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Alteration of peripheral blood monocyte gene expression in humans following diesel exhaust inhalation

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Abstract

Context—Epidemiologic associations between acutely increased cardiorespiratory morbidity and mortality and particulate air pollution are well established, but the effects of acute pollution exposure on human gene expression changes are not well understood.

Objective—In order to identify potential mechanisms underlying epidemiologic associations between air pollution and morbidity, we explored changes in gene expression in humans following inhalation of fresh diesel exhaust (DE), a model for particulate air pollution.

Materials and methods—Fourteen ethnically homogeneous (white males), young, healthy subjects underwent 60-min inhalation exposures on 2 separate days with clean filtered air (CA) or freshly generated and diluted DE at a concentration of 300 $\mu\text{g}/\text{m}^3$ $\text{PM}_{2.5}$. Prior to and 24 h following each session, whole blood was sampled and fractionated for peripheral blood mononuclear cell (PBMC) isolation, RNA extraction, and generation of cDNA, followed by hybridization with Agilent Whole Human Genome (4X44K) arrays.

Results—Oxidative stress and the ubiquitin proteasome pathway, as well as the coagulation system, were among hypothesized pathways identified by analysis of differentially expressed genes. Nine genes from these pathways were validated using real-time polymerase chain reaction (PCR) to compare fold change in expression between DE exposed and CA days. Quantitative gene fold changes generated by real-time PCR were directionally consistent with the fold changes from the microarray analysis.

Discussion and conclusion—Changes in gene expression connected with key oxidative stress, protein degradation, and coagulation pathways are likely to underlie observed physiologic

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Declaration of interest

The authors declare no conflicts of interest.

and clinical outcomes and suggest specific avenues and sensitive time points for further physiologic exploration.

Keywords

Diesel; oxidative stress; proteasome; air pollution

Introduction

There are strong epidemiologic associations between acute cardiorespiratory morbidity and mortality and particulate air pollution. These associations are strongest for fine particulate air pollutants (with median cut-point of $<2.5 \mu\text{m}$ aerodynamic diameter – $\text{PM}_{2.5}$) (Brook, 2008; Mills et al., 2009; Pope et al., 2004); however underlying mechanisms are not well established. We and others have used fresh diesel exhaust (DE), a complex aerosol of organic and inorganic gaseous and particulate emissions, as a model to study the effects of acute exposure to fine particulate air pollution (Laumbach et al., 2009; Mills et al., 2009; Peretz et al., 2007; Reed et al., 2004; Sunil et al., 2009).

The oxidant and proinflammatory nature of combustion-derived particulate matter has been established from *in vitro*, animal, and human exposure studies. These studies have suggested that generation of oxidative stress may be a mediator of human toxicity from air pollutants (Donaldson et al., 2005; Miller et al., 2009; Nel et al., 2001). Examples of experimentally induced toxicity from exposure to DE at levels occurring in urban environments include airway inflammation (Salvi et al., 1999), increased coagulation (Lucking et al., 2008), and arterial narrowing (Peretz et al., 2008), as well as changes in expression of cytokines in respiratory epithelial cells (Elder et al., 2004; Li et al., 1996; Seaton et al., 1995). Our laboratory has also recently observed changes in protein degradation activity via the ubiquitin proteasome pathway (UPP) in human volunteers immediately following exposure to fresh DE (Kipen et al., 2011).

The systemic inflammatory response following acute inhalation exposure to particulate matter (PM) can induce leukocytosis and monocyte release from the bone marrow (Fujii et al., 2002). Alveolar macrophages stimulated by PM release cytokines that promote bone marrow release of monocytes (Goto et al., 2004). These responses of peripheral blood mononuclear cells (PBMCs) and other leukocytes, as well as their accessibility, make them relevant candidates in which to explore the effects of air pollution on gene expression as it relates to cardiopulmonary toxicity from ambient air pollution.

We studied the effects of an acute inhalation of fresh DE on human PBMCs using microchip array analysis verified by real-time polymerase chain reaction (PCR). Functional analysis of gene expression was performed to identify and confirm potential biological mechanisms affected by particulate pollution. Based on the literature, we hypothesized that short-term exposure to DE would alter oxidative stress pathways, inflammatory response, vascular homeostasis, and protein degradation pathways 24 h after exposure, and we found evidence supporting these hypotheses.

Methods

In a cross-over study performed primarily to examine changes in the inflammatory phenotype response to fresh DE inhalation, 1-h exposure sessions were conducted in randomized order on 2 separate days, at least 1 week apart, with either clean filtered air (CA) or freshly generated and diluted DE at a concentration of $300 \mu\text{g}/\text{m}^3 \text{PM}_{2.5}$. All

participants gave written informed consent approved by the UMDNJ Institutional Review Board following an orientation to the procedures of the study (Laumbach et al., 2011).

Subjects

The 100 recruited individuals were healthy non-smokers between the ages of 21 and 44. RNA specimens from 14 of the individuals were selected for the pilot analyses described here. Prior to beginning the study, all subjects were screened and determined to be free of cardiovascular disease, stroke, hypertension, asthma, and cigarette smoking within 5 years. No clinically significant abnormalities of ECG, spirometry, complete blood count (CBC) with differential and platelet count, urinalysis, or standard metabolic screen of electrolytes, minerals, liver, and renal function were permitted for participation in the study. White blood cell (WBC), lymphocyte, and monocyte counts were similar and not significantly different between the 100 total subjects and the 14 selected, based on age, ethnic, and gender homogeneity, for this pilot study of gene expression. The only medications permitted were sporadic use of OTC pain relievers. Other exclusions for a 1-week window prior to an exposure session included an active respiratory infection or allergic exacerbation, or use of medications relating to allergies or other respiratory conditions.

Controlled exposure facility

The controlled exposure facility (CEF) located within the Environmental and Occupational Health Sciences Institute in Piscataway, NJ, was utilized for exposure sessions. The CEF is a 25 m³ stainless steel chamber constructed to maintain constant environmental conditions. This facility is linked to the DE generation system, which contains a diesel-fueled generator and a dilution/delivery system. DE is created using a 5500W Yanmar electricity generator, with a 406 cc displacement air-cooled engine. The engine is operated using 40-weight motor lube oil. A large amount of Number 2 undyed low sulfur on-highway fuel was purchased at the beginning of the study to minimize fuel composition variation. In order to maintain full capacity of the engine, several space heaters were utilized as a load during the sessions. The system contains two mass reduction devices including a 10-position butterfly valve that separates the exhaust between the waste pipe and the variable-speed blower. Following the mass reduction, exhaust was delivered into the CEF air stream to reach a specific concentration of particulate matter. The air stream had been filtered through activated carbon cartridges and high efficiency particulate air (HEPA) filters to minimize pollutants and ambient particles. PM_{2.5}, NO_x, and CO were monitored throughout each exposure session. Temperature was maintained at 72 ± 0.5°F and relative humidity at 40 ± 2%. Table 1 gives the composition of diluted exhaust from our diesel generator when operated at 100% of rated capacity with a target mass of 300 µg/m³.

Protocol during exposure

Each exposure session lasted 60 min and occurred at approximately the same time of day in the early morning. Research technicians verified adherence to health, medication, and dietary criteria. An ECG was recorded; blood was drawn for physiologic endpoints (to be reported elsewhere) and RNA extraction. ECG electrodes were attached for continuous monitoring during exposure.

Following check-in and pre-exposure testing, subjects walked approximately 40 yards and took a two story elevator ride to the CEF located in the same building. The diesel generator ran during all sessions, but exhaust was diverted completely outside for CA sessions. Participants were comfortably seated and asked to read or study during sessions. Subjects and real-time exposure data were continuously monitored by staff.

Blood was sampled prior to and immediately following exposure, then a third time approximately 24 h following the start of each session. Subjects were asked not to do any strenuous activity in the time period between the completion of the exposure and the 24-h post exposure blood draw. Samples from venipuncture were collected and immediately placed on ice and transferred to the Bionomics Research and Technologies Core (BRTC) at EOHSI for processing and analysis. One hundred subjects were studied in the overall protocol and had RNA extracted. However, in order to enhance any detectable changes and stay within financial constraints, we selected 14 subjects who were ethnically homogeneous (white males) and narrowly age-matched (younger than 30 years) for the present costly analyses. This paper presents only the data from the 24-h samples comparing DE with CA conditions within a subject, as cost constraints precluded analysis of pre-exposure gene expression data.

RNA extraction

Whole blood collected in BD Vacutainer Cell Preparation *Tubes* (CPTs) containing sodium heparin as an anticoagulant was processed according to the manufacturer's instructions. After blood collection and inversion, the tubes were transported immediately to the laboratory, where they were inverted eight more times before centrifugation (15 min at 1500 $\times g$) at room temperature. Approximately 2 mL of PBMC was collected and mixed with 6 mL of Trizol LS reagent (Invitrogen Inc). The cells were lysed at room temperature for 5 min and were then subjected to RNA extraction. A 1.5 mL Phase Lock Gel (Eppendorf) was used for separating the aqueous phase from the organic phase, and RNA was extracted using the RNeasy mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Quality of the RNA was assessed with use of the Agilent Bioanalyzer 2100 and spectrophotometric analysis. Quality as measured by RIN score met criteria for gene expression analysis, with scores being higher than 7.

Microarray sample processing

RNA (10 ng) from each sample was used to generate cDNA for array hybridization using the NuGEN WT-Ovation Pico RNA Amplification System. The cDNA was then labeled with Cy3 dye using a NuGEN FL-Ovation cDNA Fluorescent Module. Detailed protocols for sample processing can be found at <http://www.nugeninc.com>. The labeled cDNA was hybridized to Agilent Whole Human Genome (4X44K) arrays, which represents 43,000+ coding and non-coding human sequences. The hybridized slides were washed using a commercial kit (Agilent Technologies, Palo Alto, CA) and scanned by Agilent G2565BA microarray scanner. A total of 28 (one DE and one CA per subject) arrays were used for the study and their data set is available through Gene Expression Omnibus (GEO) at <http://www.ncbi.nlm.nih.gov/geo/> (Accession number GSE25531).

Microarray data analysis

Gene expression analysis was carried out using GeneSpring (Agilent, CA) for data normalization and statistical analysis. Data were normalized using a global scaling method. The level of fold change significance between DE and CA sessions used to identify genes for pathway construction was set at a fold change of 1.2 with $p < 0.05$, which yielded 3281 significantly up or downregulated genes. Paired t -tests were performed to determine differentially expressed genes between CA and DE exposure. Average linkage hierarchical clustering analysis was applied using Euclidean distance as the similarity measure to understand the distribution of gene intensities as a function of exposure. The differentially expressed genes were annotated and pathway analyses were completed using Ingenuity Pathway Analysis software. IPA forms pathways based on gene connections demonstrated in previously published public domain literature.

Real-time quantitative PCR

Gene expression was validated using Taqman chemistry with probes and primers designed using the Roche Universal Probe Library (UPL). Gene expression was measured in a 10 μ L PCR reaction containing 5 μ L of ABI 2X universal master mix, 0.1 μ L each of forward and reverse primers (final concentration 200 nM), 0.1 μ L of the corresponding UPL probe and RNAase/DNAase-free water. All quantitative PCR was carried out in an ABI7900 HT sequence detection system on biologic replicates leading to three QPCR data points per condition. The cycling parameters were 95°C for 15 s and 60°C for 1 min for 40 cycles. Raw data were analyzed using ABI SDS 2.3 software, while relative quantification using a comparative threshold method was performed in Microsoft Excel. A gene that showed no differential expression in the microarray data set (human beta actin) was used as the normalizer for delta Ct analysis.

Results

Gene expression profiles

Agilent Microarray gene chip analysis generated gene expression profiles on PBMCs that were sampled at 24 h post-DE and CA exposures. To understand the potential effects of DE inhalation on gene expression in PBMCs, we analyzed differential gene expression profiles using microarray analysis to evaluate the effect of DE on hypothesized biological pathways. Figure 1 shows representative examples of scatter plots of the gene expression data. Figure 1A displays the gene expression signal intensity for two subjects following a CA exposure, while Figure 1B shows gene alterations between the DE and CA exposure of one subject. A greater spread of the data points in Figure 1B demonstrates an example of where the diesel effect is greater than an individual difference effect on gene expression variation. Figure 2 shows heat maps constructed following sample analysis. The microarray pattern recognition shows the gene expression of all 14 subjects both for control CA and DE exposure days (28 columns). For 12 of 14 subjects, the overall pattern of expression for differentially regulated genes seems to be consistent with the exposure paradigm. This analysis was used to generate hypotheses for validation of specific gene products of biological interest. We used hierarchical clustering to identify genes and gene families that had similar differential gene expression patterns (Figure 2).

Differentially expressed genes from the microarray analyses were analyzed using the Ingenuity Pathway Analysis program (IPA, Ingenuity® Systems, www.ingenuity.com) in order to construct molecular pathway connections that showed the greatest expression changes, calculated for each canonical pathway as follows:

IPA forms functional pathways based on gene–protein connections demonstrated in previously published public domain literature. Canonical pathway analysis identified pathways from the Ingenuity Pathways Analysis library of canonical pathways that were most representative of the data set. Gene targets from the data set that met the $p < 0.05$ cutoff of $FC > 1.2$ and were associated with a canonical pathway in Ingenuity's Knowledge Base were considered for the analysis. The significance of the association between the data set and the canonical pathway was measured in two ways: 1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway as displayed in Figure 3 for the first 24 out of 167 canonical pathways; 2) Fisher's exact test to calculate a p value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone (IPA, Ingenuity® Systems, www.ingenuity.com). The level of significance used to identify genes for pathway construction set at $p < 0.05$ and a 1.2 fold mean change in expression, yielded 3281 significantly up or downregulated genes.

We found significant changes in canonical pathways that have been previously implicated in mechanistic studies of air pollution health effects including the NRF2-mediated oxidative stress response (183 genes with expression altered), hypoxia signaling in the cardiovascular system (172 altered genes), the coagulation system (37 altered genes), as well as other fundamental physiologic pathways (Figure 3). Additionally, the mitochondrial dysfunction pathway was particularly prominently altered and is linked to other findings discussed below. Moreover, a number of individual genes previously associated with or hypothesized to relate to acute diesel-induced phenotypic change demonstrated significant induction or repression as discussed below.

Validation of expressed genes

Following analysis in the IPA program, nine genes were chosen for further evaluation based on their ability to meet our statistical significance cutoff for differential expression, as well as their association with hypothesized and/or literature-validated mechanisms of acute air pollution-induced toxicity. We relaxed the fold change criterion for a few of these in order to focus our validation on specific mechanistic hypotheses of interest. The specific genes chosen for further validation were involved in pathways of *a priori* interest including oxidative stress (NOS2A, NOS3), coagulation (PLAU, F2R), and the ubiquitin proteasome pathway (CBL, USP10, UBR1, UBR2, CDH1) (Table 2). Real-time PCR was used to generate a quantitative fold change for the nine genes chosen. Validation was limited to genes related to our *a priori* hypotheses. Table 2 displays the average fold change of the nine genes from the microarray analysis and their corresponding real-time PCR and corresponding standard deviation.

The directionality of the microarray and real-time PCR fold changes within each of the nine genes were consistent. This confirmed that our microarray technique correctly determined the direction of change alterations in transcription following exposure and served to validate our overall observations.

Discussion

In a clean-air controlled cross-over design that analyzed 14 healthy subjects, a 1-h inhalation of fresh DE was associated with marked changes in peripheral blood mononuclear cell gene expression at 24 h after exposure. Subjects served as their own controls, thus limiting typical sources of bias and confounding. A total of 3281 genes on an Agilent array demonstrated up or downregulated differential expression (> 1.2 fold and $p < 0.05$). These genes were utilized by the Ingenuity (IPA) program to produce functional pathways. Multiple IPA pathways, both biological (canonical) and toxicological, were significantly impacted, and scatterplots demonstrated greater overall differences in gene expression between samples collected from the same individual after exposure to DE versus clean air, compared with samples collected from different individuals after exposure to only clean air. Moreover, based on patterns derived from hierarchical clustering we can differentiate between exposures on a sample by sample basis across most subjects.

Validation of expression results

Based primarily on relevance to hypothesized mechanisms for acute air pollution toxicity [hemostasis, oxidative stress, NO metabolism, and the proteasome pathway (Kipen et al., 2011)], we chose nine relevant genes as PCR validation targets, all differentially expressed ($p < 0.05$; Fold change (FC) > 1.2) between CA and DE in the same individuals. The hemostasis parameter gene F2R (thrombin receptor) increased 30 fold (prothrombotic), while PLAU (plasminogen activator) expression decreased 25 fold (also a generally prothrombotic effect based on this enzyme's role in degrading thrombus). In comparison,

one lab demonstrated increased thrombosis and platelet activation at 6 h postcontrolled human exposure to DE (Lucking et al., 2008), consistent with an observational finding that ambient air pollution increased platelet aggregation in diabetic patients (Jacobs et al., 2010). These two platelet-oriented findings may be construed as generally consistent with our findings of upregulation in the soluble coagulation system, although there is one report of null results following DE exposure in both healthy and metabolic syndrome subjects for circulating biomarkers of coagulation (d-dimer, von Willebrand factor (VWF), and plasminogen activator inhibitor-1 (PAI-1)) (Carlsten et al., 2007).

With respect to NO synthesis and metabolism, expression of NOS2A (inducible nitric oxide synthase, involved in pulmonary defense and the oxidative stress response) increased 3.76 fold while NOS3, endothelial (constitutive) nitric oxide synthase, key to endothelial function, increased 2.15 fold. Assuming that changes in PBMC reflect changes in the endothelial and pulmonary epithelial tissues of interest, this is consistent with a 24-h homeostatic response to acute depletion of NO by oxidants in diesel PM (Miller et al., 2009). Consistent with this, Kooter et al. (2005) showed a 2.6 fold increase in expression of NOS2A in whole lung tissue at 24–40 h after particulate matter instillation in rats. Further supporting the relevance of these findings to cardiopulmonary disease, we have demonstrated in humans a decrease in non-ischemically stimulated forearm venous blood levels of nitrite (the proximal metabolite of NO) 2 h after onset of fresh DE exposure in human volunteers (Gandhi et al., 2009).

Alterations of the proteasome pathway at 24 h following DE were seen through the strongly upregulated genes, USP10, UBR2, and UBR1. USP10 is a carboxy-terminal hydrolase that could play a key role in providing additional ubiquitin under conditions of oxidative stress, when increased numbers of oxidatively damaged proteins should be targeted for elimination (Obin et al., 1998; Salomons et al., 2009). Oxidative damage to mitochondria, the most prominent pathway in our array data (Figure 3), should decrease ATP production leading to further decreases in proteasome activity (Smith et al., 2005; Liu et al., 2006). Consistent with this, as well as with direct oxidative damage to the proteasome itself, we found acutely decreased proteasome activity in leukocytes among another group of human volunteers after similar exposure to DE (Kipen et al., 2011). Our observed upregulation of USP10, UBR1, and UBR2 is again consistent with a compensatory enzyme induction at 24 h in response to this decrease in activity immediately following DE exposure. Such reduced proteasome activity may actually serve to stimulate antioxidant defense (Dreger et al., 2009; Meiners et al., 2006) as well as increase eNOS expression and activity (Stangl et al., 2004).

CBL, and its endogenous human gene (c-Cbl) counterpart, is involved in protein ubiquitination by encoding ubiquitin E3 ligase. Its downregulation seems opposite to the effect of the other proteasome pathway components, but serves to underscore a complex and central role of the proteasome pathway in the acute effects of particulate air pollution on human physiology. Another gene chosen for validation, CDH1 (E cadherin), was downregulated almost 14 fold. While not related to vascular cell adhesion as are other cadherins hypothesized to play a role in inflammation-mediated effects of air pollution inhalation, E cadherin is a substrate of the proteasome and is likely to conjugate to a distinct type of multi-ubiquitin chain.

As noted above, Kooter et al. (2005) studied the time-dependent gene expression patterns in whole lung tissue of spontaneously hypertensive rats treated with an intratracheal instillation of urban particulate. From 8799 genes or expressed sequence tags, 132 were up or downregulated within 2–6 h, with 46 and 56 genes at 15–21 and 24–40 h, respectively. Upregulated genes were involved in inflammatory, oxidative stress, and cardiopulmonary responses. In addition to the well-known SOD and GSH responses, they found additional

antioxidant responses to include heme oxygenase, metallothioneins, and thioredoxin reductase. Table 3 indicates the results for these genes in our microarray analysis. Thus, there is consistency between rat lungs and human PBMCs in the MAPK pathway response to inhaled DE, also suggesting support for the use of PBMCs to infer changes of relevance to the pulmonary system and perhaps the vasculature discussed above.

Kooter et al. (2005) also reported signal transduction changes through genes such as FOS-like antigen 1 (FOSL-1, FC of 16) and jun-B oncogene (also known as AP-1, FC of 2.1), which are regulated by NF κ B. Our microarray analysis shows an increase in FOSL1 expression as well as an increase in jun-B (Table 3). Gene expression alterations, dominantly upregulated, in the MAPK pathway are seen in Table 3, further supporting the potential for air pollutants to activate proinflammatory processes.

Two additional studies have addressed the impact of PM on gene expression in mammalian systems. Peretz et al. (2007) studied two healthy humans at 22 h post-DE exposure in an exposure protocol similar to ours. 1290 out of 54,675 Affymetrix probe sets evidenced more than 1.5 fold up or downregulation ($p < 0.05$), between CA and DE. Interestingly, they also found increased expression at 22 h of oxidative stress genes (e.g. heme oxygenase-1, peroxiredoxin-1) and multiple genes involved in leukocyte migration, cell adhesion, and cell migration.

Huang et al. (2010) studied three healthy humans at 24 h postultrafine particle (UFP) inhalation and found 1020 annotatable genes to be up or downregulated by a ratio of 1.1. Pathways identified include NRF2-mediated oxidative stress response, PPAR signaling, and glucocorticoid receptor signaling. Upregulation for two of the genes was confirmed by RT-PCR.

This study has several limitations that may affect interpretation. Table 2 indicates the wide variability of these data based on a limited number of subjects and reinforces the exploratory nature of this study, seen in the large standard deviations of the real-time PCR. Figure 2 shows that 12 of 14 subjects demonstrated similar gene expression in response to DE and CA. However, 2 subjects, 72 and 80, did not follow this pattern, with 72 showing relatively little response to DE and subject 80 showing relatively unique responses to both exposures. We do not have a concise explanation for these outliers. Technical errors have been minimized by review of raw data. Both subjects received medical examinations before the study and were found to have unremarkable history, physical examination, CBC with differential, and serum chemistries. Of course it is possible that both of these individuals had significant variants in their genetic makeup (e.g. diesel-metabolizing enzymes) to account for the different outcomes, however this remains unknown and could generate hypotheses for further examination.

Additional limitations are that we were unable to analyze the baseline (pre-exposure) expression data, limiting comparisons to the post-exposure samples only, but all analyses were within subjects. We have only limited physiologic correlates to compare with the changes in gene expression although the literature provides substantial context for interpretation. We were only able to measure a single time point in relation to exposure, and the literature suggests substantial effects of timing on expression of genes as well as physiologic change following air pollution exposure. Additionally, our studies were conducted in PBMC, not the primary tissues hypothesized to be affected by air pollution. Nevertheless, we have shown expression changes that are consistent with the available limited literature in rodents and humans.

Conclusions

These data represent the largest gene expression study in humans exposed to DE in a controlled setting and demonstrate significant up or downregulation of genes in critical hypothesized pathways for acute cardiopulmonary morbidity and mortality as well as for chronic effects that may mediate cardiopulmonary and other morbidity. Pathways include the oxidative stress response, vascular homeostasis, coagulation, and the proteasome pathway, as well as mitochondrial dysfunction. Further investigation of these relationships is indicated to confirm the observations.

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Figure 1.

Sample reproducibility versus exposure effect. (A) Scatter plot of two samples from two different subjects each sampled following the control CA exposure. (B) Scatter plot of representative CA and DE exposure samples from the same subject. The intensities of all probes were included. The fold change lines represent two fold differences. The overall difference in expression is greater between post-CA and DE exposure measurements in the same individual compared with CA exposure measurements across individual subjects.

Figure 2.

Pattern recognition for CA and DE exposure samples. Hierarchical clustering analysis studies of the effect of diesel exposure. Raw data were normalized by a quantile normalization method in GeneSpring GX 10.0.1 (Agilent Technologies, Palo Alto, CA). Genes included in the hierarchical analysis were chosen by a p value cutoff of 0.05 (without multiple hypothesis adjustment) and a fold change cutoff of 1.2. Data were further transformed by calculating the z-scores. Hierarchical analysis was carried out using Euclidean distance as the similarity measure. The 14 sessions with DE exposure are coded blue at the bottom of the figure and the 14 with CA are shown in orange.

Figure 3. Canonical pathways from the IPA program with the greatest ratio of gene expression change to total pathway gene expression. The canonical pathways for signaling and metabolic activity were created using differential expression data. This figure illustrates pathways affected by differentially regulated transcripts and significance of pathway assignment is a function of the total number of eligible molecules in a given reference pathway. IPA uses the Fisher's exact test to calculate statistical significance of pathway involvement.

Table 1

Composition of CA and diluted DE from the diesel generator operated at 100% load (mean $\pm$ SD).

Analyte	Unit	Diesel exhaust concentration	Clean air concentration
PM2.5	$\mu\text{g}/\text{m}^3$	295 ± 18.0	4.8 ± 3.9
PM number	$\#/\text{cm}^3$	68263 ± 6994	3460 ± 1963
CO	ppm	3.42 ± 0.20	0.93 ± 0.36
NO	ppm	3.43 ± 0.18	0.06 ± 0.22
NO2	ppm	0.15 ± 0.05	0.19 ± 1.18

Table 2

Mean Real-time PCR and microarray fold changes in expression of nine genes.

Gene abbreviation	Ingenuity name	Microarray fold change	RT-PCR fold change $\pm$ SD
F2R	Coagulation factor II (thrombin) receptor	1.36	30.81 $\pm$ 110.98
PLAU	(Plasminogen) Urokinase	-1.24	-25.19 $\pm$ 79.17
USP10	Ubiquitin-specific peptidase 10	1.72	11.67 $\pm$ 16.03
UBR2	Ubiquitin protein ligase E3 component n-recognin 2	1.4	10.55 $\pm$ 34.37
UBR1	Ubiquitin protein ligase E3 component n-recognin 1	1.44	18.29 $\pm$ 51.87
CBL	Cas-Br-M ecotropic retroviral transforming sequence	-1.33	-5.18 $\pm$ 13.39
NOS2A	Nitric oxide synthase 2	1.12	3.76 $\pm$ 4.62
NOS3	Nitric oxide synthase 3	1.15	2.15 $\pm$ 7.37
CDH1	Cadherin-1	-1.61	-13.91 $\pm$ 27.69

Table 3

Oxidative stress genes found in Ingenuity Pathway Analysis Program.

Genes	IPA gene abbreviation	Microarray fold change
Metallothioneins	MT1G	↑1.233
	MT3	↑1.218
Thioredoxin reductases	TXNRD1	↑1.279
	TXNRD3	↑1.234
Superoxide dismutase	SOD1	↓-1.255
Glutathion-S-transferase M1	GSTM1	↑1.247
Glutathion-S-transferase M3	GSTM3	↑1.312
Glutathion-S-transferase O1	GSTO1	↓1.268
Glutathion-S-transferase O2	GSTO2	↑1.243
Glutathion-S-transferase T1	GSTT1	↑1.338
Mitogen activation protein kinase kinase kinase 1	MAP3K1	↓-1.367
Mitogen activation protein kinase kinase kinase 7	MAP3K7	↑1.230
Mitogen activation protein kinase kinase kinase 3	MAPK3	↑1.394
Mitogen activation protein kinase 8	MAPK8	↑1.248
Mitogen activation protein kinase 14	MAPK14	↑1.259
Fos-like antigen 1	FOSL1	↑1.238
Jun oncogene (also called AP-1)	JUN	↑1.294