**EHP MS #11647_revised, Gottipolu et al., 2008, Supplemental Material**

## Methods

### Diesel Exhaust Generation and Exposure Characterization

DE was generated by operating a 30 kW (40 hp) 4-cylinder indirect injection Deutz diesel engine (BF4M1008) under load of a 22.3 kW Saylor Bell air compressor. Fuel was supplied via a recirculating loop from a 208 L (55 gal drum). Engine speed was maintained at approximately 1725 rpm. We used readily available road-taxed diesel fuel (analysis not shown). Engine lubrication oil (Shell Rotella, 15W-40) was changed before the exposure tests. The engine and compressor were operated at steady-state to produce 0.8 $m^3$/min of compressed air at 400 kPa. This translates to approximately 20% of the engine's full-load rating. Fuel consumption was 7.6-11.4 L/h (2-3 gal/h). From the engine, a small portion of the exhaust was routed to a dilution system with the remainder being directed to a small baghouse where the particles were filtered before the gases were emitted to the atmosphere through a stack. The exhaust directed through the dilution system passed through two-stage air dilution and was then routed through approximately 15 m of flexible food grade polyvinyl chloride (PVC) tubing (7.62 cm inside diameter) to two Hinner exposure chambers. A third exposure chamber exposed rats to HEPA filtered room air as a control.

For these experiments approximately 1.4 $m^3$/min of dilution air was mixed with 0.043 $m^3$/min of engine exhaust for a dilution ratio of approximately 30 to 1. The dilution ratio was verified by measuring nitric oxide (NO, Thermo Electron Corp., model 42c, Franklin, MA) concentrations in both the engine exhaust and the dilution ductwork. However, the dilution air flows were periodically adjusted

to maintain desired particle concentrations in the exposure chambers as measured by tapered element oscillating microbalances (TEOMs, Rupprecht and Patashnick Co., series 1400, Albany, NY). The dilution air quality was maintained by passing it through a HEPA filter before introduction into the diluter**.**

Three Hinner exposure chambers were used (0, 500, and 2000 $\mu g/m^3$) in parallel. These chambers were made of stainless steel with glass windows and have an internal volume of 0.31 $m^3$ (11 $ft^3$). The chamber flows and pressures are controlled by a ring compressor fan. All flow exiting the chambers was vented outside the building via wall penetrations. Each chamber had a flow of around 70.8 L/min (2.5 $ft^3$/min). Two chambers pulled directly from the dilution system and contained diluted flue gas and target PM concentrations of 2000 $\mu g/m^3$, and with additional dilution at the second chamber, 500 $\mu g/m^3$. The third chamber (air control) pulled clean, filtered room air. During exposures the engine was under constant load with constant compressed air demand as described above.

In addition to NO and TEOM measurements, continuous emission monitors (CEMs) were used to measure chamber concentrations of oxygen ($O_2$, Beckman Corp. model 755, La Habra, CA), carbon monoxide (CO, Thermo Electron Corp., model 48, Franklin, MA), nitrogen dioxide ($NO_2$, Thermo Electron Corp., model 42c, Franklin, MA), and sulfur dioxide ($SO_2$, Thermo Electron Corp., model 43c, Franklin, MA). Samples were extracted through fixed stainless steel probes in the exposure chambers and filtered prior to the gas analyzers. Particle size distributions were characterized using a scanning mobility particle sizer (SMPS, TSI Inc., model 3080/3022a, St. Paul, MN) and an aerodynamic particle sizer (APS, TSI Inc., model 3321, St. Paul, MN). Chamber temperature and

relative humidity were monitored continuously. Integrated 4 h filter samples (14.1 L/min) were collected daily from each chamber and analyzed gravimetrically to determine particle concentrations. In addition, triplicate quartz filter samples were collected daily from the exposure chambers and analyzed using a thermal/optical carbon analyzer (Sunset Laboratory Inc., model 107, Tigard, OR) to determine organic carbon/elemental carbon (OC/EC) partitioning of the collected particles.

**Exposure of Rats to Air or Diesel Exhaust (DE)**

Before the start of the exposure rats were transferred to a nearby satellite animal housing facility within the same building. Rats were randomized based on weight to different exposure groups. Animals were periodically monitored for changes in breathing parameters using barometric whole body plathysmography system (Buxco Electronics, Inc.; Sharon, CT) to obtain data on pulmonary ventilation as described previously (Kodavanti et al. 2005). Diesel exposure did not cause any major change in any of the breathing parameters analyzed (See Supplemental Material, Figure 1). Before the start of the exposure, rats were transferred to stainless steel wire mesh cages and then exposure chambers. Each group of rats (n=6 for pathology and molecular analysis; n=6 for mitochondria isolation; n=3 for transmission electron microscopy [TEM]) was exposed to air or DE (500 and 2000 $\mu g/m^3$), 4 hours/day, 5 days per week for 4 consecutive weeks and necropsies were performed one day after the final exposure.

**Necropsy and Sample Collection**

At designated time points, rats were weighed and anesthetized with sodium pentobarbital (50-100 mg/kg, i.p.). Blood was collected from the abdominal aorta, directly into blood collection tubes containing EDTA (for complete blood counts), citrate (for plasma protein analysis), or in serum separator tubes without an anticoagulant for cytokine assays. In the first set of animals (n=6), the heart was removed, blotted dry, weighed and cut into two mid-longitudinal halves. One half was fixed in 10% neutral formalin for histological evaluation. From the second half, the right ventricle was discarded and portions of the left ventricle plus septum were snap-frozen in liquid nitrogen and retained for enzyme activity analysis and RNA isolation.

The trachea was cannulated and the left lung was tied. The right lung was lavaged with $Ca^{++}$/$Mg^{++}$ free phosphate buffered saline (pH 7.4) with a volume equal to 28-mL/kg body weight (approximately 75% total lung capacity) x 0.6 (right lung representing 60% of total lung mass). Three in-and-out washes were performed with the same buffer aliquot to enrich for protein and enzymes. This bronchoalveolar lavage fluid (BALF) was collected in tubes and kept on ice for further analysis. The left lung was tracheally fixed with neutral formalin for later histological evaluation.

**Bronchoalveolar Lavage Fluid Analysis**

Aliquots of bronchoalveolar lavage fluid (BALF) were used to determine total cell counts with a Z1 Coulter Counter (Coulter, Inc., Miami, FL). A second aliquot was centrifuged (Shandon 3 Cytospin, Shandon, Pittsburg, PA) to prepare cell differential slides. Slides were dried at room temperature and stained with

Leukostat (Fisher Scientific Co., Pittsburg, PA). Macrophages, neutrophils, eosinophils, and lymphocytes were counted using light microscopy. At least 300 cells were counted on each slide. The remaining BALF was centrifuged at 1500 x g to remove cells, and the supernatant fluid was analyzed for markers of lung injury. Total protein (μg/ml BALF) was analyzed using Coomassie plus Protein Assay Kit using bovine serum albumin as a standard (Pierce, Rockford, IL). BALF albumin (μg/ml BALF) was analyzed using a commercially available kit (Diasorin, Stillwater, MN). Lactate dehydrogenase (LDH) activity (U/L BALF) was determined using Kit 228 from Sigma Chemical Co. (St. Louis, MO). N-Acetyl glucosaminidase (NAG) activity (U/L BALF) was measured using a kit and standards from Roche Diagnostics (Indianapolis, IN). γ-glutamyl transferase (GGT) activity (U/L BALF) was measured using a kit from Thermo Trace Ltd (Melbourne, Australia). These assays were modified and adapted for use on the Konelab clinical chemistry analyzer (Thermo Clinical Labsystems, Espoo, Finland).

**Blood Chemistry and Cytology**

Aliquots of EDTA-collected blood were analyzed for complete blood counts by a Beckman-Coulter AcT blood analyzer (Beckman-Coulter Inc., Fullerton, California). Each blood sample containing citrate anticoagulant was centrifuged at 4500 rpm for 10 min at 4 $^{o}$C. Plasma fibrinogen, activated plasma thromboplastin time and plasma thromboplastin time were measured in the citrated plasma by the Laboratory Corporation Inc., Durham, NC. Angiotensin converting enzyme (ACE) activity was measured in citrated plasma using

reagents and controls from Sigma Diagnostics, St Louis, MO. C-Reactive protein (CRP) was measured in citrated plasma using a SPQ II kit which contained its own calibrations and controls (Diasorin Inc., Stillwater, MN). D-Dimer measurements were done using a kit obtained from Kamiya Biomedical Company (Seattle, WA). Total antioxidant status was determined using a kit from RANDOX Laboratories Ltd. (Oceanside, CA). These assays were modified and adapted for use on the KONLAB clinical chemistry analyzer (Thermo Clinical Labsystems, Espoo, Finland).

**Determination of Cytokines in Bronchoalveolar Lavage Fluid and Serum**

Serum and BALF samples were analyzed for a number of rat cytokines using the rat cytokine/chemokine Lincoplex Kit with 24 total markers. The samples were processed according to the kit protocol using Luminex 100 system (Luminex Corporation, Austin, TX). Sample values were normalized based on standard curve for each protein and data were calculated using Luminex software. Note that not all cytokines/chemokines provided positive values for the samples analyzed, and therefore, the cytokine/chemokine name is given in the Supplemental Material Table 2 if positive consistent values were obtained in the assay.

**Lung and Heart Light Microscopy and Cardiac Transmission Electron Microscopy (TEM)**

Tissues from heart and lung were processed, embedded in paraffin, sectioned at 5μ, and stained routinely with hematoxylin and eosin (H&E) for pathological analysis. Step sectioning was performed for each heart for better

sampling of the tissues. Nine sections from each heart were examined. H&E slides of the lung and the heart were blindly evaluated microscopically by a panel of expert pathologists. Any lesions seen were graded on scale of 1=minimal, 2=mild, 3=moderate, and 4=marked. Lesions were described according to a published system of grading of morphological criteria (Nyska et al. 2005).

A separate group of rats (n=3 for each control and 2000 $\mu g/m^3$ group) were anesthetized and exsanguinated via the abdominal aorta one day following final exposure. Hearts were quickly removed and sliced crosswise into 3 approximately equal-sized segments, of which the center one was used for electron microscopy. This middle segment was placed on a Petri dish in drops of modified Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4) and 1.5–2-mm tissue cubes were cut from the left ventricle (LV), interventricular septum (IS), and right ventricle (RV). The remaining portions of heart tissues were assembled in a cassette and processed for histological evaluation. These TEM specimens were stored at $4^0C$ in the fixative overnight or for several days, then rinsed in buffer, postfixed in cacodylate-buffered 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA), rinsed, dehydrated through a series of graded alcohols and acetone, and embedded in Spurr's resin (EM Sciences). One block from each region of each heart was analyzed. Semithin (1/2-$\mu m$) sections stained with 1% toluidine blue + 1% sodium borate were examined to locate regions containing longitudinal fibers. Ultrathin (90-nm) sections were cut from these regions, placed on 150-mesh copper grids with support films (EM Sciences), stained with 5% uranyl acetate followed by Reynold's lead citrate, and examined in a Tecnai

12 electron microscope (FEI Electron Optics, Eindhoven, The Netherlands) equipped with a digital Megaview III soft imaging system (SIS) and Windows 2000.

**Preparation of Cardiac Cytosol, Mitochondria and Whole Homogenates**

A separate group of rats (n=6) was anesthetized as indicated above and cardiac tissue was excised quickly. The lung tissue was processed as indicated above. The right ventricle was discarded. A small portion of the left ventricle was quick frozen for later homogenization and the remaining large portion was homogenized and processed as indicated earlier for isolation of mitochondria and cytosol (Wallenborn et al. 2008). Both of these fractions were frozen at -80$^{o}$C for later analysis. The frozen left ventricular tissues were homogenized in 10 mM tris buffer (pH 7.4) and homogenates were centrifuged at 12000 x g at 4$^{o}$C. The supernatants, which contained all cytosolic and mitochondrial soluble fractions, were termed as whole homogenates. The cytosolic, mitochondrial, and whole homogenate fractions were aliquoted and quick frozen at -80$^{O}$C until further analysis.

**Analysis of Oxidative Stress Sensitive Enzyme Markers**

Aconitase activity was measured based on the formation of NADPH from NADP+ in all three fractions using the Bioxytech Aconitase-340 Assay (Oxis International Inc., Foster City, CA). Ferritin levels were measured using the K-Assay Ferritin from the Kamiya Biomedical Company (Seattle, WA). Superoxide dismutase (SOD) activity was measured using a kit from RANSOD (Randox Laboratories, Oceanside, CA). Glutathione peroxidase activity was measured in

whole homogenate fraction indirectly through a coupled reaction with glutathione reductase. Glutathione (GSH) is oxidized to GSSG, which then acts as the substrate for glutathione reductase. The oxidation of NADPH is measured (Jaskot et al. 1983). Glutathione transferase activity was determined according to Habig et al (1974). Thioredoxin reductase activity was determined according to the method developed by Smith and Levander (2002). Isocitrate dehydrogenase (ICDH) activity was assessed in both cytosolic and mitochondria fractions by measuring reduced NADPH following the ICDH catalyzed oxidative decarboxylation of L-isocitrate to 2-oxoglutarate. Ubiquinone reductase activity was measured as described by Cormier et al (2001). These assays were modified and adapted for use on the KONLAB clinical chemistry analyzer (Thermo Clinical Labsystems, Espoo, Finland).

**Functional Roles of Selected Genes Inhibited by Diesel Exhaust (DE) Exposure in WKY and at Baseline in Spontaneously Hypertensive (SH) Rats**

A selected group of genes down regulated by DE in WKY and at base line in SH rats were reported to have a role in cardiovascular disease causation (See Supplemental Material, Table 7). Cardiovascular ischemia and myocardial infarction are associated with inhibition of thrombomodulin (Aleksic et al. 2008), TGF-b1 (Hermonat et al. 2007), fibroblast growth factor-2 (Kardami et al. 2007), and collagens (LeClair and Linder 2007). DE caused these genes to be downregulated in WKY rats. These same genes were found already downregulated in hypertensive rats without DE exposure. The lack of hepcidin gene expression is associated with iron overload, common in chronic cardiovascular disease (Nicolas et al. 2001). Dimerization of fibroblast growth factor receptor-1 reduces dilation in infarcted hearts (Stevens et al. 2007). Fibromodulin, a small leucine-rich proteoglycan, plays an important role in maintenance of matrix in mature tissues (Viola et al. 2007). The inhibition of activity of cyclin kinase inhibitor attenuates matrix protein secretion in the heart (Weiss and Randour 2002). The lack of JunD promotes apoptosis in pressure overloaded heart (Hilfiler-Kleiner et al. 2005). S100A4 protein promotes growth and survival of injured heart and is involved in tissue remodeling and ATP synthesis (Schneider et al. 2007). Mutation in fibrilin-1, a major component of extracellular microfibrils, is associated with dysregulation of matrix metabolism and TGF-beta signaling (Mizuguchi and Matsumoto 2007). These matrix genes were downregulated by DE in WKY rats. Selenoprotein W, a protein that is

normally enriched in the heart and the muscle tissue is suggested to have an antioxidant function (Whanger 2000). Down regulation of all these genes, involved in cardiac compensatory response and extracellular matrix metabolism suggests that cardiac compensatory mechanisms are impaired by DE in WKY rats without an inflammogenic response.

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Supplemental Material, Table 1. Verification of gene array expression data with that of quantitative real time PCR.

| Comparison | RT-PCR[a] - Fold Change from WKY-Air | | Gene Array - Fold Change from WKY-Air | |
|---|---|---|---|---|
| | HO-1 | Thioredoxin Reductase | HO-1 | Thioredoxin Reductase |
| WKY-Air/WKY-Air | 1.00 | 1.00 | 1.00 | 1.00 |
| WKY-DE/WKY-Air | 0.98 | 0.94 | 1.00 | 0.79 |
| SH-Air/WKY-Air | 1.94 | 1.28 | 1.34 | 1.05 |
| SH-DE/WKY-Air | 1.81 | 0.83 | 1.29 | 0.98 |

[a]The target transcripts were notmalized to β-actin expression values.

Supplemental Material, Table 2. The list of examined cardiopulmonary and systemic biomarkers.

| Tissue Examined | Toxicity Pathway | Biomarkers |
|---|---|---|
| Lung-BALF | Injury | Total protein, albumin, lactate dehydrogenase, N-acetylglucosaminidase, γ-glutamyl transferase |
| | Inflammation | Total cells, macrophages, neutrophils |
| BALF and serum | Cytokines (Luminex multiplex assay) | IL-9, IL-18, GMCSF, GRO/KC, RANTES, VEGF (MCP-1 and Leptin in serum only) |
| Blood | Hematology | RBC, WBC, HCT, HGB, platelet, lymphocytes, monocytes |
| Citrated plasma | Thrombosis, inflammation | PT, APTT, fibrinogen, ACE activity, D-dimer, total antioxidant status, CRP |
| Lung, Heart | Pathology | Light microscopy, cardiac transmission electron microscopy |
| Cardiac cytosol and mitochondria | Oxidative stress | Aconitase, isocitrate dehydrogenase, ubiquinone reductase, thioredoxin reductase activities and ferritin |
| Left ventricle, whole homogenate | Oxidative stress | Aconitase, glutathione peroxidase, glutathione reductase, glutathione transferase, and superoxide dismutase activities and ferritin |
| Left ventricular gene expression by PCR and Affymetrix array | Oxidative stress, Vasoactivation, thrombosis | Heme oxygenase-1, endothelin, plasmionogen activator inhibitor-1 |

Supplemental Material, Table 3. Cytokines levels (pg/ml) in bronchoalveolar lavage fluid (BALF) after air or diesel exhaust (DE)-exposure in Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats.[a]

| Rat Strain | DE ($\mu g/m^3$) | GMCSF | IL-9 | IL-18 | GRO/KC | RANTES | VEGF |
|---|---|---|---|---|---|---|---|
| WKY | 0 | 26.0 ± 5.2 | 24.3 ± 6.4 | 155.9 ± 40.2 | 226.3 ± 13.3 | 16.3 ± 5.1 | 162.5 ± 38.1 |
| | 500 | 19.9 ± 5.0 | 28.0 ± 9.2 | 163.7 ± 51.1 | 210.6 ± 47.5 | 15.8 ± 11.7 | 127.4 ± 25.7 |
| | 2000 | 18.0 ± 5.0 | 30.8 ± 14.0 | 154.0 ± 24.3 | 262.4 ± 32.3 | 21.5 ± 6.0 | 216.4 ± 44.6 |
| SH | 0 | 12.9 ± 3.9 | 34.4 ± 7.4 | 76.3 ± 4.9 | 378.0 ± 121 | 20.9 ± 8.0 | 99.0 ± 15.5 |
| | 500 | 16.7 ± 4.3 | 37.9 ± 15.3 | 74.7 ± 8.9 | 337.0 ± 73.4 | 20.5 ± 4.5 | 103.8 ± 38.3 |
| | 2000 | 23.5 ± 8.7 | 52.5 ± 19.3 | 185.4 ± 197 | 344.6 ± 77.3 | 24.8 ± 5.5 | 135.2 ± 35.5 |

[a]Cytokine levels in cell-free BALF were measured using Luminex multiplex rat cytokine assay kits (Luminex Corporation, Austin, TX). Note that several other cytokines included in the kit were not detectible in BALF. Values represent mean ± SD (n=4 rats/group). No significant exposure-related changes were apparent.

Supplemental Material, Table 4. Serum cytokines levels (pg/ml) in air and diesel exhaust (DE)-exposed Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats.[a]

| Rat Strain | DE ($\mu g/m^3$) | MCP-1 | GMCSF | IL-9 | IL-18 | GRO/KC | RANTES | Leptin |
|---|---|---|---|---|---|---|---|---|
| WKY | 0 | 67.6 ± 15.7 | 26.0 ± 5.2 | 58.6 ± 4.2 | 33.8 ± 24.1 | 169.2 ± 109.6 | 816.7 ± 437.7 | 410.6 ± 61.8 |
| | 500 | 82.2 ± 30.2 | 19.1 ± 5.0 | 44.3 ± 21.9 | 23.8 ± 10.9 | 225.0 ± 77.1 | 784.9 ± 402.0 | 365.3 ± 139.6 |
| | 2000 | 64.8 ± 12.2 | 18.0 ± 5.0 | 50.9 ± 9.9 | 27.4 ± 14.7 | 144.6 ± 78.9 | 927.0 ± 259.3 | 348.8 ± 108.0 |
| SH | 0 | 94.0 ± 46.3 | 12.9 ± 3.9 | 47.1 ± 8.0 | 13.3 ± 5.9 | 187.2 ± 72.3 | 1324.9 ± 562.9 | 741.8 ± 318.8 |
| | 500 | 121.3 ± 110.4 | 13.7 ± 7.0 | 42.5 ± 34.4 | 15.6 ± 9.5 | 171.6 ± 101.9 | 1236.2 ± 453.3 | 340.8 ± 164.1 |
| | 2000 | 51.1 ± 5.9 | 27.2 ± 8.3 | 51.1 ± 5.4 | 13.9 ± 15.0 | 131.0 ± 32.8 | 1118.4 ± 471.3 | 865.9 ± 256.6 |

[a]These cytokines and other biomarkers were analyzed in serum samples using Luminex Multiplex rat cytokine kits (Luminex Corporation, Austin, TX). Note that several other cytokines included in the kit were not detectible in serum. Values represent mean ± SD (n=4 rats/group). No significant exposure-related changes were apparent.

Supplemental Material, Table 5. Levels of plasma coagulation markers in air or diesel exhaust (DE)-exposed Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats.

| Rat Strain | DE ($\mu g/m^3$) | APTT[a] (Seconds) | PT[b] (Seconds) | Fibrinogen (mg/dL) |
|---|---|---|---|---|
| WKY | 0 | 16.8 ± 0.4 | 20.3 ± 1.3 | 216.2 ± 18.0 |
| | 500 | 16.8 ± 0.4 | 19.7 ± 1.5 | 225.2 ± 17.1 |
| | 2000 | 16.8 ± 1.0 | 19.5 ± 1.9 | 224.3 ± 25.0 |
| SH | 0 | 16.8 ± 0.5 | 20.8 ± 4.0 | 250.4 ± 14.7 |
| | 500 | 17.0 ± 0.5 | 20.2 ± 1.8 | 256.3 ± 14.2 |
| | 2000 | 16.8 ± 0.4 | 19.7 ± 1.3 | 264.9 ± 21.0 |

[a]Activated partial thromboplastin time. [b]Prothrombin time. These coagulation biomarkers were analyzed in citrated plasma samples. Values represent mean ± SD (n=9 rats/group). No significant exposure-related changes were apparent.

Supplemental Material, Table 6. Blood count and plasma markers of systemic diesel exhaust (DE) effects in Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats.[a]

| Rat Strain | DE ($\mu g/m^3$) | Red Blood Cells x $10^9$/ml | White Blood Cells x $10^6$/ml | HCT[b] % | Hb[c] g/dL | Platelets X $10^9$/ml | Lymphocytes X $10^6$/ml | TAC, mmol/L | CRP, $\mu$g/ml | D-dimer, $\mu$g/L | ACE activity, U/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WKY | 0 | 7.8 ± 0.2 | 3.6 ± 0.4 | 40.2 ± 1.1 | 14.2 ± 0.6 | 950 ± 83 | 2.4 ± 0.4 | 1.19 ± 0.06 | 106 ± 19 | 0.93 ± 0.52 | 63.3 ± 4.2 |
| | 500 | 7.9 ± 0.3 | 3.8 ± 1.2 | 40.8 ± 1.5 | 14.2 ± 0.5 | 928 ± 93 | 2.6 ±0.9 | 1.20 ± 0.07 | 98 ± 5 | 0.85 ± 0.37 | 69.4 ± 8.2 |
| | 2000 | 7.9 ± 0.2 | 4.0 ± 0.9 | 40.6 ± 1.3 | 14.1 ± 0.5 | 953 ± 97 | 2.6 ± 0.3 | 1.19 ± 0.05 | 107 ± 20 | 0.71 ± 0.30 | 64.2 ± 5.3 |
| SH | 0 | 8.8 ± 0.3 | 2.4 ± 0.7 | 43.7 ± 1.7 | 14.8 ± 0.5 | 1247 ± 116 | 1.8 ± 0.5 | 1.23 ± 0.08 | 163 ± 45 | 0.76 ± 0.32 | 72.9 ± 9.5 |
| | 500 | 9.0 ± 0.3 | 3.4 ± 1.4 | 44.7 ± 1.3 | 15.2 ± 0.5 | 1298 ± 112 | 2.1 ± 0.6 | 1.20 ± 0.12 | 119 ± 32 | 0.36 ± 0.09 | 73.0 ± 7.5 |
| | 2000 | 9.0 ± 0.4 | 3.7 ± 2.7 | 44.6 ± 2.0 | 15.2 ± 0.4 | 1278 ± 73 | 2.0 ± 1.0 | 1.26 ± 0.13 | 141 ± 43 | 0.72 ± 0.50 | 70.4 ± 6.1 |

[a]Blood collection tubes containing EDTA were used for complete blood count (CBC). Total antioxidant capacity (TAC), D-Dimer, angiotensin converting enzyme (ACE) activity, and C-reactive protein (CRP) determinations were done in citrated plasma samples. Values represent mean ± SD (n=9 rats/group for CBC and n=4 rats/group for TAC, D-Dimer, ACE activity and CRP). No significant exposure-related changes were noted.

[b]Hematocrit

[c]Hemoglobin

Supplemental Material, Table 7. Notable examples of downregulated genes in the hearts of Wistar Kyoto (WKY) rats following diesel exhaust (DE) exposure (2000 $\mu g/m^3$) and in control SH rats at baseline without DE exposure.[a]

| Category/ function | UniGene ID | Gene Symbol | Gene Name |
|---|---|---|---|
| Stress/ oxidative | 22614 | *Stip1* | stress-induced phosphoprotein 1 |
| | 20155 | *Hspb3* | heat shock protein 3 |
| | 37192 | *Sepw1* | selenoprotein W, muscle 1 |
| | 10358 | *Sod3* | superoxide dismutase 3, extracellular |
| | 28532 | *Rarres2* | retinoic acid receptor responder (tazarotene induced) 2 |
| | 17145 | *Ctgf* | connective tissue growth factor |
| Growth/ Extracellular matrix | 8778 | *Fmod* | fibromodulin |
| | 7018 | *Tgfb3* | transforming growth factor, beta 3 |
| | 35769 | *Dynll1* | dynein light chain LC8-type 1 |
| | 107239 | *Col1a2* | collagen, type I, alpha 2 |
| | 2458 | *Tubb5* | tubulin, beta 5 |
| | 154431 | *Tubg1* | tubulin, gamma 1 |
| | 37227 | *Egfr* | epidermal growth factor receptor |
| | 8531 | *P4ha1* | procollagen-proline, 2-oxoglutarate 4-dioxygenase, alpha 1 polypeptide |
| | 203012 | *Igfbp7* | insulin-like growth factor binding protein 7 |
| | 1046 | *Tgfbi* | transforming growth factor, beta induced |
| | 99346 | *Emilin1_ predicted* | elastin microfibril interfacer 1 (predicted) |
| | 2090 | *Arpc1b* | actin related protein 2/3 complex, subunit 1B |
| | 207203 | *Fgfr1* | Fibroblast growth factor receptor 1 |
| | 2953 | *Col1a1* | collagen, type I, alpha 1 |
| | 35666 | *Vcan* | versican |
| | 104497 | *Tagln2* | transgelin 2 |
| | 97792 | *Ecm1* | extracellular matrix protein 1 |
| Mitochondria Function | 1608 | *Mrpl23* | mitochondrial ribosomal protein L23 |
| | 220 | *Comt* | catechol-O-methyltransferase |
| | 11359 | *Por* | P450 (cytochrome) oxidoreductase |
| Other | 88295 | *Thbd* | thrombomodulin |
| | 73051 | *Ptgis* | prostaglandin I2 (prostacyclin) synthase |
| | 2549 | *Ces3* | carboxylesterase 3 |
| | 14532 | *Arntl* | aryl hydrocarbon receptor nuclear translocator-like |
| | 32351 | *Apoe* | apolipoprotein E |
| | 22279 | *Ccnd1* | cyclin D1 |
| | 2305 | *Atp2a2* | ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 |
| | 10089 | *Cdkn1a* | cyclin-dependent kinase inhibitor 1A |
| | 11207 | *Thbs4* | thrombospondin 4 |
| | 16993 | *Serpinf1* | serine (or cysteine) peptidase inhibitor, clade F, member 1 |
| | 11881 | *Jund* | Jun D proto-oncogene |
| | 395 | *S100a4* | S100 calcium-binding protein A4 |
| | 1378 | *Fbn1* | fibrillin 1[b] |
| | 11515 | *Hamp* | hepcidin antimicrobial peptide[b] |
| | 6940 | *Sln* | sarcolipin[b] |
| | 3715 | *NOs3* | nitric oxide synthase 3, endothelial cell[b] |

[a]These genes were selected from 377 genes affected by DE inhalation in WKY rats and also found downregulated at baseline in SH rats without DE exposure.

[b]Although appeared to be inhibited; these genes did not reach significance ($P \leq 0.01$) between WKY-DE and WKY-air, but were significantly different between SH-air and WKY-air groups.

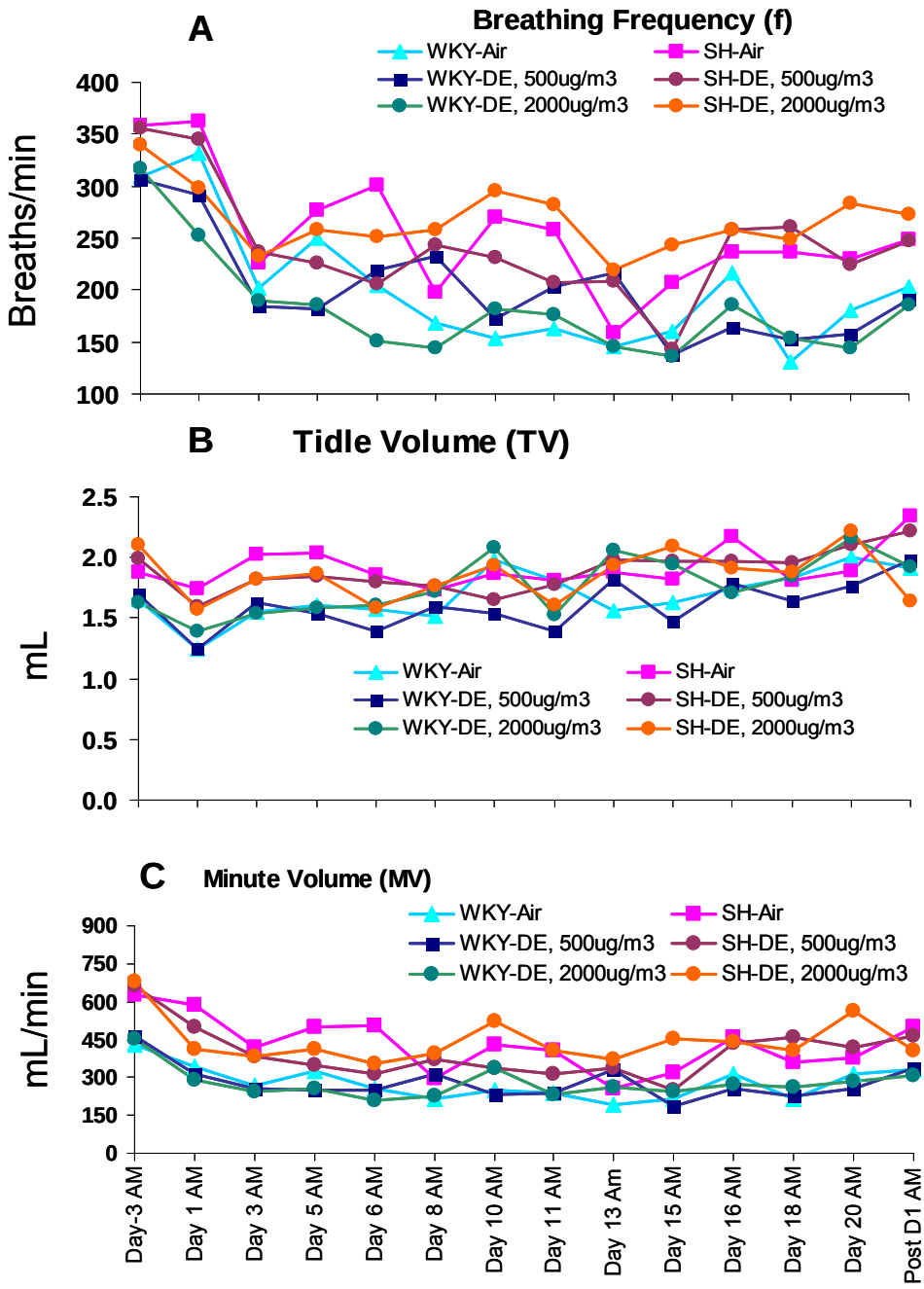
**Supplemental Material, Figure Legends**

**Supplemental Material, Figure 1**. Breathing parameters (A-F) recorded prior to and during 4-week diesel exhaust (DE) exposure in Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats. All values reflect the measurements done during morning hours (between 7:30-8:30 am) by whole body plethysmography using Buxco system. WKY and SH rats were exposed to either 0.0 (air), 500 or 2000 $\mu g/m^3$ DE, 5 hours/day, 5 days/week for 4 consecutive weeks (excluding weekends). Day 1 measurement shows the value prior to exposure. Note that no exposure-related changes were apparent but the strain-related differences readily apparent. No error bars are given for clarity. Each value represents the mean ± SD for 4 animals.

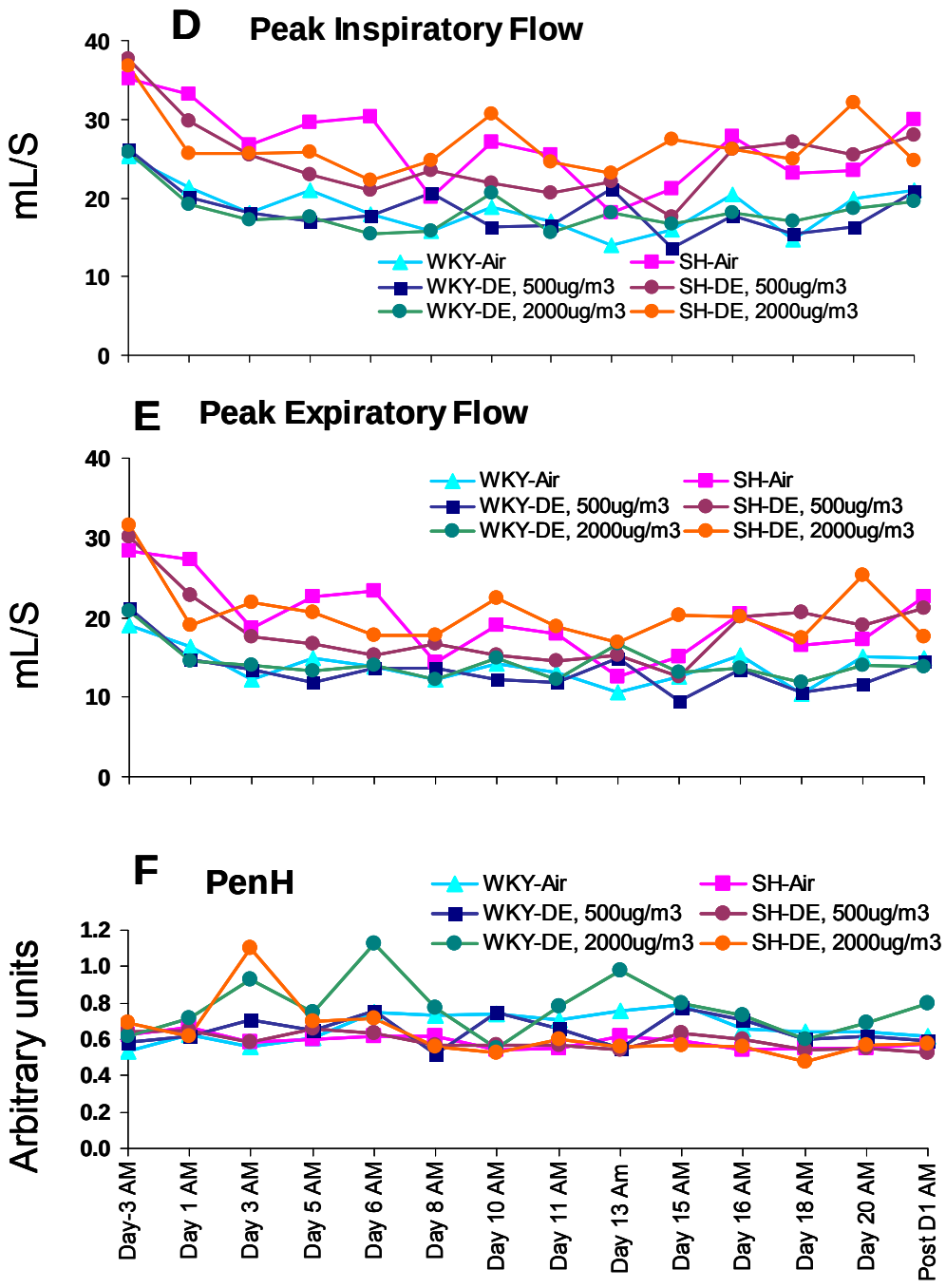
**Supplemental Material, Figure 2.** Activities of glutathione peroxidase (A), glutathione transferase (B), and superoxide dismutase (C) in left ventricular homogenates obtained from air or DE-exposed Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats. These values represent the data collected from whole homogenates, which contained cytosolic and also mitochondrial matrix enzymes. Note that no exposure-related changes were apparent but the strain-related differences are evident. Each bar represents the mean ± SD for 6 animals. † Indicate significant strain effect.

**Supplemental Material, Figure 3.** Cardiac mitochondrial (A) and cytosolic ferritin (B) levels in air or diesel exhaust (DE)-exposed Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats. Note that no exposure-related changes were apparent but the strain-related differences are evident. Each bar represents the mean ± SD for 6 animals. † Indicate significant strain effect.

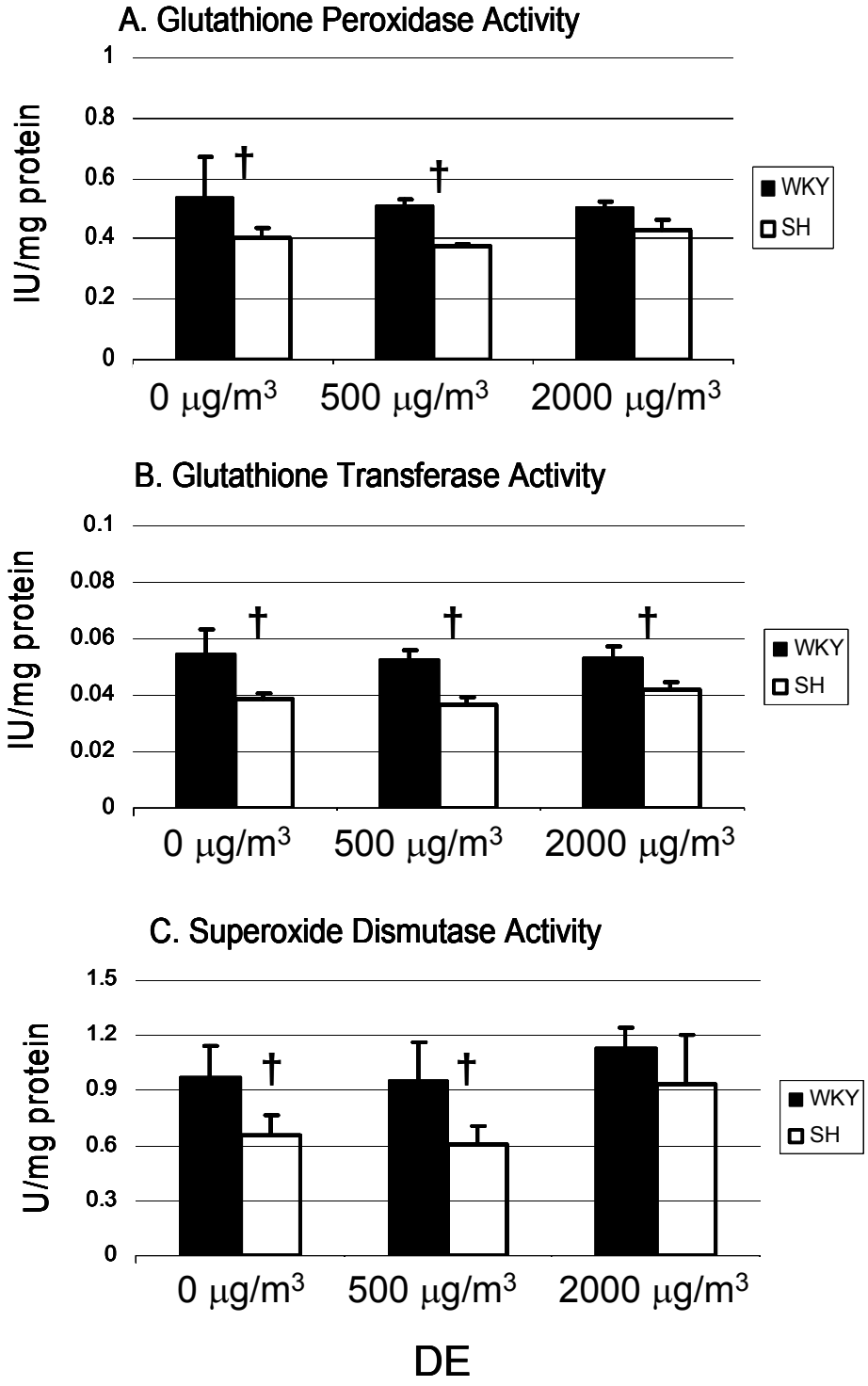
Supplemental Material, Figure 1



Supplemental Material, Figure 1 (continued)



Supplemental Material, Figure 2



Supplemental Material, Figure 3