HHS Public Access

Author manuscript

Inhal Toxicol. Author manuscript; available in PMC 2024 June 11.

Published in final edited form as:

Inhal Toxicol. 2024 March ; 36(3): 189–204. doi:10.1080/08958378.2024.2327364.

Biological effects of diesel exhaust inhalation. III cardiovascular function

Kristine Krajnak[a], **Hong Kan**[b], **Janet A. Thompson**[b], **Walter McKinney**[a], **Stacey Waugh**[a], **Tim South**[a], **Dru Burns**[e], **Ryan Lebouf**[e], **Jared Cumpston**[c], **Theresa Boots**[d], **Jeffrey S. Fedan**[b]

[a]Physical Effects Research Branch, National Institute for Occupational Safety and Health, Morgantown, WV, USA

[b]Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, Morgantown, WV, USA

[c]Animal Facilities, National Institute for Occupational Safety and Health, Morgantown, WV, USA

[d]Risk Evaluation Branch, National Institute for Occupational Safety and Health, Morgantown, WV, USA

[e]Respiratory Health Division, National Institute for Occupational Safety and Health, Morgantown, WV, USA

Abstract

Objective: Inhalation of diesel exhaust (DE) has been shown to be an occupational hazard in the transportation, mining, and gas and oil industries. DE also contributes to air pollution, and therefore, is a health hazard to the general public. Because of its effects on human health, changes have been made to diesel engines to reduce both the amounts of particulate matter and volatile fumes they generate. The goal of the current study was to examine the effects of inhalation of diesel exhaust.

Materials and Methods: The study presented here specifically examines the effects of exposure to 0.2 and 1.0mg/m^3 DE or filtered air (6h/d for 4 d) on measures of peripheral and cardio-vascular function, and biomarkers of heart and kidney dysfunction in male rats. A Tier 2 engine used in oil and gas fracking operations was used to generate the diesel exhaust.

CONTACT Kristine Krajnak ksk1@cdc.gov Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1000 Frederick Lane, Morgantown, West Virginia, 26508, USA.

Disclosure statement

The authors declare that they have no conflicts of interest in relation to this publication.

Disclaimer

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/08958378.2024.2327364.

Results: Exposure to 0.2 mg/m^3 DE resulted in an increase in blood pressure 1d following the last exposure, and increases in dobutamine-induced cardiac output and stroke volume 1 and 27d after exposure. Changes in peripheral vascular responses to norepinephrine and acetylcholine were minimal as were changes in transcript expression in the heart and kidney. Exposure to 1.0 mg/m^3 DE did not result in major changes in blood pressure, measures of cardiac function, peripheral vascular function or transcript expression.

Discussion and Conclusions: Based on the results of this study, we suggest that exposure to DE generated by a Tier 2 compliant diesel engine generates acute effects on biomarkers indicative of cardiovascular dysfunction. Recovery occurs quickly with most measures of vascular/cardiovascular function returning to baseline levels by 7d following exposure.

Keywords

Blood pressure; cardiac function; vascular function; particulate; diesel exhaust

Introduction

Workers in a number of different occupations, including but not limited to mining, oil and gas exploration and refining, long-haul truck drivers, and operators of other earth moving equipment can regularly be exposed to diesel exhaust (Hart et al. 2012; Harrison et al. 2018). Workers exposed to diesel exhaust may develop health problems that are similar to those seen in persons living in areas with high levels of air pollution (Langrish and Mills 2013; Robertson and Miller 2018; Rider and Carlsten 2019). These health problems include an increased risk for developing asthma, cardiovascular disease, reproductive problems and certain cancers (Mills et al. 2011; Robertson et al. 2012; Nemmar et al. 2016; Mookherjee et al. 2018). Workers exposed to diesel exhaust and people living in areas with high levels of air pollution may develop similar health problems because diesel particulate and fumes are major contributors to air pollution (Robertson et al. 2012; Hankey and Marshall 2017; Robertson and Miller 2018). Because diesel exhaust affects functioning of a number of physiological systems, and serves as a risk factor for a number of diseases, diesel engines have been improved or modified in an attempt to reduce and alter their emissions (What You Should Know About EPA Diesel Emissions Tiers - General Power Limited; genpowerusa.com). The particulate matter generated by diesel engines and concentrations of volatile compounds emitted vary from engine type to engine type. Therefore, characterizing the emissions released by a specific engine, and the physiological, cellular and molecular effects of diesel exhaust inhalation on various target organs is critical for identifying exposure-induced biological and physiological markers of disease or dysfunction.

As mentioned above, people exposed to high levels of pollution and/or high levels of diesel exhaust at work are at an increased risk of developing cardiovascular disease (Langrish and Mills 2013; Liu et al. 2017; Wilson et al. 2018; Rider and Carlsten 2019). Changes in cardiovascular function associated with exposure to fumes or aerosols containing particulate matter may be the result of the particulate matter entering the lung and inducing systemic inflammation that then affects other organs in the body, including the cardiovascular system (Brito et al. 2010; Kooter et al. 2010; Tong et al. 2019). An increased risk of cardiovascular disease may also be the result of translocation of particulate matter from the lungs into the

cardiovascular circulation where it can be carried to the heart, through the blood vessels, or to the kidneys altering kidney function, which in turn can also affect blood pressure and cardiovascular function (Brito et al. 2010; Langrish and Mills 2013; Nemmar et al. 2016). The effects of diesel emissions on health may also be due to the inhalation of other toxic components in the exhaust, e.g. volatile gases, which can be absorbed through the respiratory system and directly affect blood vessels, kidneys or the heart.

High levels of diesel exhaust have been measured at sites where hydraulic fracturing (i.e. fracking) occurs and in communities near fracking sites (Litovitz et al. 2013; Tsrebotnjak and Rotkin-Ellman 2014; Esswein et al. 2018). Diesel engines at fracking sites provide power to operate equipment used to construct fracking wells (e.g. mud pumps, winches) and to equipment used during the fracking process (e.g. sand moving machines, transport belts, frac pumps). Most of the trucks that carry supplies and water also have diesel engines (Litovitz et al. 2013; Tsrebotnjak and Rotkin-Ellman 2014; Esswein et al. 2018). Because of the large number of diesel engines in use at fracking sites, there is concern about workers and people in the surrounding communities being exposed to levels of diesel exhaust that may result in health problems. The National Institute for Occupational Safety and Health (NIOSH)has measured diesel particulate levels at a number of oil and gas sites. Exposure levels of diesel particulate in workers personal breathing zonesvaried depending upon their job, with the arithmetic mean for transportation workers being 10 $\mu g/m^3$ (range 0.1 to 52 $\mu g/m^3$) and for workers performing service or drilling jobs the arithmetic means were between 5.4 and 11.9 $\mu g/m^3$(Esswein et al. 2018). These investigators also found that when total carbon in the samples was measured at fracking sites, total carbon levels were between 100 and 1000 $\mu g/m^3$.

Because workers in a number of sectors, including workers on fracking sites, are exposed to high levels of diesel exhaust, and based on the results of the studies cited above demonstrating that inhalation of diesel exhaust (DE) affects peripheral and cardiovascular function, we used an animal inhalation model to determine how exposure to exhaust from a Tier 2 diesel engine affected peripheral vascular function, cardiovascular function, blood pressure, and the expression of markers in heart and kidney tissue that are associated with inflammation, oxidative stress or cardiovascular dysfunction. A Tier 2 engine was chosen to for the exposure because it is often used at fracking sites not only to provide power for equipment used in well drilling, but also for powering equipment used in the actual fracking process. The levels and size of particulate matter and of volatile compounds were measured so that it could be determined how exposure to these emissions may be associated with changes seen in measures of vascular or cardiovascular dysfunction (Mehus et al. 2015; Schisler et al. 2015; Brower et al. 2016; Nemmar et al. 2016). Based on measurements of total carbon in emissions collected at fracking sites (Esswein et al. 2018), we chose to expose animals to 2 doses of diesel, 0.2 and 1.0 mg/m^3, one at the lower range of measured exposures and the other at the upper range.

Methods

Animals:

Male Sprague-Dawley rats ([H1a: (SD) CVF, n = 8 rats/group, 225–250 g at arrival or 6–8 weeks of age) were obtained from Hilltop Lab Animals, Inc. (Scottdale, PA). All animals were free of viral pathogens, parasites, mycoplasm, *Heliobacter* and cilia-associated respiratory bacillus. The rats were acclimated to the facilities for 1 week after arrival and housed in cages ventilated with HEPA (high efficiency particulate air)-filters under climate-controlled conditions and a 12-h light/12-h dark cycle (lights on 0600 h). Food (Teklad 7913, Invigo; Madison, WI) and tap water were provided *ad libitum*. The animal facilities are pathogen-free, environmentally controlled, and accredited by AAALAC International. All procedures were approved by the CDC Morgantown Animal Care and Use Committee and were in compliance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

Inhalation exposure system:

An eight-kilowatt (kW) diesel generator (Onan QD 8000, part number 8HDKAK11451J, Cummins Inc., Columbus, IN) was used to produce diesel exhaust in real time during inhalation exposures. This diesel engine was Tier 2 EPA compliant. Tier 2 compliant engines were built between 2001 and 2005 and emit reduced levels of carbon monoxide, unburned hydrocarbons, nitrogen oxides and sulfur emission (What You Should Know About EPA Diesel Emissions Tiers - General Power Limited (genpowerusa.com)). A load bank was connected to the generator and set to 4 kW (50% generator load) for all inhalation exposures. Mobil 1Delvac 1300 Super Motor Oil 15w40 was used as the engine oil. The fuel used in the generator was ultra-low sulfur (15 ppm or less sulfur), No. 2 dyed winter blend from Jacobs petroleum products (Waynesburg PA).

A custom exposure system with software written in LabVIEW automatically controlled chamber air flows, particle concentration, and exposure duration. Several combustion gasses were monitored and recorded from inside the exposure chamber during each inhalation exposure run by using a multi-gas analyzer (Model PG-350Z, Horiba Instruments Inc., Irvine CA). These concentrations of gases were SO_2, NO, CO_2 and O_2, and were recorded every 2 s. Exposure chamber humidity and carbon monoxide levels were higher during the 1.0 mg/m^3 than 0.2 mg/m^3 exposure. The exhaust was further analyzed by GC/MS from samples collected over a 2–6 h collection periods (samples were collected into cannisters, and there were 1–2 cannisters used/exposure period at each dose of diesel). The measurement of volatile organic compoundswas conducted using a method published in the NIOSH Analytical Methods Manual (2018; 3900: Volatile organic compounds (VOC), C1 to C10 canister method (https://www.cdc.gov/niosh/nmam/default.html). A portion of the diesel exhaust was directed into the exposure chamber, and a computer-controlled ball valve determined the amount. Clean dry dilution air (80 L/min) from a mass flow controller was added to the diesel exhaust delivery line before it entered the top of the exposure chamber.

The aerosol mass concentration inside the exposure chamber was continuously monitored with a Data RAM (DR-40000 Thermo Electron Co.) and gravimetric determinations (37 mm

cassettes with 0.45 μm pore-size Teflon filters (2 L/min. sample flow) were used to calibrate and verify the Data RAM readings during each exposure run. For this study the target diesel concentration was either 0.2 mg/m^3 or 1.0 mg^3 over 6-hr long exposures each day. Rats (8 per group) were placed in the custom-made, whole-body exposure chamber. After each exposure, animals were returned to their home cage in the animal facility.

Particle size distribution inside the exposure chamber (count based) was collected (SMPS Model 3081, TSI Inc., Shoreview, MN). Two methods were used to measure particulate diameter, electric and aerodynamic mobility. The count median electric mobility diameter was 120 nm. Mass-based aerodynamic particle size distribution was determined in the exposure chamber by using a Micro-Orifice Uniform Deposit Impactor (MOUDI, MSP Model 110, MSP Corporation, Shoreview, MN). The mass median aerodynamic diameter was found to be 170 nm. Table 1 provides the measured exposure chamber conditions for all exposures used in this study. Gravimetric filter data was used for the particle concentration data and combustion gasses were monitored in real time inside the exposure chamber.

Microvessel physiology:

Animals were euthanized by i.p. injection of sodium pentobarbital solution (100–300 mg/kg) and exsanguination. Tails were dissected from rats after exsanguination and placed in cold Dulbecco's modified Eagle's medium with glucose (Invitrogen/Gibco; Carlsbad, CA). Ventral tail arteries from the C18–20 region of the tail were dissected, mounted on glass pipettes in a microvessel chamber (Living System; Burlington, VT), and perfused with bi-carbonated HEPES buffer (130 mM NaCl, 4 mM KCl, 1.2 mM $MgSO_4$, 1.8 mM $CaCl_2$, 10 mM HEPES, 1.80 mM KH_2PO_4, 0.03 mM EDTA, plus 10% glucose) warmed to 37 °C. Arteries were pressurized to 60 mm Hg and allowed to equilibrate for approximately 1 hr. After an hour acclimation period, the chamber buffer was replaced with fresh HEPES buffer and responsiveness of the arteries to phenylephrine (PE)-induced vasoconstriction and acetylcholine (ACh)-induced re-dilation was measured. All chemicals for microvessel exposures were purchased from Sigma (Indianapolis, IN) unless otherwise noted. To assess the effects of treatment on sensitivity to α_1-adrenoreceptor-mediated vasoconstriction, PE was added to the chamber so that changes in the concentration occurred in half-log increments (−9.0 to −5.5 M) and the internal diameter of the artery was recorded after the arteries stabilized (approximately 5 min between concentrations). After measuring the dose-dependent vasoconstriction that occurred in response to PE, the chamber buffer, was removed and replaced with fresh, oxygenated HEPES buffer. After rinsing in oxygenated HEPES buffer for 15 min, arterial diameter returned to near baseline levels. Because ventral tail arteries usually display little basal tone, endothelial-mediated re-dilation was assessed after arteries were pre-constricted to approximately 50% of their baseline diameters with PE. In pilot work, we demonstrated that re-constricting arteries with PE did not affect subsequent responses to ACh or other vasomodulating factors. To assess the dilatory effects of ACh, the agonist was added cumulatively in half-log increments (−10.0 to −5.0 M) and changes in the internal diameter of the vessel were measured as described for PE.

Procedures for in vivo hemodynamic measurements—To obtain *in vivo* measurements of cardiac function and reactivity to adrenoreceptor agonistsat 1, 7 and 27

d after DE exposure, rats were anesthetized with 3% isoflurane mixed with1 L/minoxygen in an induction chamber and maintained at 1–2% isoflurane and 0.5 l/min of oxygen during surgery to instrument the animals. Cardiopulmonary responses [heart rate (HR); breathing rate and depth] and toe pinch spinal reflex were examined as intra-operative monitoring techniques. The concentration of isoflurane was adjusted to maintain the proper depth of anesthesia. A temperature-controlled heating pad was used to regulate normal body temperature, and temperature was monitored *via* an anal probe during the entire procedure. Before the surgery, surgical instruments and supplies were autoclaved, and the catheter was cold-sterilized using Cidex (Physician Sales and Services, Inc.; Jacksonville, FL) prepared according to the manufacturer's instructions and flushed with sterile saline solution. The rat was placed in dorsal recumbent position, and the incision sites were clipped and the aseptically prepared with povidone-iodine, followed by 70% alcohol. Mikro-Tip® ultra-miniature PV loop catheters (SPR-901; Millar, Inc., Houston, TX) were inserted into the left ventricle through the carotid artery. The correct position of the catheter tip in the left ventricle was confirmed by the waveform of a pressure-volume loop visualized on a computer monitor. After stabilization for 20 min, signals were continuously recorded at a sampling rate of 1000 samples/sec using a PV conductance system (MPVS-Ultra; Millar, Inc. Houston, TX) connected to the PowerLab4/30 data acquisition system (AD Instruments; Colorado Springs, CO), stored and displayed on a personal computer using the LabChart7 Software System (AD Instruments). Increasing doses of norepinephrine or dobutamine (Hospira, Inc.; Lake Forest, IL) were administrated through a catheter (polyurethane, 3 French size) that had been placed in the right jugular vein. Systolic (SBP), diastolic (DBP), mean arterial blood pressure (MAP) and left ventricular function were measured. Parallel conductance volume was calculated by the software and used for the correction of the cardiac mass volume, and each rat was euthanized by exsanguination under deep anesthesia at the conclusion of the experiment.

Measuring oxidative stress: Heart and kidney tissues were homogenized in 1 ml of 0.1 M phosphate buffered saline (PBS) with protease inhibitors. The supernatant was removed and protein concentrations (absorbance ration of λ260/λ280nM) were measured using the BCA protein assay (Fisher, Pittsburgh, PA). Concentrations of reactive oxygen species (ROS) were measured using 2′7′-dichlorofluorescein diacetate (DCFH-DA; Sigma-Aldrich, St. Louis, MO), or 2′7′-dichlorofluorescein (Sigma-Aldrich). When these dyes are added to tissue homogenates they fluoresce (i.e. higher levels of fluorescence indicate greater levels of ROS). To perform this assay, duplicates of the supernatant from each pellet (10 μl) were pipetted into a 96-well plate. Re-constituted dye was diluted so that the final concentration was 1 mM, and 50 μl was added per well. Plates were incubated in the dark for 45 min and then fluorescence was measured at 490–540 nM using a Synergy H1 all in one microplate reader (Biotek; Winooski, VT). Background measures (wells with dye plus PBS) were subtracted from each sample, and fluorescence/μg tissue was calculated and analyzed as described below.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR): qRT-PCR was performed to determine if exposure to DEinhalation resulted in changes in transcript levels in the heart and kidney using the methods described in Hughes et al. (Hughes et al.

2009). Because the goal of these studies was to determine if exposure to DE inhalation affected the cardiovascular system, we examined transcripts in the heart and kidney (these tissues are involved in blood pressure regulation). We examined transcripts that were indicative of changes in the expression of inflammatory cytokines, apoptotic factors, and factors involved in vasomodulation, vascular remodeling and ionic status in the heart and kidney. The specific transcripts measured were chosen based on the results of previous studies examining the effects of inhaled particulate matter on the cardiovascular system and the kidneys (Krajnak et al. 2011; McKinney et al. 2012; Roberts et al. 2014; Krajnak et al. 2020). The transcripts measured in the heart included interleukin-1β (*Il-1 β*), endothelin (*Et1)*, factors involved in regulating apoptosis (*Bax, Bad* and *Bcl2*), hemeoxygenase (*Hmox*), hypoxia induced factor 1α (*Hif1-α*), inducible and endothelial nitric oxide synthase (*iNos*and *eNos*, respectively) and tumor necrosis factor α (*Tnf*-α). Kidney transcripts examined included angiotensin converting enzyme (*Ace*), *Il1-β*, *Il-6*, tissue inhibitor of metalloproteinases (*Timp)*, tumor necrosis factor (*Tnf)-α*, *nNos*, *iNos2,eNos3* and vascular endothelial growth factor (*Veg-f*). RNA was isolated from the tissue using RNAeasy lipid Miniprep kits (Qiagen; Valencia, CA), and first strand cDNA was synthesized from 1 mg of total RNA using a Reverse Transcription System (Invitrogen; Carlsbad, CA). Melt curves were run for each transcript using each tissue. Samples that did not show a single defined melt peak in the 80 °C range were not included in the data set. To determine if the treatment resulted in changes in transcript levels, fold changes from the same day controls were calculated.

Superoxide dismutase concentrations: Because previous studies had demonstrated that the primary effects of inhalation to toxins are changes in tissue levels of oxidative stress, cytokines and concentrations of anti-oxidant enzymes, an ELISA was used to measure super-oxide dismutase-2 (SOD2, R&D Systems Inc.; Minneapolis, MN). Proteins concentrations in tissues were measured using the manufacturer's protocols.

Analyses: Microvessel data were analyzed using repeated measures analysis of variance (ANOVAs) to determine if there were treatment differences between the control and DE-treated groups. Significant pairwise interactions were probed with one-way ANOVAs or student's *t*-tests. Non-linear regression analyses using a log-linear transformation were performed to determine the effective dose 50 (ED50) for each artery. The ED50s were then analyzed using Student's *t*-tests (air vs. diesel at 1, 7 and 27 d). ROS, tissue protein concentrations and transcript levels were analyzed using a 2-way (treatment × time) ANOVAs. Post-hoc comparisons were made using Student's *t*-tests. Changes in cardiac physiology collected through telemetry, or *in vivo* PV-loop measurements were analyzed using 2 (treatment) × 3 (days of recovery) repeated measures ANOVAs. Subsequent pairwise comparisons were tested using Fishers LSD. All telemetry and PV loop data were analyzed using R (Team RC 2023); other data were analyzed using Jmp (15.1.01). The values in the figures were expressed as the mean ± SEM. Differences with $p \leq 0.05$ were considered statistically significant.

Results

Body weight:

Body weights in air controls and DE-exposed were similar after exposure to 0.2 or 1 mg/m^3 DE (Figure 1A and B, respectively).

Microvessel responsiveness:

Figures 2 and 3 show the dose-dependent vasoconstriction in response to PE (1 d: A, 7 d: C, and 27 d: E), and the dose-dependent re-dilation to ACh (1 d: B, 7 d: D, and 27 d: F), in air- and DE-exposed rats. Exposure to 0.2 mg/m^3DE did not alter the dose-dependent responses to PE or ACh (Figure 2A–F). Exposure to 1 mg/m^3DE resulted in a reduced responsiveness of arteries to PE-induced vasoconstriction 1d after the exposure, at the $10^{-6.5}$M concentration (Figure 3A), but did not affect responses to PE-induced vasoconstriction at the other time points, or ACh-induced re-dilation (Figure 3B–F). Exposure at the low (0.2 μg/m^3) and high (1.0 μg/m^3) did not result in significant differences in the ED50 or in the dose-dependent vasoconstriction or re-dilation induced by any concentration of PE or ACh, respectively.

Low dose (0.2 mg/m^3)PV loop:

Exposure to the low dose of DE did not affect NE-induced changes in any measures of blood pressure (Figure 4A–C). However, there was an increase in baseline measures of systolic blood pressure (SBP) and mean arterial blood pressure (MAP) in DE-exposed animals 1d following exposure (Brito et al. 2010). There was also an increase in diastolic blood pressure (DBP) in animals exposed to DE 27 d following the exposure (Table 2). Dobutamine-induced changes in cardiac output (CO) and cardiac stroke volume (SV)were greater in animals exposed to DE than in air controls (Table 3). In contrast, dobutamine-induced reductions in the end systolic pressure (PES) and in the end diastolic pressure (PED) were greater in DE-exposed animals than in air controls.

High dose (1.0 mg/m^3) PV loop:

Baseline measures of blood pressure were not affected by exposure to the high dose of DE (Table 4). However, norepinephrine(NE)-induced increases in SBP were greater in DE-exposed compared to air-exposed animals 1 and 7d following exposure, and MAP was greater in DE than air exposed 7d following exposure (Figure 5). Dobutamine-induced increases in cardiac stroke volume tended to be greater in DE than air control animals 7 d followingexposure (Table 5).

Oxidative stress:

ROS levels were significantly greater in heart tissue from rats exposed to DE (0.2 μg/m^3) than from rats exposed to air 7 d after exposure (Figure 6A). There were no significant differences between the groups on 1 or 27d after exposure. Exposure to DE resulted in a significant decrease in ROS in the kidneys 1 d after exposure (Figure 6B), but ROS concentrations returned to air control levels 7 d after exposure.Exposure to the high dose of DE did not affect ROS concentrations in the heart or kidney (Figure 6C, D). Concentrations

of the anti-oxidant enzyme, superoxide dismutase (SOD)2, in the heart, were not affected with low dose exposure to DE (Figure 7A), but with exposure to the high dose, it was significantly lower 27 d after exposure to DE 1 d after exposure (Figure 7C). SOD2 concentrations in the kidney were significantly reduced 7d after exposure to the low dose of DE as compared to air controls (Figure 7B). Exposure to the high dose of DE resulted in a reduction in SOD2 27d after exposure as compared to 1d after exposure (Figure 7D).

Quantitative RT-PCR:

Exposure to the low dose of DE resulted in a significant increase in the expression of *Bcl2*, *Et1, Timp-1* and *Hif-1* in heart tissue and a reduction in *Hmox*1d after exposure to DE. There was also an increase in *Bcl2*, *Timp-1* and *Hif-1* 7d after the last exposure and a reduction in *Nos2* expression 27d after exposure (Table 6). In the kidney, there was a significant increase in superoxide-dismutase S*od2*1d following exposure to DE (Table 7). Seven days after DE exposure, there was an increase in *Il6* in the kidney, and 27d after exposure there were reductions in *Il1β*, *Nos3 Sod2* and *Tnfα* in the kidney.

Exposure to the high dose of diesel resulted in reductions in *Il1β*, *Nos2* and *Nos3* transcript levels and an increase in *Timp-1* transcript levels in heart tissue 1d following the exposure (Table 8). There was also a reduction in *Cat* transcript levels 7d after exposure to DE and a reduction in *Bad* and increase in *Il6* 27d following exposure to DE. In the kidney, exposure to the high dose of DE resulted in a reduction in *Il6* and *Ace* 1d following exposure, a reduction in *Nos1* 7d after exposure, and a reduction in *Ace* 27d after exposure (Table 9).

Discussion

Exposure to DE has been associated with respiratory and reproductive problems, an increased incidence of cancer, and an increased incidence of cardiovascular disease (Langrish and Mills 2013; Robertson and Miller 2018; Rider and Carlsten 2019). In this study, animals were exposed to DE for 4d at a dose of 0.2 mg/m^3 or 1 mg/m^3 generated by a Tier 2 compliant diesel engine. There was not a significant change in ventral tail artery responsiveness to vasoconstricting or dilating factors. However, there were changes in baseline measures of SBP and MAP 1d following exposure to the low dose of DE, and changes in SBP in response to NE treatment after exposure to the high dose of DE. Exposure to low doses of DE also were associated with transient changes in oxidative stress, factors involved in vascular modulating and remodeling, anti-oxidant enzymes and inflammation in heart and kidney tissues.

In previous studies, changes in peripheral vascular responsiveness to vasoconstricting and/or vasodilating agents were associated with changes in measures of blood pressure or cardiac output (Krajnak et al. 2011; 2020). In the current studies, exposure to *in vivo* and *in vitro* measures of blood pressure and vascular responsiveness were consistent. Vascular responsiveness to NE was minimally affected by diesel exposure but baseline measures of blood pressure were, especially at the 0.2 mg/m^3 dose. However, exposure to the high dose of DE did not affect peripheral vasculature responsiveness to the α_1-adrenoreceptor agonist, PE, but did result in an increase in blood pressure in response to NE-treatment when measured in cardiac blood vessels. The inconsistent responses between the peripheral

and cardiovascular tissues may have been due to different receptors being activated; PE specifically acts at a single receptor, while the endogenous neurotransmitter NE acts on multiple adrenergic receptors (Ruffolo and Hieble 1994; McGrath 2015; Michel and Balligand 2016). The different responses seen in the ventral tail artery and cardiac tissues may also have been due to the fact that the distribution of receptors for various modulating factors is different in the heart and other vascular beds of the body (Ruffolo and Hieble 1994). For example, findings from other studies have demonstrated that exposure to diesel exhaust has significant effects on endothelial cell function, and responsiveness of arteries to ACh and the nitric oxide donor, S-nitroso-N-acetylpenacillamine (SNAP; (Robertson et al. 2012; Schisler et al. 2015; Törnqvist et al. 2007). It's also possible that changes in responsiveness to PE or AChin peripheral arteries weren't seen in the current study because the exposure length or dose weren't high enough. The fact that there was no difference between the 0.2 and 1 mg/m^3 exposures also suggests that a longer exposure may be needed to see more pronounced changes in cardiovascular function. Additional studies examining the effects of DE exposure on other blood vessels may provide a more complete picture of the effects of DE on peripheral vascular function and its relationship to cardiovascular function.

There were also dobutamine-induced changes in measures of cardiac function. Dobutamine acts at the β_1-adrenoreceptor and can induce vasodilation and increase cardiac output (Grose et al. 1986; Ahonen et al. 2008). Therefore, it is possible that the DE-induced changes in heart rate and cardiac output seen in this study may have been due to DE exposure altering dobutamine's function at the receptor or altering tissue responses to dobutamine treatment (Michel and Balligand 2016). In addition, previous studies have shown that exposure to particulate matter might have prolonged effects of dobutamine-induced increases in heart rate and cardiac output (Krajnak et al. 2020). These changes, which were apparent 7 and 27 d after exposure may be the result of an increased response of neural inputs (i.e. autonomic inputs) to the heart after DE exposure, or the direct result of particles or other components of the exhaust on cardiovascular tissues (Kan et al. 2018). These data are consistent with epidemiological findings in humans showing that exposure to DE has effects on the cardiovascular system, and that these changes are in part due to changes in the sensitivity of the cardiovascular system to adrenergic modulation (Törnqvist et al. 2007).

Changes in kidney function can also affect blood pressure (Li et al. 2018). Previous studies demonstrate that angiotensin release and fluid regulation affect cardiovascular function and blood pressure (Barboza et al. 2018). In the current experiments, exposure to the high dose of DE resulted in a reduction in transcript expression for angiotensin converting enzyme (*Ace*) 1 and 27d following exposure. The change in *Ace* expression in the kidney with the high dose exposure is consistent with the changes in NE-induced blood pressure seen in exposed animals. In contrast, the low-dose DE exposure was associated with a reduction in measures of ROS1 d after the exposure. The dye used to detect ROS emits a fluorescent signal in response a number of different ROS, including nitric oxide [NO; (Gomes et al. 2005)]. It is possible that the DE-induced reduction in ROS was associated with a reduction NO which may have acutely affected blood flow in the kidney (Barboza et al. 2018). However, this reduction was transient and not associated with changes in Sod2 enzyme concentrations or transcript levels of the anti-oxidant enzymes *Sod2* or *Cat* in the kidney,

suggesting that if the DE exposure resulted in changes in oxidative stress in the kidney, they were transient and not associated with changes in anti-oxidant enzymes. There also wasn't any indication of inflammation as measured by RNA or protein concentrations in kidney tissue. However, it is possible that DE inhalation affected blood pressureby regulating fluid and electrolyte levels in the circulation and the synthesis and release of angiotensin (Ozkayar et al. 2016; Barboza et al. 2018). Changes in cardiac function, or damage to endothelial cells in the kidney can also result in changes in the release of angiotensin or other vascular regulating factors that regulate blood pressure (Gottipolu et al. 2009; Thompson et al. 2019). Additional studies examining the effects of DE inhalation on the interaction between renal and cardiovascular function are warranted.

In the heart, exposure to the low dose of DE was associated with an increase in the expression of the anti-apoptotic gene *Bcl2* 1 and 7d after exposure. It also resulted in an increase in *Et1*and *Hif1* expression and reduction in *Hmox* expression. These changes are consistent with a reduction in blood flow to the heart which could have induced hypoxia. The increase in *Et1* suggests that there could have been vascular remodeling in animals exposed to DE. Interestingly, there were fewer changes in transcript expression with exposure to the high dose of DE. There were reductions in inflammatory markers and in the expression of both endothelial and inducible NOS. These changes were associated with changes in NE-mediated blood pressure and a reduction in *Ace* expression in the kidney. Therefore, it's possible that altered response of blood pressure to NE may have been the result of changes in kidney-induced blood pressure and cardiovascular function as mentioned above. Additional studies with longer exposures will help determine the mechanisms underlying DE-induced changes in cardiovascular and renal function.

The fact that *in vivo* measures of blood pressure, and changes in transcript levels in the heart and kidney did not show a response may have been the result of difference on carbon monoxide (CO) levels.

Exposure to DE has been associated with an increased risk for developing a lung, colon, bladder, and potentially other cancers (Guo et al. 2004; Kachuri et al. 2016). It has also been associated with an increased risk of developing cardiovascular disease (Brito et al. 2010; Wilson et al. 2018; Phillippi et al. 2022). Epidemiological studies examining the effects of DE exposure on the kidney have produced mixed findings, with some studies showing that exposure to DE inhalation results in an increased risk for developing kidney cancer, while others showing that change in risk is marginal (Guo et al. 2004; Peters et al. 2018). This discrepancy may be the result of genetic mutations that regulate cell cycle and carcinogenesis (Roth et al. 1997). Future studies looking at diesel with various additives, and the effects of exposure to emissions from newer diesel engines that produce less particulate, may provide new information regarding a worker's risk of developing cancers or other diseases when exposed to DE. Studies examining longer term exposures can also provide additional information on how DE exposure may induce peripheral and cardiovascular disease.

Funding

This work was funded by a National Occupational Research Agenda Award from the National Institute for Occupational Safety and Health awarded to Jeffrey Fedan 6927ZLDC.

Data availability statement

Once published, data presented in tis manuscript will be available through the National Institute for Occupational Safety and Health.

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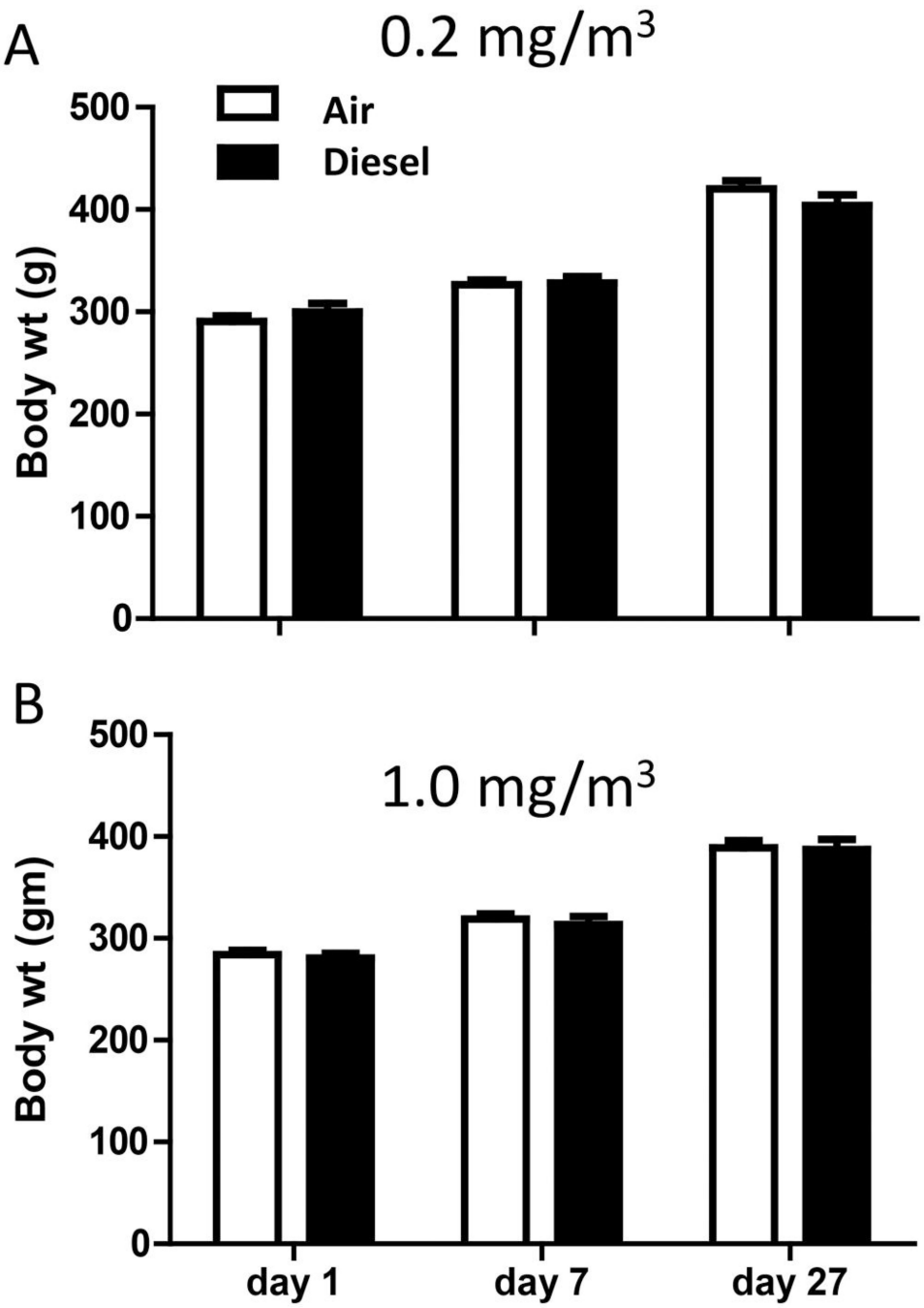

Figure 1.

Body weights (g) of rats 1, 7 or 27 d following exposure to filtered air or a low (A) or high (B) dose of DE. All animals gained weight over the experiment and there were no significant differences in body weight between air-exposed or DE-exposed animals.

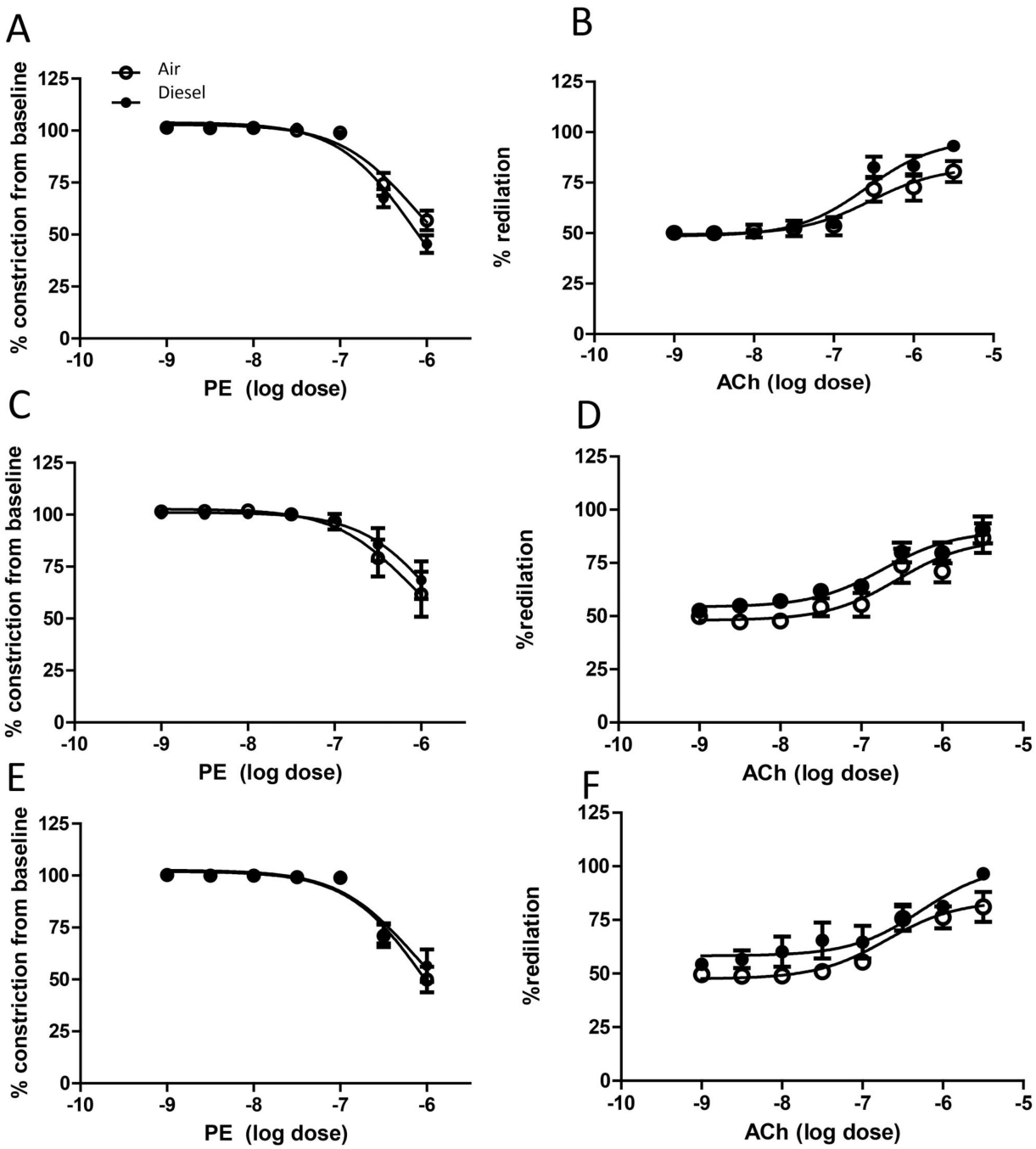

Figure 2.

Dose response curves to phenylephrine (PE; A, C, E) or acetylcholine (ACh; B, D, F) of ventral tail arteries from air- or low dose DE-exposed animals 1, 7 and 27 d following exposure. There were no significant differences between air- and DE-exposed arteries to PE-induced vasoconstriction or ACh-induced re-dilation.

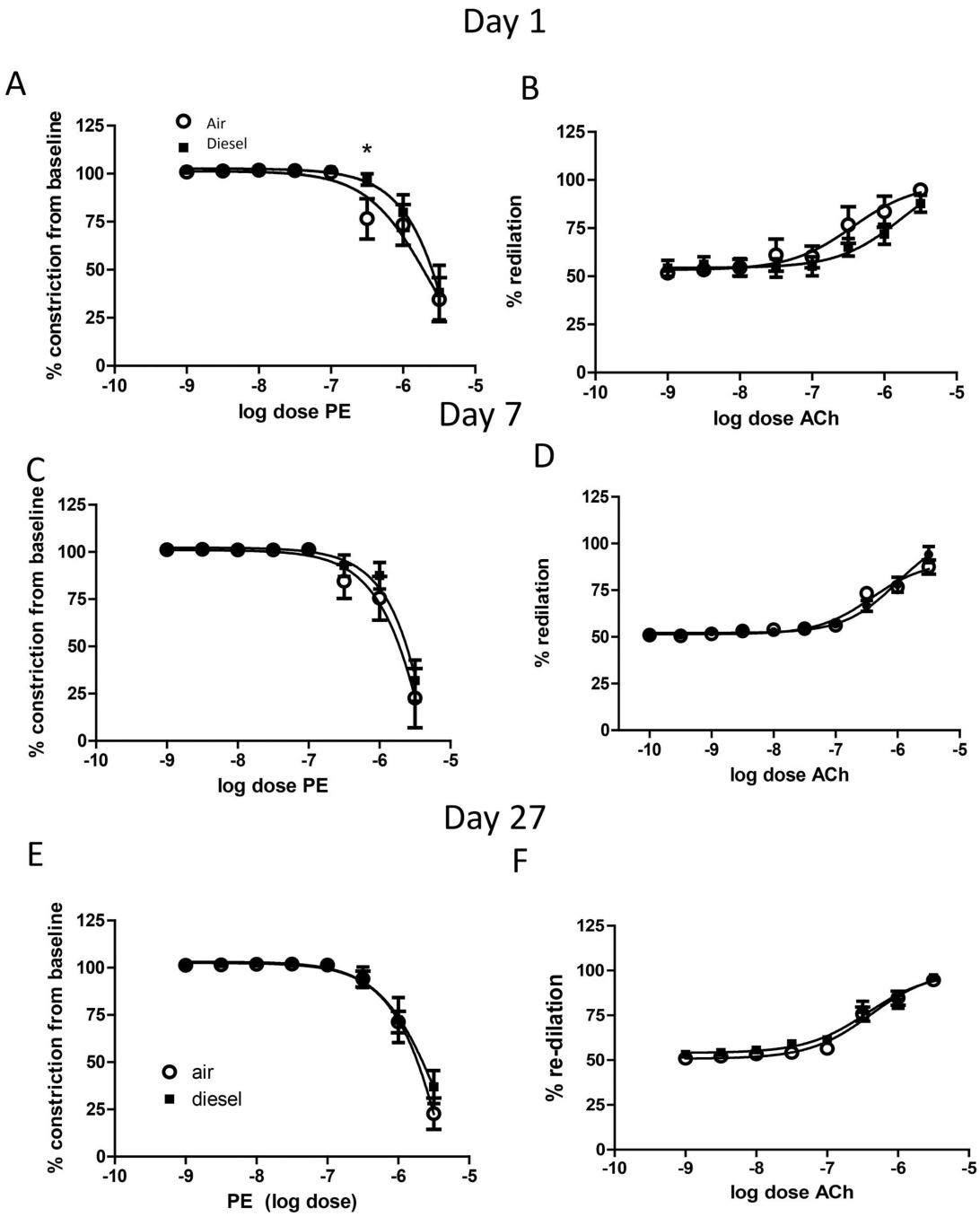

Figure 3.

Dose response curves to phenylephrine (PE; A, C, E) or acetylcholine (ACh; B, D, F) of ventral tail arteries from air- or high dose DE-exposed animals 1, 7 and 27 d following exposure. Arteries from DE-exposed animals showed a small reduction in sensitivity to PE-induced vasoconstriction 1d following exposure (* $p < 0.05$). There were no treatment-associated changes in ACh-induced re-dilation.

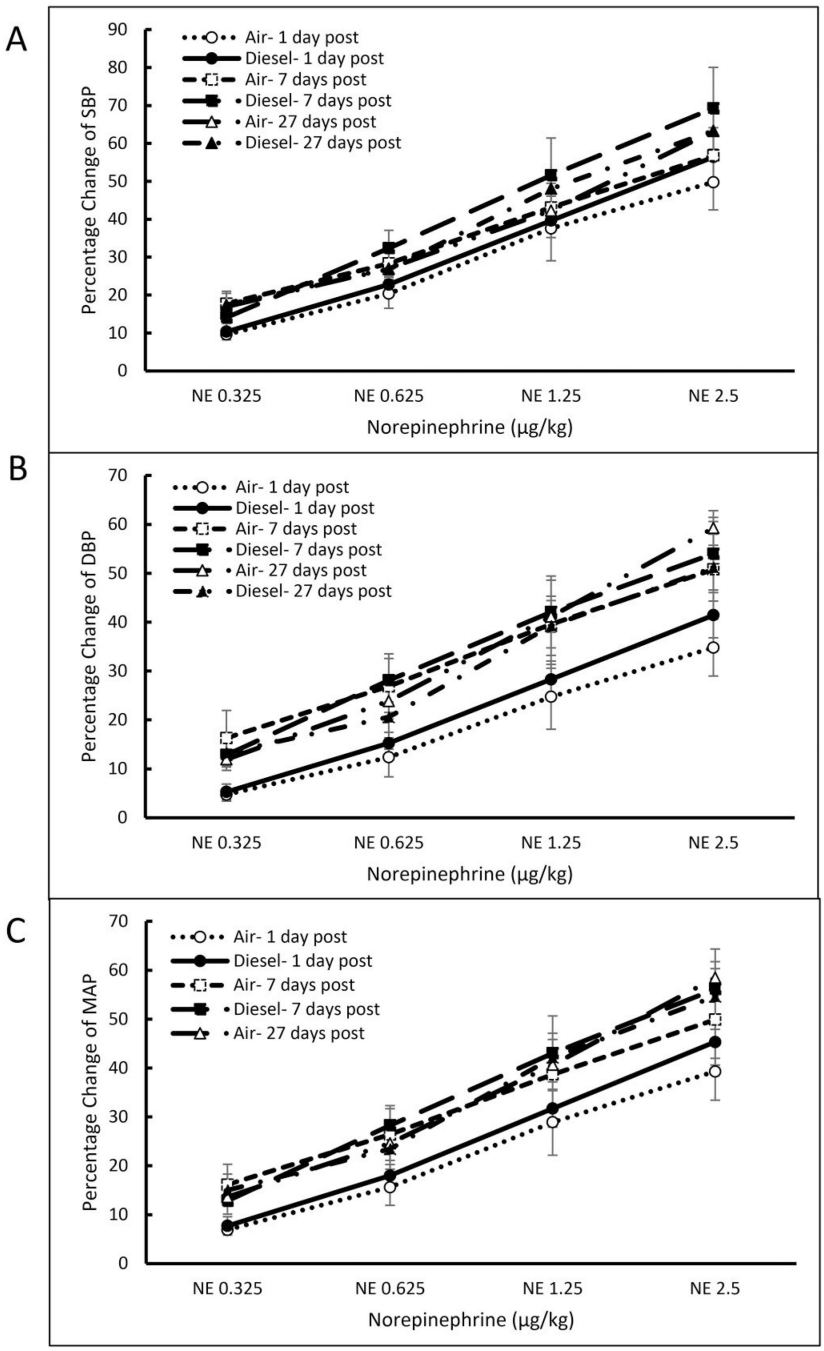

Figure 4.

Norepinephrine (NE)-induced changes in systolic (SBP, a), diastolic (DBP, B) or mean arterial (MAP, C) blood pressure 1, 7 or 27 d after exposure to filtered air or low dose DE. Although baseline measures of SBP, DBP and MAP were affected by DE exposure (see Table 2), there were no treatment-associated changes in SBP, DBP or MAP in response to NE.

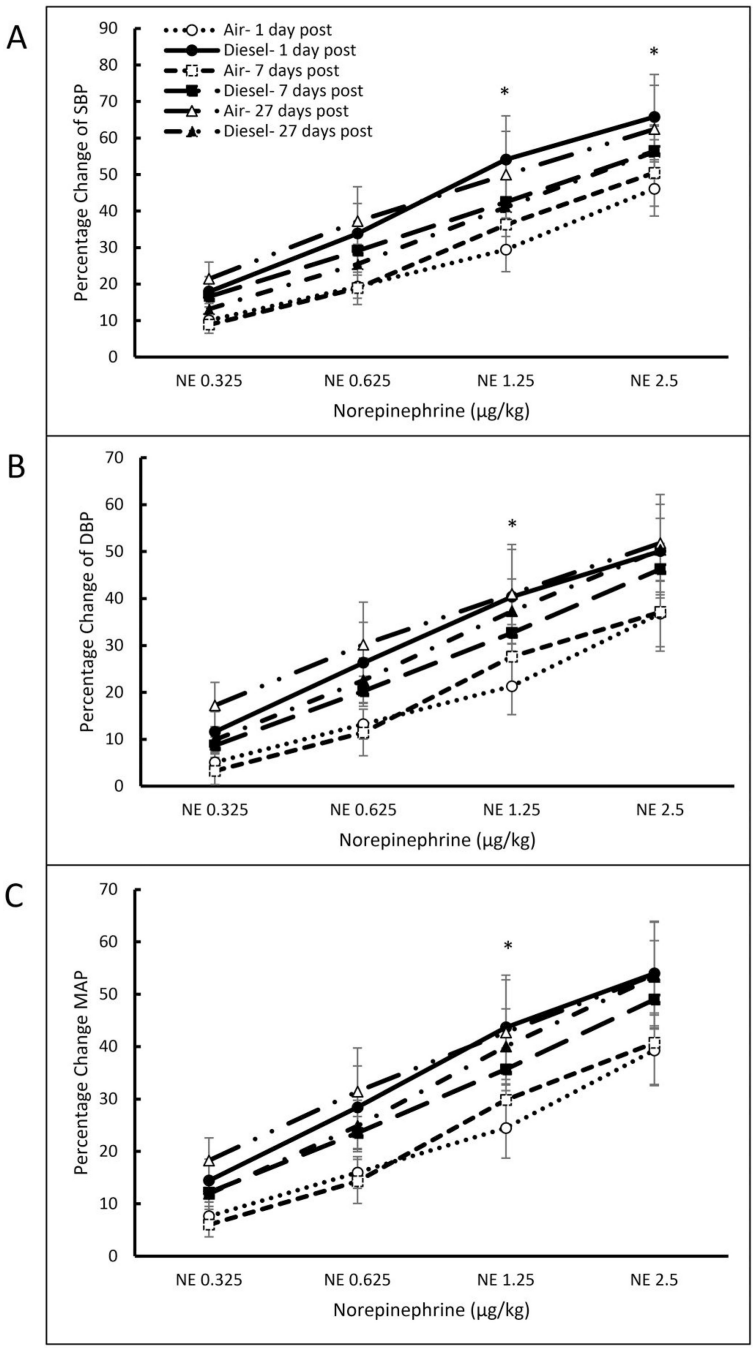

Figure 5.

Norepinephrine (NE)-induced changes in systolic (SBP, a), diastolic (DBP, B) or mean arterial (MAP, C) blood pressure 1, 7 or 27 d after exposure to filtered air or high dose DE. There were no changes in baseline blood pressure measures (Table 4). However, NE-induced changes in SBP, DBP and MAP were significantly greater in DE-exposed than air-exposed animals 1d following exposure. These differences were significant at the higher doses of NE (*1d air different than 1d DE-exposed, $p < 0.05$).

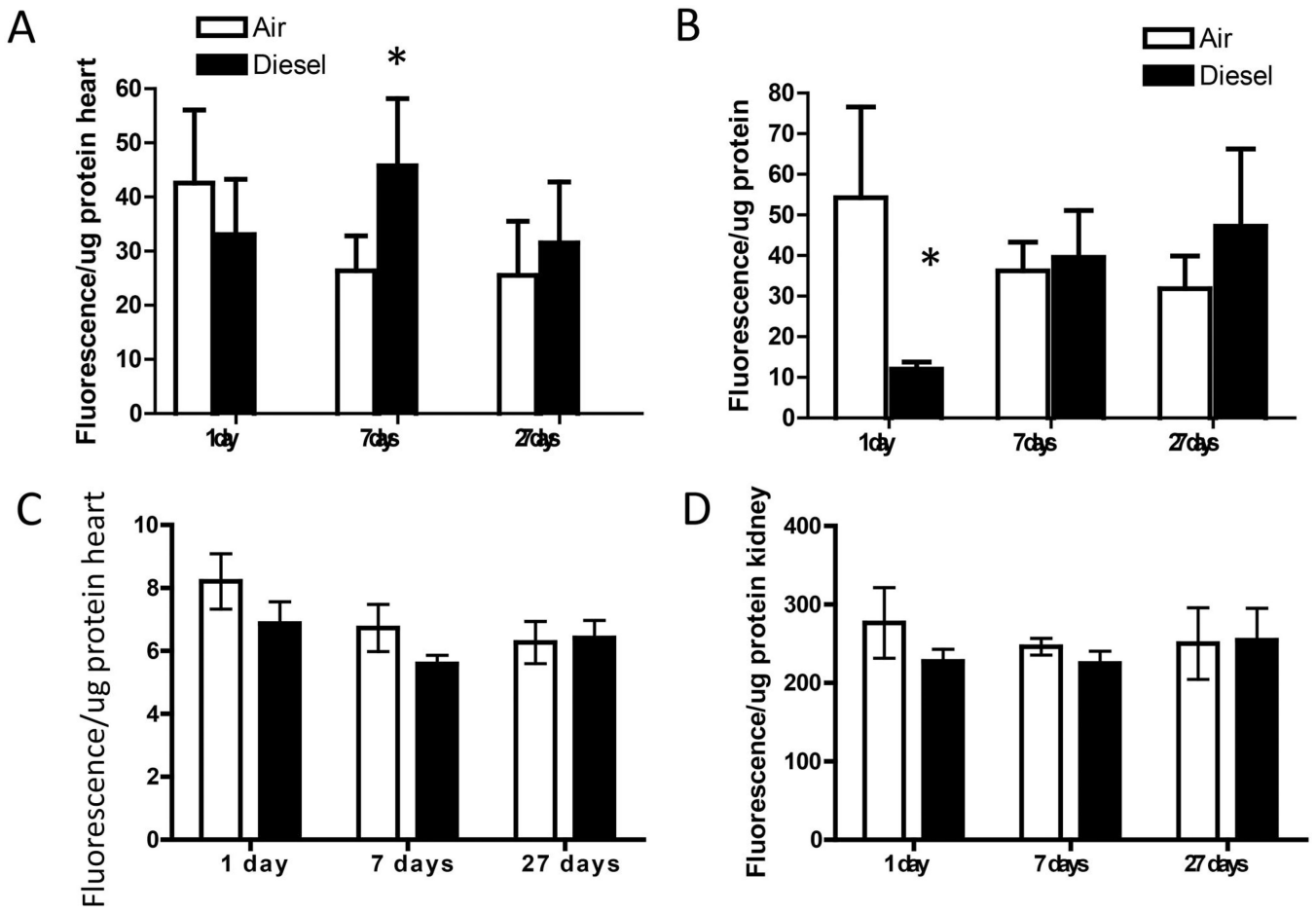

Figure 6.

Average concentrations of ROS (fluorescence/ug tissue) in the heart (A and C) and kidney (B and D) tissues of animals exposed to filtered air or a low or high dose of DE. ROS was significantly increased 7d following exposure to the low dose of DE in heart tissue (A), and reduced 1d following exposure in kidney tissue (B). There were no significant changes in ROS in the heart or kidney after exposure to the high dose of DE.

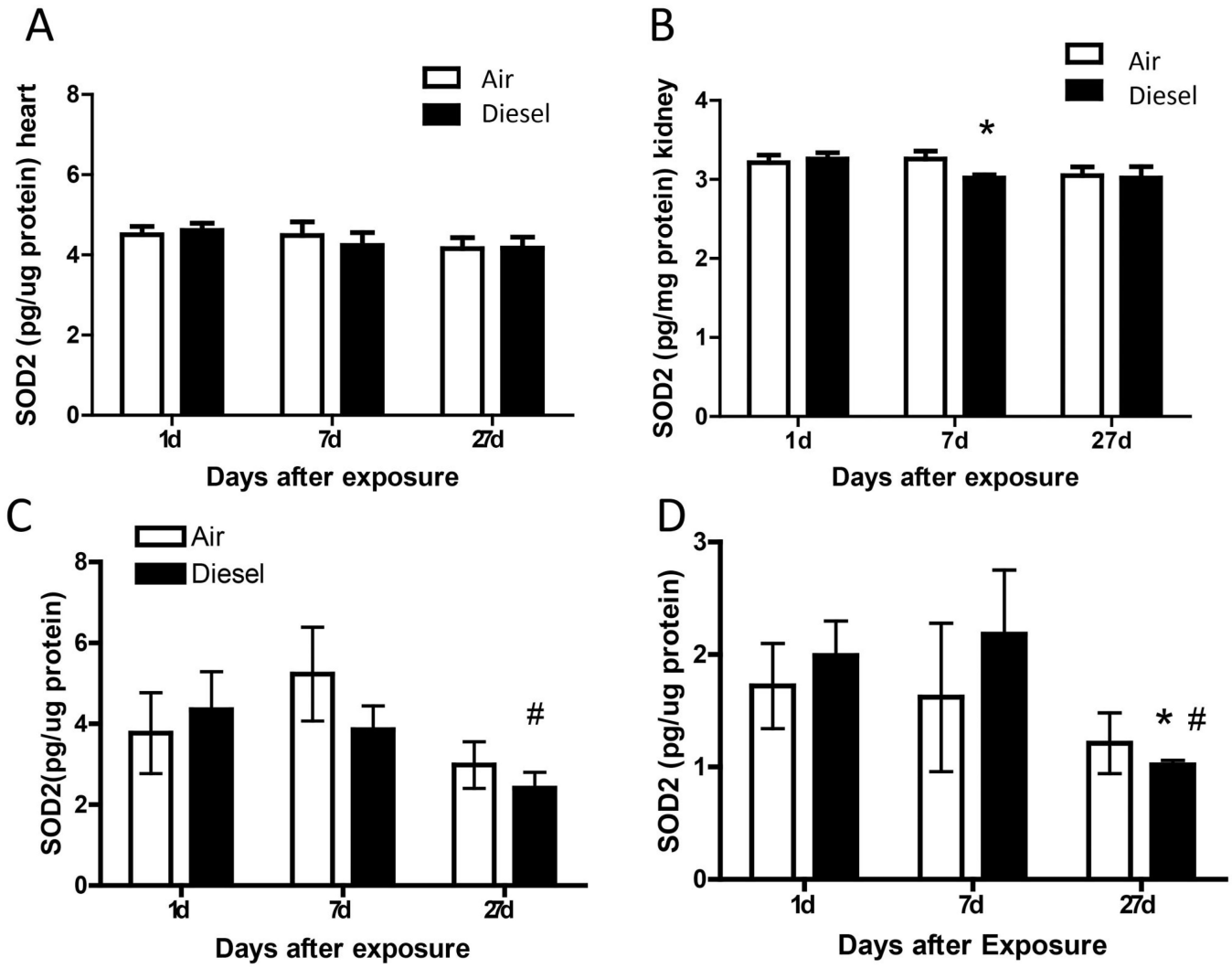

Figure 7.

Average superoxide dismutase concentrations (SOD; pg/μg protein) 1, 7 or 27 d after exposure to filtered air or a low or high dose of DE in the heart (A and C, respectively) and (B and D, respectively). Exposure to the low dose of DE did not alter SOD in heart tissue (A), but did result in an increase in SOD in the kidney 7d after exposure (B, $*p < 0.05$). Exposure to the high dose of DE resulted in a gradual decrease in SOD in both the heart (C) and kidney (D) from 1d to 27d following exposure ($\#p < 0.05$). SOD concentrations in the kidney also were lower in DE-exposed than air-exposed tissue 27d after exposure ($*p < 0.05$).

Table 1.

Concentrations of gases and other volatile chemicals in samples of diesel exhaust (DE) collected at diesel particulate concentrations of 0.2 and 1.0 mg/m^3 over a 6 h period.

	0.2 mg/m^3 DE Analyte concentrations PPM (mean ± sd)	1.0 mg/m^3 DE Analyte concentrations (mean ± sd)	NIOSH RELs (PPM)
Carbon dioxide	3550 (580)	3611 (1100)	5000
Nitric oxide	8 (2)	4 (4)	25
Sulfur dioxide	<LOD	1 (1)	2
Carbon Monoxide	4 (2)	18 (2)	35
2,3-Butanedione	<LOD	<LOD	.005
2,3-Hexanedione	<LOD	<LOD	10
2,3-Pentanedione	0.006 (0.001)	0.001 (0.0001)	.009
Acetone	0.028 (.024)	0.028 (.056)	250
Acetonitrile	0.003 (.002)	0.005 (0.003)	20
Benzene	0.014 (.003)	0.029 (.044)	8
Carbon dioxide	3550 (580)	3611 (1100)	5000
Carbon monoxide	4 (2)	18 (2)	35
Chloroform	<LOD	<LOD	2
D-Limonene	0 (0)	0 (0)	No REL
Ethanol	0.008 (0.013)	0.005 (0.009)	1000
Ethylbenzene	<LOD	0.002 (0.0005)	800
Isopropyl Alcohol	<LOD	<LOD	100
Methyl Methacrylate	<LOD	0.0006 (0.0001)	100
Methylene Chloride	<LOD	0.002 (0.001)	None established
Styrene	<LOD	<LOD	50
Sulfur dioxide	<LOD	1 (1)	2
Toluene	0.615 (0.164)	0.734 (1.145)	100
alpha-Pinene	<LOD	<LOD	No REL
m,p-Xylene	0.001 (0.00)	0.004 (0.001)	100
n-Hexane	0.0007 (0.001)	0.003 (0.0004)	50
o-Xylene	0.002 (0.0003)	0.003 (0.0006)	100
Diesel Particulate (mg/m^3)	0.20 (0.01)	0.94 (0.08)	
Oxygen (%)	21 (0.8)	20 (0.01)	
Temperature (F)	75 (1)	74 (1)	
Relative Humidity (%)	30(6)	45(8)	

The NIOSH recommended exposure limits (RELs) for the various chemicals are presented in the far right column to demonstrate that the components of the diesel exhaust were below the RELs.

Table 2.

PV loop blood pressure measures in animals exposed to 0.2mg/m^3 diesel exhaust; baseline measures and measures in response to varying levels of NE.

	Baseline			% change from baseline 0.325 mg NE			% change from baseline 0.625 mg NE			% change from baseline 1.25 mg NE			% change from baseline 2.5 mg NE		
Measure	**Id**	**7d**	**27d**	**Id**	**7d**	**27d**	**Id**	**7d**	**27d**	**Id**	**7d**	**27d**	**Id**	**7d**	**27d**
SBP Air	**114.4 (2.6)**	103.2 (6.1)	97.1 (4.0)	9.6 (0.8)	28.3 (0.5)	16.9 (1.2)	20.4 (4.2)	28.3 (5.1)	27.0 (2.3)	37.6 (0.8)	43.1 (7.4)	42.3 (4.8)	49.8 (7.9)	56.9 (0.3)	63.2 (6.7)
Diesel	**122.7 (2.6)**	101.7 (4.4)	99.4 (3.0)	10.3 (2.1)	14.1 (2.7)	17.4 (2.7)	22.8 (2.3)	32.4 (4.7)	26.8 (4.6)	39.7 (4.5)	51.6 (9.9)	48 (4.4)	56.4 (6.1)	69.3 (10.8)	63.3 (5.1)
DBP Air	66.3 (0.09)	70.2 (5.7)	*61.7 (2.2)*	4.7 (1.4)	16.3 (6.5)	12.0 (1.6)	12.4 (4.3)	26.9 (7.7)	23.9 (2.3)	24.7 (6.6)	39.6 (10.4)	23.7 (17.2)	34.8 (5.8)	50.8 (11.3)	59.3 (3.6)
Diesel	71.2 (1.50)	70.3 (2.7)	*68.9 (3.3)*	5.3 (1.6)	12.8 (3.2)	13.3 (3.6)	15.2 (2.2)	28.1 (3.2))	20.6 (6.6)	28.3 (3.4))	42.1 (7.3)	39.3 (6.1)	41.5 (4.3)	54.0(7.4)	51.2 (6.8)
MAP Air	**97.9 (2.12)**	87.5 (5.8)	79.6 (2.7)	6.9 (1.2)	16.1 (4.8)	13.6 (1.3)	15.6 (4.0)	26.4 (6.1)	24.6 (1.8)	28.9 (7.3)	38.7 (8.3)	40.6 (2.8)	39.3 (6.4)	49.9 (9.1)	58.8 (3.3)
Diesel	**105.4 (2.07)**	87.2 (3.4)	84.9 (3.3)	7.7 (1.9)	12.8 (2.7)	15.0 (3.3)	18.0 (2.5)	28.3 (4.0)	23.4 (5.5)	31.7 (3.8)	43 (7.6)	42.1 (5.0)	45.3 (4.7)	56.1 (8.2)	54.6 (5.7)
Air-CO_2(µl/min)															
Air	24803 (3941)	21669 (3226)	22062 (2720)	*1.07 (3.7)*	8.4 (3.2)	*0.2 (4.6)*	**0.37 (5.6)**	37.7 (11.9)	7.0 (4.7)	16.2 (7.7)	65.9 (25.4)	30.0 (13)	**23.3 (7.3)**	82.9 (29.1)	84.5 (0.28)
Diesel	25778 (3375)	23461 (2319)	28776 (2967)	*11.9 (4.3)*	11.6 (4.3)	*11.1 (3.2)*	**11.9 (4.3)**	19.2 (9.3)	19.8 (6.7)	32.0 (7.5)	36.2 (10.4)	33.1 (7.7)	**51.3 (9.8)**	64.0 (12.8)	47.4 (10.1)

Measures were collected 1, 7 and 27days after exposure. Cells that are highlighted and bold designate air significantly different than DE ($p < 0.05$) and cells that are highlighted and in italics designate air different than DE ($p < 0.06$).

Table 3.

PV loop measures of cardiac function in animals exposed to 0.2 mg/m^3 dobutamine; baseline measures and measures in response to varying levels of NE.

	Baseline			Dobutamine 1.25 μg/kg			Dobutamine 2.50 μg/kg			Dobutamine 5.0 μg/kg			Dobutamine 10.0 μg/kg		
Measure	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**
CO2 (μl/min)															
Air	24803 (3941)	21669 (3226)	22062 (2720)	*1.07 (3.7)*	a8.4 (3.2)	*0.2 (4.6)*	**0.37 (5.6)**	37.7 (11.9)	7.0 (4.7)	16.2 (7.7)	65.9 (25.4)	30.0 (13)	**23.3 (7.3)**	82.9 (29.1)	84.5 (0.28)
Diesel	25778 (3375)	23461 (2319)	28776 (2967)	*11.9 (4.3)*	11.6 (4.3)	*11.1 (3.2)*	**11.9 (4.3)**	19.2 (9.3)	19.8 (6.7)	32.0 (7.5)	36.2 (10.4)	33.1 (7.7)	**51.3 (9.8)**	64.0 (12.8)	47.4 (10.1)
SV (μl/min)															
Air	140.3 (7.2)	143.7 (22.1)	151.7 (23.0)	**2.8 (2.8)**	10.3 (3.0)	*−0.4 (1.3)*	**1.6 (4.4)**	29.6 (10.4)	**2.4 (4.6)**	15.6 (6.1)	48.8 (20.3)	21.2 (11.9)	**17.4 (6.5)**	56.2 (23.1)	63.7 (30.8)
Diesel	159.5 (19.4)	152.92 (14.9)	179.6 (18.3)	**10.0 (2.5)**	7.9 (5.6)	*12.5 (3.1)*	**14.7 (3.2)**	10.1 (5.3)	**17.9 (4.1)**	23.5 (4.4)	22.4 (7.2)	27.5 (5.8)	**34.2 (5.7)**	44.8 (17.8)	36.9 (8.7)
Pes (mmHg)															
Air	90.8 (3.5)	95.2 (5.7)	95.9 (4.0)	2.3 (6.0)	7.1 (5.0)	1.7 (1.4)	**9.2 (5.2)**	5.7 (2.7)	−0.1 (2.8)	9.0 (7.8)	3.5 (2.8)	−3.8 (3.2)	**8.8 (9.5)**	1.4 (3.6)	−6.0 (4.7)
Diesel	102.5 (2.3)	101.8 (3.3)	98.0 (2.7)	−1.3 (2.2)	−1.4 (3.2)	3.5 (1.0)	**−3.6 (2.8)**	−1.2 (4.8)	−1.5 (1.8)	−6.5 (3.5)	−4.2 (4.9)	−5.7 (2.5)	**−12.9 (3.4)**	−10.2 (4.1)	−8.6 (2.7)
Ped (mmHg)															
Air	−8.6 (1.4)	−3.0 (4.1)	−8.7 (2.3)	−17.8 (17)	**−5.0 (4.7)**	−2.7 (3.2)	**−12.7 (13**	**−5.2 (4.4)**	**−11.8 (4.4)**	−18.3 (19)	−17.8 (8.4)	**−12.6 (3.9)**	**−5.2 (4.4)**	−19.7 (8.3)	−16.3 (4.8)
Diesel	−3.2 (4.1)	−3.2 (2.3)	−4.6 (1.6)	−49.8 (9.9)	**−44.9 (15**	−112 (7)	**−67 (7.1)**	**−42.9 (15**	**−28.2 (6.3)**	−64.5 (12)	−55 (19.8	**−32.4 (7.9)**	**−44.9 (15)**	−57.8 (21)	−31.2 (8.4)
Hr (bpm)															
Air	372.2 (20.0)	335.33 (13.4)	326.7 (24.3)	−0.9 (3.1)	0.1 (0.3)	**1.3 (0.7)**	0.7 (2.4)	1.3 (0.3)	1.7 (0.5)	2.1 (4.0)	4.5 (1.52)	5.4 (1.0)	6.9 (3.2)	10.3 (3.4)	11.8 (2.1)
Diesel dP/dt max (mmHg/s)	346.1 (6.9)	345 (19.3)	307.4 (19.2)	1.6 (2.0)	−0.9 (0.1)	**−0.1 (0.4**	3.5 (2.5)	−0.1 (0.3)	−2.4 (3.3)	6.7 (2.9)	2.6 (0.99)	3.3 (1.3)	12.8 (3.2)	8.1 (3.1)	8.2 (1.9)
Air	7313 (530.7)	6776 (660)	6631 (474)	32.7 (14.7)	18.7 (6.8)	16.6 (3.4)	34.2 (10.4)	34.1 (7.2)	31.8 (7.2)	44.0 (10.)	55.2 (8.9)	54.1 (11.0)	57.5 (10.7)	68.7 (14.2)	82.3 (19.2)
Diesel dP/dt min (mmHg/s)	7431 (148.3)	7465 (549)	7079 (571)	20.2 (3.7)	19.5 (8.1)	27.2 (3.7)	31.6 (2.2)	34.9 (6.9)	40.3 (5.5)	48.6 (6.1)	54.2 (11.2)	56.0 (8.3)	52.6 (8.9)	56.9 (13.5)	68.4 (8.2)

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	Baseline			Dobutamine 1.25 μg/kg			Dobutamine 2.50 μg/kg			Dobutamine 5.0 μg/kg			Dobutamine 10.0 μg/kg		
Measure	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**
Air	−7526 (563.7)	−7272 (735)	−7089 (578)	−0.3 (9.8)	−7.2 (2.8)	−11.8 (6)	−10 (0.87)	−10.9 (6.8)	−11.6 (6)	−6.5 (9.1)	−12.4 (7.7)	−4.3 (5.5)	−0.8 (9.5)	**−7.2 (10.7)**	5.8 (6.8)
Diesel	−8635 (1272)	−8498 (424)	−7953 (547)	−0.9 (4.5)	1.8 (5.0)	−9.6 (2.6)	−1.4 (6.6)	0.2 (9.6)	−8.0 (4.0)	0.5 (12.9)	5.6 (9.6)	5.6 (5.1)	13.9 (10.3)	**17.9 (8.8)**	12.6 (6.2)
RR Interval															
Air	0.2 (0.01)	0.19 (0.01)	0.19 (0.01)	−2.4 (2.0)	−0.3 (0.3)	−2.1 (1.4)	−2.6 (1.8)	−2.6 (2.5)	−2.04 (1.9)	−3.9 (2.9)	−8.7. (3.2)	−4.1 (1.7)	−6.8 (2.8)	−12.6 (2.3)	−8.2 (3.0)
Diesel	0.2 (0.01))	0.14 (0.01)	0.18 (0.01)	−0.9 (0.9)	−3.4 (1.2)	−1.4 (0.5)	−1.9 (2.2)	−5.2 (2.2)	0.80 (3.5)	−3.4 (2.0)	−8.6 (2.6)	−0.9 (4.1)	−9.7 (2.3)	−9.9 (4.0)	3.9 (6.0)
QTc Interval															
Air	0.134 (0.01)	0.141 (0.01)	0.106 (0.01)	7.6 (2.0)	6.5 (1.9)	3.4 (1.4)	11.2 (4.0)	14.9 (4.7)	8.7 (1.0)	14.5 (5.2)	18 (4.7)	9.7 (3.3)	19.8 (6.4)	14.4 (3.9)	9.7 (3.3)
Diesel	0.149 (0.01)	0.118 (0.02)	0.106 (0.01)	2.9 (1.4)	9.0 (2.4)	5.5 (3.7)	5.4 (1.5)	18.2 (11.7)	8.4 (4.4)	10.3 (2.5)	18.8 (11.7)	12.5 (9.5)	12.8 (2.1)	10.6 (10.9)	7.2 (13.9)

Measures were collected 1, 7 and 27days after diesel exhaust or air exposure. Cells that are high, lighted and bold designate air significantly different than DE ($p < 0.05$) and cells that are highlighted and in italics designate air different than DE ($p < 0.06$). CO_2 is cardiac output, SV is stroke volume, Pes is pressure at end systolic, Ped is pressure at end diastolic, Hr is heart rate, dp/dt max: slope of maximum derivative of change in systolic pressure over time, dP/dtmin = minimum derivative of change in diastolic pressure over time, RR interval is the RR interval of heart rate readings and QTc are changes in the QT interval of heart rate.

Table 4.

PV loop blood pressure measures in animals exposed to 1.0mg/m^3 diesel exhaust; baseline measures and measures in response to varying levels of NE.

Measure	Baseline			% change from baseline 0.325 mg NE			% change from baseline 0.625 mg NE			% change from baseline 1.25 mg NE			% change from baseline 2.5 mg NE		
	1	7	27	1	7	27	1	7	27	1	7	27	1	7	27
SBP Air	105.4 (6.2)	103.4 (3.2)	100.9 (5.4)	*10.1 (1.7)*	**8.9 (2.6)**	21.4 (4.9)	19.3 (3.1)	18.8 (4.8)	37.2 (10.1)	**29.4 (6.0)**	36.2 (7.3)	50 (12.7)	46.1 (7.4)	50.4 (9.8)	62.4 (12.9)
Diesel	100.0 (14.6)	98.2 (2.8)	102.5 (4.2)	*17.9 (3.9)*	**16.6 (2.0)**	13.2 (3.7)	33.8 (7.7)	29.1 (4.5)	25.4 (5.8)	**54.1 (11.1)**	42.5 (6.6)	41.1 (8.5)	65.8 (10.8)	56.4 (7.4)	56.2 (7.8)
DBP Air	73.9 (5.4)	71.1 (3.3)	66.3 (5.0)	5.1 (2.4)	3.3 (3.1)	17.2 (5.3)	13.2 (3.2)	11.5 (5.4)	30,2 (9.7)	31.3 (6.0)	27.6 (7.4)	41 (11.3)	36.8 (7.1)	37.1 (9.0)	51.2 (11)
Diesel	69.3 (10.3)	66.9 (3.0)	71.4 (3.4)	11.6 (4.0)	8.7 (2.0)	9.9 (3.)	26.3 (8.0)	20.3 (3.5)	22.6 (5.1)	40.4 (9.3)	32.7 (4.2)	37.3 (7.4)	50.1 (9.2)	46.3 (5.9)	50.4 (7.2)
MAP Air	90.7 (5.7)	88.2 (3.1)	82.0 (5.2)	7.6 (1.9)	**6.0 (2.5)**	18.3 (4.6)	16.0 (3.0)	14.3 (4.5)	31.4 (8.9)	**24.5 (5.7)**	29.8 (6.5)	42.7 (11)	39.3 (6.7)	40.7 (8.5)	53.7 (11)
Diesel	86.1 (12.6)	83.5 (2.9)	88.1 (3.7)	14.4 (3.8)	**12.2 (2.0)**	11.9 (3.1)	28.4 (7.3)	23.5 (3.4)	24.9 (5.2)	**43.7 (9.2)**	35.7 (4.4)	40 (7.7)	54.0 (9.3)	49.0 (6.1)	53.3 (7.4)

Measures were collected 1, 7 and 27days after exposure. Cells that are highlighted and bold designate air significantly different than DE ($p < 0.05$) and cells that are highlighted and in italics designate air different than DE ($p < 0.06$).

Table 5.

PV loop measures of cardiac function in animals exposed to 1.0mg/m^3 dobutamine; baseline measures and measures in response to varying levels of NE.

	Baseline			Dobutamine 1.25 μg/kg			Dobutamine 2.50 μg/kg			Dobutamine 5.0 μg/kg			Dobutamine 10.0 μg/kg		
Measure	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**
CO2 (μl/min)															
Air	28734 (2877)	26721 (4842)	23526 (1271)	3.5 (5.0)	2.5 (2.3)	17.3 (3.2)	15.9 (11.5)	7.4 (4.0)	26.1 (7.4)	32.4 (14.8)	21.0 (7.1)	42.9 (7.4)	51.2 (20.2)	**33.4 (9.4)**	63.8 (9.7)
Diesel	33144 (4807)	30568 (4052)	29962 (2959)	8.6 (4.0)	12.6 (6.7)	7.8 (4.4)	12.6 (4.8)	22.3 (8.9)	10.2 (13.9)	33.2 (8.6)	41.4 (11.2)	37.8 (18.8)	58.8 (14.2)	**65.4 (15)**	54.3 (22.9)
SV (μl/min)															
Air	84.8 (9.4)	80.7 (16.1)	73.7 (4.3)	3.0 (4.5)	2.2 (2.2)	15.9 (3.5)	13.3 (11)	*4.9 (4.2)*	24.0 (7.1)	24.7 (12.9)	*13.3 (7.6)*	35.9 (7.4)	35.5 (16.2)	**18.9 (10)**	47.3 (8.9)
Diesel	95.9 (13.8)	87.1 (10.1)	98.7 (10.8)	10.2 (3.7)	13.5 (6.0)	7.8 (4.3)	13.0 (5.0)	*22.5 (8.6)*	11.7 (13.5)	30.0 (8.8)	*36.9 (9.8)*	33.8 (19.4)	47.0 (12.8)	**51.6 (11)**	42.4 (20.9)
Pes (mmHg)															
Air	102.3 (6.6)	98.4 (4.5)	99.5 (4.4)	2.4 (1.7)	1.3 (0.9)	2.5 (1.7)	4.5 (3.2)	2.8 (2.5)	3.7 (3.8)	4.7 (2.9)	−0.5 (1.9)	1.0 (3.5)	3.0 (3.5)	−2.6 (1.5)	−3.0 (4.9)
Diesel	98.6 (14.2)	95.9 (3.2)	83.5 (11.6)	2.9 (0.7)	0.5 (1.4)	2.5 (1.6))	0.8 (1.2)	−1.7 (1.5)	5.5 (2.7)	−2.0 (1.9)	−3.7 (1.9)	2.4 (2.2)	−4.3 (2.7)	−5.3 (3.0)	−2.1 (2.3)
Ped (mmHg)															
Air	−0.5 (6.7)	−5.7 (4.8)	−5.0 (3.1)	−3.0 (3.4)	−5.2 (4.4)	*−7.1 (2.4)*	4.8 (11.0)	−7.1 (12.5)	*−7.2 (4.0)*	2.6 (13.5)	−9.1 (16.4)	−7.6 (8.7)	3.2 (12.1)	−17.7 (18)	−10.9 (9.1)
Diesel	−1.55 (3.2)	−3.1 (3.2)	−9.0 (1.3)	−10.2 (2.7)	−44.9 (15)	*0.0 (1.7)*	−3.4 (18.1)	−8.9 (1.3)	*−3.7 (3.8)*	21.7 (48.2)	−14.2 (9.5)	−1.5 (7.9)	20.9 (51.4)	−10.4 (1.6)	−6.8 (8.3)
Air-Hr (bpm)															
Air	342 (12.5)	335 (12.4)	326.7 (21.1)	0.4 (0.6)	0.1 (0.4)	1.3 (0.6) −0.1 (0.3)	0.21 (0.9)	1.3 (0.9)	1.7 (0.5)	5.7 (1.7)	4.5 (1.2)	5.4 (0.90)	10.7 (2.1)	10.3 (1.8)	11.8 (1.9)
Diesel	346 (50.6)	345 (17.9)	307.4 (16.4)	−1.5 (0.6)	−0.9 (1.1)		−0.20 (1.1)	−0.1 (1.0)	−2.4 (3.0)	3.3 (2.0)	2.6 (1.7)	3.3 (1.2)	8.6 (2.3)	8.1 (2.5)	8.2 (1.6)
dP/dt max (mmHg/s)															
Air	6912 (376)	7215 (405) 7164 (427)	6404 (578)	−3.0 (3.4)	14.7 (2.0)	21.7 (3.0)	4.8 (11.0)	30.7 (3.9)	37.4 (4.9)	2.6 (13.5)	52.4 (5.0)	63.3 (8.1)	3.2 (12.1)	72.0 (7.4)	84.4 (15.4)

	Baseline			Dobutamine 1.25 μg/kg			Dobutamine 2.50 μg/kg			Dobutamine 5.0 μg/kg			Dobutamine 10.0 μg/kg		
Measure	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**	**1**	**7**	**27**
Diesel	6637 (1000)		5967 (1117)	−10.2 (2.7)	17.1 (4.5)	14.1 (2.8)	−3.4 (18.1)	28.6 (5.6)	29.4 (12.1)	21.7 (48.3)	49.0 (8.3)	69.5 (14.7)	20.9 (51.4)	65.4 (11)	89.9 (17.8)
dP/dt min (mmHg/s)															
A	−7512 (523)	−7230 (702)	−6510 (766)	13.3 (1.8)	3.1 (8.0)	14.7 (6.6)	30.5 (3.5)	3.5 (8.5)	−16.6 (13)	55.8 (6.1)	2.0 (6.9)	−16.1 (16)	72.0 (9.6)	3.3 (8.4)	−12.0 (15)
D	−7225 (1120)	−7549 (309)	−5681 (1139)	14.7 (3.1)	−2.4 (2.9)	−8.6 (3.7)	24.0 (7.7)	0.0 (3.8)	−6.3 (10.2)	42.3 (13.5)	1.4 (5.4)	−20.4 (12)	53 (17.6)	3.6 (8.2)	−26.5 (16)
RR Interval A															
D	0.18 (0.01)	0.18 (0.01)	0.19 (0.01)	−4.4 (2.2)	−06 (0.3)	*−1.2 (0.9)*	−5.6 (3.4)	−1.3 (0.8)	−1.5 (0.6)	−5.9 (4.5)	−3.6 (1.3)	−3.6 (1.1)	2.0 (3.7)	−7.8 (2.)0)	−6.5 (2.5)
	0.18 (0.01)	0.17 (0.01)	0.20 (0.01)	−6.2 (1.7)	0.1 (0.4)	*−1.5 (0.6)*	−5.3 (2.6)	0.04 (0.9)	2.6 (2.1)	−3.1 (4.4)	−0.24 (1.0)	−0.7 (1.8)	−0.2 (5.6)	−6.0 (1.6)	−2.3 (3.1)
QTc Interval															
Air	0.10 (0.01)	0.11 (0.01)	0.12 (0.01)	0.1 (1.1)	5.3 (2.1)	4.0 (1.9)	−1.3 (1.3)	7.7 (8.8)	4.7 (1.5)	*−1.4 (4.9)*	12.3 (3.8)	3.6 (4.9)	*−8.7 (2.4)*	16.6 (3.0)	8.6 (6.3)
Diesel	0.13 (0.03)	0.10 (0.01)	0.11 (0.01)	1.4 (0.49)	4.6 (1.6)	3.6 (4.9)	2.0 (1.7)	6.8 (3.6)	9.2 (6.5)	*3.1 (2.2)*	6.4 (2.1)	17.0 (6.3)	*0.5 (2.6)*	13.5 (1.8)	20.5 (5.1)

Measures were collected 1, 7 and 27days after diesel exhaust or air exposure. Cells that are highlighted and bold designate air significantly different than DE ($p < 0.05$) and cells that are highlighted and in italics designate air different than DE ($p < 0.06$). CO_2 is cardiac output, SV is stroke volume, Pes is pressure at end systolic, Ped is pressure at end diastolic, Hr is heart rate, dp/dt max: slope of maximum derivative of change in systolic pressure over time, dP/dtmin = minimum derivative of change in diastolic pressure over time, RR interval is the RR interval of heart rate readings and QTc are changes in the QT interval of heart rate.

Table 6.

Fold changes in transcript levels (mean ± sem) in heart tissue from animals exposed to air or 0.2 mg/m^3 diesel exhaust for 1, 7 or 27d.

Transcript	Air 1d Diesel 1d	Air 7d Diesel 7d	Air 27d Diesel 27d
18s	12.25 (0.14)	13.74 (0.36)	12.97 (0.24)
	12.58 (0.13)	13.68 (0.17)	12.33 (0.35)
Bad	1.11 (0.17)	1.24 (0.04)	1.25 (0.36)
	1.35 (0.06)	1.24 (0.04)	1.86 (0.53)
Bcl2	**1.37 (0.32)**	**1.31 (0.30)**	1.40 (0.56)
	3.29 (1.17)	**2.80 (0.80)** *	1.67 (0.24)
Cat	1.19 (0.29)	1.37 (0.41)	1.09 (0.20)
	0.82 (0.10)	1.84 (0.39)	1.30 (0.23)
Et1	**1.19 (0.27)**	1.16 (0.21)	1.09 (0.17)
	2.08 (0.51)	1.34 (0.28)	1.26 (0.14)
Hif1a	1.14 (0.20)	**0.51 (0.09)**	0.83 (0.05)
	1.17 (0.32)	**0.78 (0.14)**	0.81 (0.12)
Hmox	**1.11 (0.18)**	1.02 (0.21)	0.99 (0.15)
	0.63 (0.08) *	1.24 (0.22)	0.88 (0.11)
Il1β	0.91 (0.23)	1.00 (0.24)	1.17 (0.24)
	1.20 (0.32)	2.00 (0.88)	1.99 (0.84)
Il6	1.15 (0.23)	1.03 (0.08)	1.04 (0.11)
	0.98 (0.14)	1.32 (0.23)	1.02 (0.13)
Nos2	1.20 (0.23)	1.22 (0.27)	**1.02 (0.05)**
	1.32 (0.50)	1.67 (0.38)	**0.53 (0.06)** *
Nos3	1.18 (0.23)	1.20 (0.27)	1.00 (0.04)
	1.33 (0.40)	1.58 (0.31)	0.92 (0.10)
Sod2	1.24 (0.27)	1.33 (0.35)	1.05 (0.10)
	1.39 (0.51)	2.23 (0.57)	1.21 (0.14)
Timp	0.96 (0.32)	**1.06 (0.28)**	1.29 (0.36)
	1.27 (0.31)	**2.51 (0.78)** *	1.86 (0.53)
Tnfα	1.19 (0.23)	1.34 (0.30)	1.20 (0.32)
	0.82 (0.26)	1.60 (0.26)	0.95 (0.30)

Abbreviations: *18s*, ribosomal 18s; *Bad, Bcl2* associated signal of cell death; *Bcl2*, β-cell lymphoma 2; *Cat,* catalase; *Et1* endothelin, *Hif1α* hypoxia-induced factor α, *Hmox* heme-oxygenase, ww*Il1β* interleukin 1β, *Il6* Interleukin 6; *Nos2* nitric oxide synthase 2 (inducible); *Nos3* nitric oxide synthase 3 (endothelial); *Sod2* superoxide dismutase 2; *Timp* tissue inhibitor of metalloproteinases; *Tnfα*, tumor necrosis factor α.

* Bold are different than same day controls ($p < 0.05$), bold only different than same day controls ($p < 0.07$).

Table 7.

Fold changes in transcript levels (mean ± sem) in kidney tissue from animals exposed to air or 0.2 mg/m^3 diesel exhaust for 1, 7 or 27d.

Transcript	Air 1d Diesel 1d	Air 7d Diesel 7d	Air 27d Diesel 27d
18s	7.52 (0.08)	8.09 (0.13)	7.92 (0.15)
	7.84 (0.15)	8.07 (0.12)	7.61 (0.14)
Ace	1.76 (0.57)	1.07 (0.15)	1.04 (0.11)
	0.95 (0.27)	1.12 (0.25	0.88 (0.23)
Il1β	(0.33)	1.30 (0.33)	**1.57 (0.60)**
	1.01 (0.56)	1.18 (0.27)	**0.27 (0.05)** *
Il6	1.20 (0.37)	**0.85 (0.16)**	0.93 (0.21)
	0.68 (0.30)	**1.81 (0.63)**	0.63 (0.14)
Nos1	1.01 (0.30)	1.25 (0.27)	1.21 (0.26)
	1.08 (0.53)	1.26 (0.38)	0.84 (0.22)
Nos2	3.09 (1.18)	1.72 (0.57)	1.12 (0.19)
	3.27 (1.08)	1.63 (0.63)	0.83 (0.28)
Nos3	1.09 (0.38)	1.20 (0.27)	**1.21 (0.28)**
	0.90 (0.29)	1.52 (0.40)	**0.72 (0.17)**
Sod2	0.89 (0.16)	1.18 (0.26)	**1.13 (0.24)**
	1.35 *0.57)	1.19 (0.27)	**0.65 (0.12)** *
Tnfα	1.34 (0.56)	1.25 (0.28)	**1.73 (0.66)**
	1.36 (0.53)	0.83 (0.28)	**0.26 (0.08)** *
Vegf	1.10 (0.34)	1.08 (0.19)	1.12 (0.17)
	1.33 (0.27)	0.89 (0.12)	0.80 (0.15)

Abbreviations: *18s*, ribosomal 18s; *Il1β* interleukin 1β, *Il6* Interleukin 6; *Nos1* nitric oxide synthase 1 (neuronal), *Nos2* nitric oxide synthase 2 (inducible); *Nos3* nitric oxide synthase 3 (endothelial); *Sod2* superoxide dismutase; *Tnfα*, tumor necrosis factor α; *Vegf* vascular derived endothelial growth factor.

* Bold different than same day control ($p < 0.05$). Bold only different than same day control ($p < 0.07$).

Table 8.

Fold changes in transcript levels (mean ± sem) in heart tissue from animals exposed to air or 1.0 mg/m^3 diesel exhaust for 1, 7 or 27d.

Transcript	Air 1d Diesel 1d	Air 7d Diesel 7d	Air 27d Diesel 27d
18s	9.86 (0.13)	9.69(0.15)	10.17 (0.25)
	9.73 (0.15)	9.93 (0.19)	10.25 (0.21)
Bad	1.30 (0.24)	1.03 (0.10)	**1.03 (0.13)**
	1.02 (0.21)	0.90 (0.10)	**0.72 (0.07)** *
Bcl2	1.24 (0.29)	1.26 (0.21)	0.92 (0.14)
	1.32 (0.27)	0.99 (0.15)	0.72 (0.07)
Cat	1.50 (0.84)	**1.66 (0.12)**	1.23(0.61)
	1.52 (1.02	**1.19 (0.12)** *	1.52 (1.02)
Et1	1.13 (0.21)	1.05 (0.39)	1.29 (0.31)
	1.31 (0.24)	1.39 (0.49)	0.98 (0.32)
Hif1a	1.34 (0.25)	1.03(0.10)	1.07 (0.16)
	0.89 (0.12)	0.97 (0.08)	0.91 (0.12)
Hmox	1.17 (0.22)	1.19 (0.24)	1.15 (0.22)
	0.83 (0.11)	0.94 (0.18)	1.33 (0.25)
Il1β	**1.90 (0.49)**	1.18 (0.24)	1.37 (0.40)
	0.94 (0.21)	0.91 (0.17)	1.34 (0.31)
Il6	1.56 (0.64)	1.21 (0.49)	**1.35 (0.82)**
	2.64 (0.68)	6.69 (4.33)	**5.53 (2.47)** *
Nos2	1.19 (0.10)	1.06 (0.14)	1.27 (0.34)
	0.76 (0.14) *	0.88 (0.09)	1.05 (0.24)
Nos3	1.01 (0.13)	1.18 (0.24)	0.79 (0.15)
	0.46 (0.11) *	0.96 (0.15)	0.76 (0.06)
Sod2	0.86 (0.20)	0.97 (0.09)	1.06 (0.12)
	1.27 (0.22)	0.93 (0.07)	1.08 (0.11)
Timp	1.07 (0.26)	1.09 (0.18)	1.12 (0.21)
	1.71 (0.31)	1.17 (0.20)	1.00 (0.18)
Tnfα	0.82 (0.06)	1.16 (0.24)	1.56 (0.50)
	0.89(0.17)	0.97 (0.15)	1.69 (0.40)

Abbreviations: *18s*, ribosomal 18s; *Bad, Bcl2* associated signal of cell death; *Bcl2*, β-cell lymphoma 2; *Cat,* catalase; *Et1* endothelin, *Hif1α* hypoxia-induced factor α, *Hmox* heme-oxygenase, *Il1β* interleukin 1β, *Il6* Interleukin 6; *Nos2* nitric oxide synthase 2 (inducible); *Nos3* nitric oxide synthase 3 (endothelial); *Sod2* superoxide dismutase 2; *Timp* tissue inhibitor of metalloproteinases; *Tnfα*, tumor necrosis factor α.

* Bold different than same day control ($p < 0.05$). Bold only different that same day control ($p < 0.07$).

Table 9.

Fold changes in transcript levels (mean ± sem) in kidney tissue from animals exposed to air or 1.0 mg/m^3 diesel exhaust for 1, 7 or 27d.

Transcript	Air 1d Diesel 1d	Air 7d Diesel 7d	Air 27d Diesel 27d
18s	9.54 (0.06)	9.55 (0.05)	9.53 (0.08)
	9.52 (0.07)	9.91 (0.33)	9.64 (0.11)
Ace	**1.16 (0.40)**	1.15 (0.21)	**1.26 (0.32)**
	0.54 (0.08) *	**1.30 (0.20)** ^	**0.53 (0.01)** *
Il1β	1.25 (0.29)	1.11 (0.17)	1.15 (0.22)
	1.67 (0.24)	1.06 (0.27)	0.97 (0.24)
Il6	**1.69 (0.62)**	1.46 (0.43)	1.57 (0.42)
	0.54 (0.12) *	1.77 (0.37)	1.21 (0.43)
Nos1	1.14 (0.23)	**1.04 (0.10)**	1.14 (0.22)
	1.59 (0.24)	**0.75 (0.15)**	1.16 (0.17)
Nos2	1.43 (0.66)	1.49 (0.71)	1.28 (0.44)
	1.03 (0.41)	0.72 (0.26)	1.22(0.78
Nos3	1.05 (0.17)	1.05 (0.12)	1.64 (0.88)
	1.34 (0.95)	0.86 (0.13)	0.97 (0.19)
Sod2	1.44 (0.46)	1.25 (0.36)	1.13 (0.20)
	1.22 (0.45)	1.36 (0.46)	1.14 (0.42)
Tnfα	1.17 (0.27)	1.08 (0.15)	1.21 (0.28)
	1.41 (0.26)	0.99 (0.16)	0.83 (0.15)
Vegf	1.06 (0.16)	1.18 (0.26)	1.29 (0.18)
	1.52 (0.60)	0.96 (0.16)	1.49 (0.30)

Abbreviations: *18s*, ribosomal 18s; *Il1β* interleukin 1β, *Il6* Interleukin 6; *Nos1* nitric oxide synthase 1 (neuronal), *Nos2* nitric oxide synthase 2 (inducible); *Nos3* nitric oxide synthase 3 (endothelial); *Sod2* superoxide dismutase; *Tnfα*, tumor necrosis factor α; *Vegf* vascular derived endothelial growth factor.

* Bold significantly different than same day air control ($p < 0.05$)

^ bold significantly different than 1d and 27d diesel exposed ($p < 0.05$).