren: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Project administration. **Mitch J. Duncan:** Conceptualization, Writing - review & editing, Supervision. **Fiona Coulson:** Writing - review & editing, Supervision. **Andrew Fenning:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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