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Cardiovascular Effects of Diesel Exhaust Inhalation: Photochemically Altered Versus Freshly Emitted in Mice

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Abstract

This study was designed to compare the cardiovascular effects of inhaled photochemically-altered diesel exhaust (aged DE) to freshly-emitted DE (fresh DE) in female C57Bl/6 mice. Mice were exposed to either fresh DE, aged DE, or filtered air (FA) for 4 hr using an environmental irradiation chamber. Cardiac responses were assessed 8 hr after exposure utilizing Langendorff preparation with a protocol consisting of 20 min of perfusion and 20 min of ischemia followed by 2 hr of reperfusion. Cardiac function was measured by indices of left-ventricular developed pressure (LVDP) and contractility (dP/dt) prior to ischemia. Recovery of post-ischemic LVDP was examined on reperfusion following ischemia. Fresh DE contained 460 $\mu\text{g}/\text{m}^3$ of particulate matter (PM), 0.29 ppm of nitrogen dioxide (NO_2) and no ozone (O_3), while aged DE consisted of 330 $\mu\text{g}/\text{m}^3$ of PM, 0.23 ppm O_3 and no NO_2 . Fresh DE significantly decreased LVDP, dP/dt_{max}, and dP/dt_{min} compared to FA. Aged DE also significantly reduced LVDP and dP/dt_{max}. Data demonstrated that acute inhalation to either fresh or aged DE lowered LVDP and dP/dt, with a greater fall noted with fresh DE, suggesting that the composition of DE may play a key role in DE-induced adverse cardiovascular effects in female C57Bl/6 mice.

Keywords

diesel exhaust; photochemistry; cardiac function

INTRODUCTION

Air pollution is among the leading top 10 risk factors for mortality globally (Collaborators 2017). Diesel exhaust (DE) is an important source of traffic-related ambient air pollution which is composed of a complex mixture of chemicals, including gases, volatile organics,

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and particulate matter (PM) (Geller et al. 2005, Lanki et al. 2006). Epidemiological studies suggested that traffic-derived air pollution may produce cardiovascular effects than individual air pollutants, such as particulate matter (Brook et al. 2010, Laden et al. 2000, Stupfel 1976, Beckerman et al. 2012, Chiu, Tsai, and Yang 2017, Yang et al. 2004). Therefore, DE is considered an important contributor to mortality and morbidity associated with exposure to traffic-derived air pollution (EPA 2009).

Primary pollutants are converted to secondary pollutants by a complex series of photochemical reactions in the atmosphere. In “real world” environments, fresh DE undergoes photochemical reactions in the ambient atmosphere to produce photochemically-altered (aged) DE. In this study, an environmental irradiation chamber was used to generate an aged DE atmosphere in the presence of a synthetic urban mixture (SynUrb54), as previously described (Lichtveld et al. 2012, McIntosh-Kastrinsky et al. 2013). Employing this approach, McIntosh-Kastrinsky et al. (2013) previously showed that exposure to photochemically-altered, particle-free SynUrb54 at concentrations that produce only minimal adverse pulmonary effects significantly affected cardiac function, including decreased left ventricular developed pressure and contractility.

Experimental studies investigating the adverse health effects attributed to photochemically aged vehicle exhaust are still sparse. Lichtveld et al. (2012) noted that aged DE generated from the same outdoor environmental irradiation chamber used in the current study induced significant inflammatory responses *in vitro*. Diaz et al. (Diaz et al. 2012) found that aged vehicular exhaust emissions enhanced the inflammatory responses compared to fresh vehicular emissions. However, using the same approach, studies conducted by the same group evaluating the adverse health effects of realistic emissions from power plant PM aerosols detected no marked or relatively mild responses (Diaz et al. 2011, Godleski, Diaz, et al. 2011, Godleski, Rohr, et al. 2011). The discrepancy in the above studies might be due to differences in chemical composition of the emissions.

The air quality index (AQI) is used to report daily air quality that focuses on the influence of air pollution on healthy and susceptible populations. The AQI is calculated from single pollutant of 5 major criteria air pollutants regulated by the Clean Air Act: ground level ozone (O₃), PM, carbon monoxide (CO), nitrogen dioxide (NO₂), and sulfur dioxide (SO₂). These criteria pollutants were found to exert adverse health impacts (Newell et al. 2018, Gryparis et al. 2004, Chen et al. 2011, Katsouyanni et al. 1997, Kan et al. 2010, Samoli et al. 2006, Brook et al. 2010, Li et al. 2018, Chen and Yang 2018). A health-based integrated air quality index (AQHI) developed by Environment Canada predicts the health impact of multiple air pollutants mixtures based upon the combined 3 hr average concentrations of O₃, NO₂, and PM_{2.5} (Stieb et al. 2008). AQHI assumes that the health impacts from these three air pollutants are additive and postulate to produce a more conservative approach to public health warnings. It is important to note that both the AQI and AQHI are tools that are aimed to take the complex atmospheric environment containing hundreds of chemical components and diurnal changes and provide a simplistic snapshot of the possible health impacts that can be easily understood. Different days can be designated as having the same AQI or AQHI value, yet the pollutants driving those values can differ.

The aim of the present study was to investigate the cardiovascular effects attributed to exposure to aged DE in mice. Studies were undertaken to examine whether inhaled exposure to DE that was photochemically modified by multi-pollutant gaseous mixtures in urban environments might affect cardiovascular responses and compared these cardiovascular effects to those observed with freshly emitted DE. The freshly generated DE contained more PM mass and NO₂ but no O₃, while the photochemical reacted (aged) DE produced substantial amount of O₃ and secondary organic aerosol but no NO₂ and less PM mass. Comparison of experimental air pollution atmospheres based upon AQI informs risk assessment of these mixtures.

MATERIALS AND METHODS

Experimental Animals

All experimental procedures were performed in compliance with protocols approved by the Institutional Animal Care and Use Committee of the University of North Carolina (UNC) at Chapel Hill according to NIH guidelines. Mice were maintained at 22°C with a 12-hr light/dark cycle and free access to food (ProLab RMH 3000) and water. Twenty-four female C57Bl/6 mice (5 months old, average weight 25.6 ± 0.6 g) were purchased from Jackson Laboratory (Bar Harbor, ME) and acclimated for 1 month prior to use.

Generation of Fresh and Aged Diesel Exhaust Atmospheres

UNC-Chapel Hill's outdoor environmental irradiation chamber (smog chamber) was employed to generate exposure atmospheres similar to those previously described (Lichtveld et al. 2012, McIntosh-Kastrinsky et al. 2013). Freshly emitted DE emissions from a 36-kW diesel generator (Model 3W991A, Dayton Electric MFG. CO.) operated at idling mode with ultra-low sulfur diesel (ULSD) fuel were injected into the smog chamber until the desired 300 µg/m³ particle concentration, as measured with a scanning mobility particle sizer (SMPS; TSI Inc., Shoreview, MN), was achieved. The dose of 300 µg/m³ of DE was chosen based on a study showing that this dose produced significant cardiovascular effects in a controlled human exposure study (Tong et al. 2014). Fresh DE was injected into the smog chamber at sunset to eliminate any photochemistry from occurring; exposures to the fresh DE were conducted after sunset. To investigate the effects of aged DE, emissions from the diesel generator were injected in the smog chamber before sunrise, followed by injections of the SynUrb54 (Scott Specialty Gases, Plumsteadville, PA), a VOC mixture (Jeffries 1995), and NO_x (nitric oxide and nitrogen dioxide). SynUrb54 is a particle-free urban mixture that contains 55 different hydrocarbons at specific ratios representing chemicals present in urban atmospheres (Sexton et al. 2004). The DE and SynUrb54 were mixed in the chamber and allowed to photochemically react throughout the day; exposures to the aged DE were conducted after sunset. Photochemically reacting the mixtures of DE and SynUrb54 creates multiple generations of oxidized products typically found in urban environments (Lichtveld et al. 2012).

DE particle size was measured using an SMPS (TSI Inc., Shoreview, MN) on a 3 min-per-sample cycle during exposure, while Teflon membrane filters (47 mm diameter; Pall Life Sciences, Ann Arbor, MI) were used to measure the DE concentration in the chamber over

the 4-hr exposure period. NO and NO₂ levels were measured every min using a Teledyne model 9841 NO_x analyzer (Teledyne Monitor Labs, Englewood, CO). O₃ was measured every min with a Teledyne model 9811 monitor. Concentrations of these compounds were averaged during the exposure period.

Animal Exposure

Since the photochemical reactions depend on the weather conditions (i.e. sunlight intensity), the multi-pollutant mixtures generated are not reproducible, and therefore mice were exposed in groups. Three groups of mice were exposed to freshly-emitted DE (fresh DE; n=8), photochemically-aged DE (aged DE; n=8) or filtered air (FA; n=8) for 4 hr (20:00–24:00 EDT) with lighting on three separate days as described previously (McIntosh-Kastrinsky et al. 2013). For exposure, mice were placed in individual stainless-steel whole-body animal cages with bedding and allowed freedom of movement and access to food and water. Animal cages were custom-made at the Environmental Sciences and Engineering Design Center at UNC-Chapel Hill. These were placed in staggered levels, with each individual cage housing a mouse facing the inlet of the ventilated gas mixture. The cages were held inside a larger stainless-steel chamber (also manufactured in the Design Center), large enough to hold 27 mice. The chambers were ventilated at a flowrate of 10 L/min controlled by mass flow controllers (Aalborg, Orangeburg, NY) and approximately 45 min were required to achieve a steady state concentration of the exposure concentration.

Cardiac Function

As described previously (Tong et al. 2009), 8–11 hr after exposure, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight). After intravenous heparin (100 units) injection the hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. The aortas were cannulated and perfused retrograde at constant pressure of 100 cmH₂O. The non-recirculating perfusate was a Krebs-Henseleit buffer containing (in mmol/L) 120 NaCl, 5.9 KCl, 1.2 MgSO₄, 1.75 CaCl₂, 25 NaHCO₃, and 11 glucose. The buffer was aerated with 95% O₂ and 5% CO₂, and maintained at pH 7.4 and 37°C.

For assessment of contractile function, a latex balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a pressure transducer (Argon Medical Devices, Athens, TX) at the same height as the heart. The pressure of the left ventricular balloon was inflated to 0–5 cmH₂O. A PowerLab system was used to collect and process the heart rate, left ventricular developed pressure (LVDP=LV peak minus end-diastolic pressure), and contractility (dP/dt) data (AD Instruments, Milford, MA). All hearts were perfused for 20 min when the baseline measurements were taken prior to initiating 20 min of global no-flow ischemia followed by 2 hr reperfusion. Onset of ischemic contracture was detected when the left ventricular pressure began to increase during ischemia. Recovery of LVDP, expressed as % of initial pre-ischemic LVDP was measured at 60 min of reperfusion after 20 min of ischemia.

Cardiac necrosis was evaluated as described previously (Tong et al. 2010, Tong et al. 2009), at the end of 2 hr of reperfusion. Hearts were perfused with 15 ml of 1% solution of 2,3,5-

triphenyltetrazolium chloride (TTC) dissolved in Krebs-Henseleit buffer, then incubated in 1% TTC at 37°C for 10 min, and then fixed in formalin. The area of necrosis was measured by taking cross-sectional slices through the ventricles, which were then photographed using a digital camera mounted on a stereo-microscope. The resulting images were quantified by measuring the areas of stained (viable tissue) versus unstained tissue (infarct) with the use of Adobe Photoshop. Infarct size was expressed as a percentage of the total ventricular section and averaged from four images.

Statistical Analysis

Data are expressed as means $\pm$ SEM. Comparisons among the fresh DE, aged DE, and FA control group were performed by ANOVA followed by Tukey's multiple comparison test. The statistical significance levels were set at $p < 0.05$.

RESULTS

Composition of Particle Photochemically Generated Multi-Pollutant-Mixtures

Photochemical reactions of the SynUrb54 generate more than 300 multiple generations of carbonyl products mixtures (Sexton et al. 2004). The primary hydrocarbons are predominantly consumed by the end of day and oxidized resulting in secondary organics. Previously McIntosh-Kastrinsky et al. (2013) demonstrated that mean chamber NO₂ and nitrogen oxide (NO) levels decreased throughout the day, and levels of secondary chemical products such as O₃ increased. The average concentrations of particle mass and gaseous components of NO, NO₂, O₃, and particle size during the exposures are presented in Table 1. The PM concentration in fresh DE was numerically higher than in aged DE although an attempt was made to match these two test atmospheres. The particle size is in the fine PM range. The chemical composition of sunlight irradiated chamber mixtures of DE and SynUrb54 were previously reported (Ebersviller et al. 2012). AQI was similar between fresh and aged DE but AQHI in the fresh DE was higher than in aged DE.

Based on the mathematical model by Hsieh et al. (Hsieh, Yu, and Oberdorster 1999) lung dose of DE is estimated to be approximately 0.017 and 0.012 ug/g body weight for fresh and aged DE, respectively.

Cardiac Effects

When compared to FA control, the baseline heart rate before ischemia was unchanged in the hearts of mice exposed to fresh DE or aged DE (reacted with SynUrb54) (Table 2 and Figure 1). No marked differences in baseline coronary artery flow rate at constant pressure were observed among the groups. However, compared to FA group (146.4 $\pm$ 14.8 cm H₂O), markedly lower baseline LVDP was noted in hearts from the fresh DE group (79.7 $\pm$ 10.7 cm H₂O) and aged DE group (102.5 $\pm$ 11.2 cm H₂O). Baseline LVDP in fresh DE group was not significantly different from aged DE mice (Figure 1). Exposure to fresh DE or aged DE decreased baseline left ventricular contractility. Compared to FA group (5421 $\pm$ 868 cm H₂O/s), the baseline rate of contraction (dP/dt_{max}) was significantly lower in fresh DE (3127 $\pm$ 561 cm H₂O/s) and aged DE mice (3725 $\pm$ 447 cm H₂O/s) (Figure 2A). There were no significant differences among the groups exposed to these two test atmospheres although

$dP/dt_{\max}$ was numerically lower in the fresh DE group. The baseline rate of relaxation ($dP/dt_{\min}$) was significantly reduced by exposure to fresh DE exposure (-2300 ± 353 cm H₂O/s) compared to FA control (-4023 ± 588 cm H₂O/s) (Figure 2B). Aged DE exposure did not significantly lower $dP/dt_{\min}$ (-3367 ± 369 cm H₂O/s) compared to FA animals.

Compared to FA group (14.5 ± 1.2 min), time to ischemic contracture was not markedly affected by exposure to fresh DE (16.1 ± 1.3 min) and aged DE (15.4 ± 2.1 min) during 20 min of ischemia (data not shown). Post-ischemic recovery of LVDP (% LVDP) at 60 min after reperfusion appeared better in fresh DE exposure but did not reach significance compared to FA control (Figure 3A). There was no significant difference of %LVDP in aged DE group compared to FA mice. There was no marked difference between fresh DE and aged DE groups (Figure 3A). Infarct size was not different markedly among the groups (Figure 3B).

DISCUSSION

Humans are exposed to ambient air pollution mixtures of gases and particles in the real world and the challenge facing current air pollution health research is how to address these mixtures. In the current study photochemically-altered DE was generated using an environmental irradiation reaction chamber for assessing the cardiac responses to inhalation of “real world” DE in mice. Data demonstrated that short-term inhalation of freshly-emitted DE or photochemically-altered aged DE depressed cardiac function and cardiac contractility in isolated female mouse perfused hearts, and fresh DE produced greater adverse cardiac effects compared to aged DE.

Short-term exposure to PM has been linked with cardiovascular mortality, including myocardial ischemia and atherosclerosis (Brook et al. 2010, Chang, Chen, and Yang 2015, Tsai, Tsai, and Yang 2018). Huang et al. (Huang et al. 2010) demonstrated that acute exposure to DE particles by intra-tracheal instillation produced significant myocardial dysfunction in rats, including decreased left ventricular systolic and diastolic contractility. In addition, exposure to DE particles *in vitro* resulted in cardiomyocyte contractile dysfunction and reduced calcium handling (Gorr et al. 2015). In agreement with these studies, our study found that DE exposure reduced cardiac function and cardiac contractility in isolated perfused hearts. Further the cardiac effects from inhalation of photochemically altered aged DE compared to fresh DE noted that fresh DE exhibited a trend of producing greater cardiac effects than aged DE. Efforts were made to match the PM concentrations in fresh DE and aged DE experiments; however, the PM concentration in fresh DE was numerically higher than aged DE. The difference in cardiac effects might be partly attributed to higher PM mass in fresh DE compared to aged DE; however, other components in the DEs may also play important roles.

It was postulated that gaseous components in the DE mixtures might mediate cardiac responses (Chen and Yang 2018). Increasing evidence suggests that gaseous components modify the biological effect induced by ambient pollutants (National Research Council, 2001). It was reported that increased diastolic blood pressure was observed in young adults co-exposed to concentrated ambient PM and O₃ but not to concentrated ambient PM alone (Fakhri et al. 2009). In a clinical study Madden et al. (Madden et al. 2014) showed that the

combination of O₃ and DE co-exposure produced greater than additive of effects of O₃ and DE on lung function decrements in healthy adults, suggesting that a potential interaction between the gaseous and particulate pollutants inducing greater than additive effects. In addition, animal studies demonstrated that the cardiovascular effects of atmosphere mixtures exposure were dominated by the gaseous components (McIntosh-Kastrinsky et al. 2013, Tong et al. 2018, Hazari et al. 2018). However, Kurhanewicz et al. (Kurhanewicz et al. 2014) found that co-exposure of O₃ with fine or ultrafine PM produced varied cardiac responses, suggesting that cardiovascular effects of particle-gas co-exposures are not simply additive or even generalizable.

The toxicity of cultured lung cells exposed to aged DE generated from the same irradiation chamber was variable depending upon the exposure method. Lichtveld et al. (2012) reported that aged DE produced significantly lower inflammatory responses when cultured lung cells were exposed under submerged conditions where aged DE was collected on a filter followed by particle resuspension in liquid medium compared to cells exposed at the air-liquid interface (ALI), due to lack of carbonyl compounds from filter samples. Similarly, animal studies have shown that the instillation of PMs from filter samples had no effects on pre-ischemic cardiac function in isolated Langendorff perfused hearts (Tong et al. 2010, Holland et al. 2017). Further, using the same irradiation chamber and ALI *in vitro* exposure systems, it was demonstrated that gas-phase-products increased the toxicity of the nontoxic PM, indicating that gaseous components of air pollution might be driving the adverse health effects as they partition on and off the PM (Ebersviller et al. 2012). Our previous study with particle-free aged SynUrb54 generated in the same irradiation chamber showed that inhalation of mixtures significantly decreased LVDP, dP/dt_{max} , and dP/dt_{min} in isolated hearts (McIntosh-Kastrinsky et al. 2013). In addition, our recent study demonstrated that inhalation of simulated smog atmospheres consisting of more gaseous and less PM components generated from photochemistry decreased cardiac function in Langendorff perfused hearts. The same may be true in the current animal study. Therefore, higher PM mass in fresh DE compared to aged DE may not be the only driving force to initiate adverse cardiac effects.

AQI is an index of air quality for human health. In this study, the total sum of individual AQI values from O₃, NO₂, and PM_{2.5} were similar between fresh and aged DE, as presented in Table 1, and total AQI value was dominated by PM. PM AQI was hazardous (code brown) in both fresh and aged DE. O₃ AQI was unhealthy (code purple) in aged DE but good (code green) in fresh DE. NO₂ AQI was unhealthy for sensitive groups (code orange) in fresh DE and good (code green) in aged DE. Taking the similar AQI values between these two DEs, one would assume similar adverse health effects would be observed. However, fresh DE displayed lower trend of cardiac function compared to aged DE. One explanation might be fresh DE contained more PM mass than aged DE. However, it is interesting to note that fresh DE contained significant levels of NO and NO₂ that were not present in aged DE, whereas O₃ was present only in aged DE. Both PM and NO₂ are criteria air pollutants that exert health impacts. To differentiate the health impact of these two pollutants, a systemic review and meta-analysis quantitatively assess daily NO₂ and mortality and hospital admissions of various cardiovascular or respiratory diseases which also controlled for PM and demonstrated that the association between short-term exposure to NO₂ and adverse health

outcome is largely independent of PM mass (Mills et al. 2016). This finding strengthens NO₂ exhibiting a causal role in health effects (Mills et al. 2016). Indeed, ambient NO₂ levels have been associated with cardiopulmonary mortality (Chen et al. 2012, Samoli et al. 2006, Chen et al. 2018, Eum et al. 2019, Chiusolo et al. 2011). In particular, epidemiological studies found a linear and positive association between short-term exposure to NO₂ and total, cardiovascular, and respiratory mortality in several Chinese cities (Chen et al. 2012, 2018). Positive associations of outdoor NO₂ levels with hospital admissions of all cardiovascular causes, ischemic heart disease and stroke, and respiratory diseases were also found in American older adults (Eum et al. 2019) and in Netherlands residents (Dijkema et al. 2016). Taken together, NO₂ in fresh DE might contribute to more adverse cardiac effects in the current study. Future experimental studies are warranted to examine the cardiovascular effects attributed to NO₂.

In a recent study, Health Canada's Air Quality Health Index (AQHI) was evaluated for predicting the mutagenicity of smog atmospheres (Zavala et al. 2018). The AQHI is a no-threshold index developed to assess adverse health risks of acute pulmonary exposures at ambient concentrations based upon the combination of PM_{2.5}, O₃, and NO₂ concentrations measured (Stieb et al. 2008). The AQHI values were calculated based upon our measurements for this study (Table 1). Interestingly, an AQHI value of 52 was calculated for fresh DE, while the aged DE displayed an AQHI value of only 29.8 even though they have similar values of total AQI. NO₂ was only present in the fresh DE and was the dominant factor for the calculated AQHI value. AQI reports an index value based on the highest single pollutant which may at times inadequately communicate the full adverse health risks of air pollution (Perlmutter and Cromar 2019). Based upon our study results, the AQHI may be a more reliable indicator than individual AQI values of adverse health effects. These studies suggest that individual criteria pollutant AQI values may be inadequate for estimating adverse health effects.

It is important to note that the AQI and AQHI values from the fresh and aged DE atmospheres tested here can be 2 to 10 times higher than what can be typically observed in real world environments. For example, in the very polluted regions of Beijing, Tianjin, and Hebei in China, driven by PM levels, their average O₃, NO₂, and PM_{2.5} levels (Ma et al. 2019) were used to calculate a PM_{2.5} AQI level of 163 (code red; unhealthy) and an AQHI of 7.8 (high risk). When events such as wildfires take place, which are occurring more frequently, the surrounding areas can experience very high AQI/AQHI values for several days as presented here, thus using these indicators as a simplistic tool for evaluating health impacts can be useful.

It has been demonstrated that co-exposure of rats to O₃ (0.38 ppm) and DE (2.2 mg/m³) in a regimen of 5 hr/day, 1 day/week for 16 weeks produced less cardiovascular effects relative to exposure to O₃ (0.4 ppm) or DE (2.1 mg/m³) alone (Kodavanti et al. 2011), suggesting that air pollutant interactions might change chemical composition during atmospheric aging process. Indeed, chemical reactions occurring on aerosol particles in the atmosphere might transform hazardous components and increase or decrease their potential for adverse health effects (Poschl 2002). Chemical composition of PM might be efficiently changed by interaction with gases such as O₃ and NO₂ (Poschl 2002). In agreement with these

observations in this study; aged DE produced less cardiac effects compared to fresh DE even though aged DE contained significant amount of O₃.

The reduced LVDP and cardiac contractility following DE exposure may indicate altered intracellular calcium regulation in the myocardium. It is known that adverse cardiovascular responses of air pollution are mediated by oxidative stress and inflammation (Brook et al. 2010). The pro-redox components of DE may modulate cardiomyocyte calcium regulation by reducing intracellular calcium or changing sensitivity of contractile proteins to calcium, resulting in diminished cardiac contractility. Gorr et al. (Gorr et al. 2015) demonstrated that reduced calcium handlings from DE treatment in cardiomyocytes was mediated by cellular oxidative stress. The preserved contractility during reperfusion in hearts exposed to fresh DE could indicate altered intracellular Ca²⁺ regulation in the myocardium. On the other hand, the reduced intracellular Ca²⁺ overload during ischemia is associated with preservation of mitochondrial function and adenosine triphosphate stores (Kowalchuk and Nesto 1989), suggested by the fresh DE had better recovery of post-ischemic LVDP during reperfusion. Future mechanistic studies are needed to better understand the role of intracellular calcium regulation in initiating cardiovascular effects following air pollution exposure.

There are limitations in this study. In this study a perfused isolated heart model was used to evaluate the ventricular function. However, isolated heart models lack innervation and blood supply and therefore lack influence by autonomic and hormonal control, which could alter findings in intact hearts. In addition, to exclude the differential impacts from sex only female mice were used in this study.

In conclusion, evidence indicated that inhalation of freshly-emitted DE and photochemically-altered aged DE depressed cardiac function and cardiac contractility in isolated mouse hearts. The cardiac effects of fresh DE and aged DE exposure and the components in these two DEs were compared and found that the fresh DE produced more adverse cardiac effects, indicating that NO₂ appears responsible for the effects on myocardial mechanical performance in this study which warrants future investigation. AQI and AQHI values were calculated for our test atmospheres and demonstrated that AQHI values might be more reliable predictors of adverse health effects. Future studies are needed to confirm these observations.

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A

B

Figure 1.

Heart rate and cardiac function in isolated perfused murine hearts before ischemia. Left ventricular developed pressure (LVDP) (A) and heart rate (B) at baseline prior to ischemia in murine hearts isolated 8 hr after inhalation exposure to filtered air (FA), freshly-emitted diesel exhaust (fresh DE), or photochemically-altered diesel exhaust (aged DE) for 4 hr as described in METHODS. $n = 8$ in the FA, fresh and aged DE groups. * $p < 0.05$; compared with FA control group.

Figure 2. Cardiac contractility in isolated, perfused murine hearts after exposures. The cardiac contractility assessed by maximum (A) and minimum (B) dP/dt at baseline prior to ischemia in murine hearts isolated 8 hr after inhalation exposure to filtered air (FA), freshly-emitted diesel exhaust (fresh DE), and photochemically-altered diesel exhaust (aged DE) for 4 hr as described in METHODS. $n = 8$ in the FA, fresh and aged DE groups. * $p < 0.05$; compared with FA control group.

Figure 3. Recovery of post-ischemic cardiac function and infarct size in murine hearts after exposure. Recovery of left ventricular developed pressure (LVDP), expressed as a percentage of the initial baseline pre-ischemic LVDP was measured after 60 min of reperfusion (**A**) and infarct size measured using TTC staining at the end of 2 hr of reperfusion (**B**) in mouse hearts isolated 8 hr after inhalation exposure to filtered air (FA), freshly-emitted diesel exhaust

(fresh DE), and photochemically-altered diesel exhaust (aged DE) for 4 hr as described in METHODS. $n = 8$ in the FA, fresh and aged DE groups.

Table 1.

Average particle mass and gas concentrations during 4 hours of exposure.

	Filtered Air	Fresh DE	Aged DE
PM mass concentration ($\mu\text{g}/\text{m}^3$)	0	460	330
NMD (μm)		0.249	0.271
MMD (μm)		0.337	0.344
Ozone (ppb)	0	0	237
NO (ppb)	0	225	0
NO ₂ (ppb)	0	292	0
NO _x (ppb)	0	517	0
PM AQI	0	473	380
Ozone AQI	0	0	217
NO ₂ AQI	0	137	0
Total AQI		610	597
AQHI	0	52	29.8

DE, diesel exhaust; NO, nitrogen oxide; NO₂, nitrogen dioxide; NMD, particle number median diameter; MMD, particle mass median diameter; Individual AQI was calculated using Air Now formula; AQHI value was calculated based on its formula (Stieb et al. 2008).

Table 2.

Hemodynamic measures of isolated mouse hearts at baseline before ischemia and reperfusion.

Time Points	Parameters	Filtered Air (n=8)	Fresh DE (n=8)	Aged DE (n=8)
Baseline	HR (bpm)	323±20	304±28	315±30
	LVDP (cmH ₂ O)	157.7±21.3	79.7±10.7 *	102.5±11.2 *
	dP/dt _{max} (cmH ₂ O/sec)	5421±868	3127±561 *	3725±295 *
	dP/dt _{min} (cmH ₂ O/sec)	-4023±588	-2300±353 *	-3367±369
	Flow Rate (mL/min)	1.7±0.2	1.6±0.3	2.0±0.2
20 min of reperfusion	HR (bpm)	294±20	286±19	288±16
	LVDP (cmH ₂ O)	55±16	52±10	45±18
	dP/dt _{max} (cmH ₂ O/sec)	2047±624	1798±356	1726±700
	dP/dt _{min} (cmH ₂ O/sec)	-1570±424	-1331±271	-1179±533
	Flow Rate (mL/min)	1.8±0.3	1.6±0.2	2.3±0.4
40 min of reperfusion	HR (bpm)	292±20	279±12	279±23
	LVDP (cmH ₂ O)	63±20	61±12	46±14
	dP/dt _{max} (cmH ₂ O/sec)	2375±735	2287±433	1899±628
	dP/dt _{min} (cmH ₂ O/sec)	-1814±491	-1554±378	-1432±467
	Flow Rate (mL/min)	1.7±0.3	1.5±0.2	2.3±0.4
60 min of reperfusion	HR (bpm)	285±17	278±14	300±12
	LVDP (cmH ₂ O)	57±15	63±13	44±9
	dP/dt _{max} (cmH ₂ O/sec)	2413±676	2314±426	1829±499
	dP/dt _{min} (cmH ₂ O/sec)	-1771±444	-1589±365	-1169±57
	Flow Rate (mL/min)	1.6±0.3	1.4±0.2	1.9±0.4

Values are means ± SE. HR=heart rate, LVDP=left ventricular developed pressure; dP/dt_{max}=maximum 1st derivative of the change in left ventricular pressure/time; dP/dt_{min}=minimum 1st derivative of the change in left ventricular pressure/time; Flow rate, coronary flow rate.

* $p < 0.05$ compared with FA group at same time point.