Comparison of Acute Health Effects From Exposures to Diesel and Biodiesel Fuel Emissions

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Objective: To investigate the comparative acute health effects associated with exposures to diesel and 75% biodiesel/25% diesel (B75) blend fuel emissions.

Methods: We analyzed multiple health endpoints in 48 healthy adults before and after exposures to diesel and B75 emissions in an underground mine setting—lung function, lung and systemic inflammation, novel biomarkers of exposure, and oxidative stress were assessed. Results: B75 reduced respirable diesel particulate matter by 20%. Lung function declined significantly more after exposure to diesel emissions. Lung inflammatory cells along with sputum and plasma inflammatory mediators increased significantly to similar levels with both exposures. Urinary 8-hydroxydeoxyguanosine, a marker of oxidative stress, was not significantly changed after either exposure.

Conclusions: Use of B75 lowered respirable diesel particulate matter exposure and some associated acute health effects, although lung and systemic inflammation were not reduced compared with diesel use.

Diesel engines are widely used in on- and off-road applications including personal vehicles, trucks, buses, trains, ships, underground mining, construction, and agriculture. Exposure to diesel engine emissions is associated with chronic bronchitis, respiratory tract infections, asthma exacerbation, and increased cardiovascular morbidity and mortality, and in 2012 diesel emissions were classified by the International Agency for Research on Cancer as a group 1 carcinogen in humans. Given the health effects of diesel emissions and ubiquitous environmental exposures, reducing engine emissions has become a public health priority.

In recent years, alternative fuels such as biodiesel have been introduced in attempts to reduce diesel particulate matter (DPM) emissions. Often used as a blend with diesel to facilitate use in current engines, biodiesel has been shown to reduce respirable particulates, dependent on fuel formulation, pollution control devices, and engine operating conditions. Despite the increasing usage of biodiesel, there is a lack of information on the human health effects of exposure to these emissions, and recent in vitro and in vivo studies suggest that exposure to biodiesel particulates may be more toxic than diesel particulates at equivalent concentrations.

This study was conducted to compare the acute human health effects related to exposures to emissions from diesel and a 75% biodiesel/25% diesel (B75) blend fuel in an underground mining setting, where DPM concentrations are among the highest reported. The null hypothesis was that switching to B75 would not reduce the adverse health effects compared with exposures to diesel emissions.

Learning Objectives

- Discuss the use of biodiesel-containing fuel blends as a potential approach to reducing health harms by lowering respirable diesel particulate matter.
- Summarize the effects of exposure to B75 fuel blend versus diesel on emissions, lung function, and inflammatory biomarkers.
- Discuss the research and practical implications for the concept of using biodiesel fuel blends to reduce diesel particulate exposure and adverse health effects.

MATERIALS AND METHODS

Subjects

Human subject recruitment and testing procedures were approved by the University of Arizona (UA) institutional review board. Subjects were recruited from the UA campus. Inclusion criteria included being at least 18 years of age. Exclusion criteria included recent (within 4 days) diesel exhaust or other significant occupational inhalation exposure, smoking, a diagnosis of asthma, heart disease, diabetes, hypertension, renal or hepatic failure, a difference in blood pressure greater than 15 mm Hg between the arms, baseline forced expiratory volume in one second (FEV1) divided by forced vital capacity (FVC) less than 0.7, or current respiratory illness.

Load-Haul-Dump Training and Baseline Testing

After written consent was obtained, subjects were scheduled for load-haul-dump (LHD) vehicle driver’s training a minimum of 96 hours prior to baseline testing, which was completed at least 72 hours prior to the first emissions exposure. Baseline testing consisted of blood pressure measurement, phlebotomy, pulmonary function testing, and sputum induction. Blood pressure was measured in both arms using an automated sphygmomanometer (OMRON, Bannockburn, IL). Blood samples were collected in serum clot activator, heparin, sodium citrate, and ethylenediaminetetraacetic acid tubes. The serum tube was allowed to clot for 30 minutes at room temperature prior to centrifugation. All of the tubes were initially centrifuged at 1000 × g for 15 minutes. The heparin and sodium citrate tubes were decanted and a second 10-minute centrifugation step at 10,000 × g was added to obtain complete platelet removal. Serum and plasma were decanted and stored immediately at −80 °C until assayed. Pulmonary function testing was performed following American Thoracic Society (ATS) standards in a sitting position using an EasyOne spirometer (ndd Medical Technologies, Andover, MA). Forced ex-

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piratory volume in one second, FVC, and age- and height-adjusted percentage predicted values were recorded. A minimum of three trials were performed, with a maximum of eight trials at any one sitting. Testing was continued until the two largest FEV₁ values were within 0.150 L of each other, with the same process followed for FVC. Sputum induction was performed using DeVilbiss Ultra-Neb 991HD ultrasonic nebulizers (Somerset, PA) with 3% saline for 30 minutes, as previously described.14 Samples were diluted with 10% Sputolysin (Calbiochem, San Diego, CA) in phosphate buffered saline and incubated at room temperature for 15 minutes with gentle mixing by inversion every 5 minutes. A 500 μL aliquot was removed prior to centrifugation; 50 μL of sample was mixed with 50 μL Trypan Blue stain (Sigma Chemical Co, St Louis, MO) prior to total cell counting performed using a hemocytometer. The remaining aliquot was mixed with an equal amount (450 μL) of preservative (Histochrome, AM-RESCO, Solon, OH), and 500 μL of the mixture was cytocolntrifuged, stained with Diff-Quik (Dade Behring AG, Switzerland), and analyzed using the first 100 white cells counted, excluding epithelial cells. The remaining sample was centrifuged at 1900 rpm for 20 minutes, and the supernatant and cell pellet were stored at −80°C until assayed.

Preexposure Testing on Emissions Exposure Day

On the emissions exposure day, subjects were instructed to fast for at least 6 hours prior to arriving at the UA San Xavier Mining Laboratory. First morning void urine samples and all subsequent voids continuing through the end of the day’s testing were refrigerated at 4°C until processing. Baseline exhaled carbon monoxide (CO), expressed as percentage carboxyhemoglobin (%COHb), and baseline fraction of exhaled nitric oxide (FENO) testing were performed following the ATS recommendations and standards, respectively, using the microCO Breath Carbon Monoxide Monitor (Micro Direct, Lewiston, ME) and a NIOX MINO (Aerocrine, Inc, New Providence, NJ). After a 20-minute rest in a supine position and ultrasound testing for brachial artery flow-mediated dilation (results to be reported separately),15 subjects were supplied a carbohydrate meal (cereal, 2% milk, and energy bars) and water.

Fuels, Equipment, and Study Site

The fuels, equipment, and study site description have previously been reported.16 Briefly, ultra-low sulfur #2 diesel (Arizona Petroleum, Tucson, AZ) was used for diesel exposure testing, and B75 was prepared by mixing the aforementioned diesel fuel at 25% by volume with a soy methyl ester biodiesel fuel (ASTM D6751-compliant; Arizona Petroleum, Tucson, AZ). Exposure to vehicle emissions was evaluated during mucking activities in the San Xavier Mining Laboratory. A 2005 Wagner B10-203 LHD vehicle with open cab and diesel oxidation catalyst, but no diesel particulate filter, was used for best fit, as determined by the BioTek KC4 automated software program (Winooski, VT) following manufacturer’s recommendations. The resulting peptide samples were desalted using solid-phase extraction and concentrated to dryness prior to analysis by high-resolution liquid chromatography–mass spectrometry/mass spectrometry for protein identification using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate biomarkers were identified using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate biomarkers were identified using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate biomarkers were identified using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate biomarkers were identified using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY). Three proteins (GRO-α, MMP-8, and TN-C) considered prime candidate biomarkers were identified using a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with an Advion nanomate ESI source (Advion, Ithaca, NY).
further validated in the entirety of the sputum and plasma samples using ELISA.

**Statistical Analysis**

All manual results were double-entered into a spreadsheet and the results checked for accuracy. Industrial hygiene measurements were time weighted over an 8-hour exposure period (TWAs), as previously described. The TWAs exposure means were first compared across fuel types using the Kruskal-Wallis rank test with the Bonferroni correction (STATA 12.0, StataCorp, College Station, TX). Those analytes with significant differences across post-diesel and post-B75 sampling were further analyzed using the Wilcoxon signed-rank test for paired analysis. Health data (exhaled CO and nitric oxide [NO], cell counts, and ELISAs) were also compared for statistical significance using the Wilcoxon signed-rank test. Spirometry data were normally distributed; therefore, a paired t test was used for statistical analysis. Data were analyzed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, www.graphpad.com). Statistical differences were measured for post-diesel or post-B75 compared with baseline values and also post-diesel compared with post-B75 values. For all analyses, statistical significance was taken as a two-tailed P value of P < 0.05. Data were expressed as median (interquartile range) or mean ± standard deviation, as appropriate.

**RESULTS**

**Study Participants**

Characteristics of the 48 study participants, predominantly UA students, are summarized in Table 1. The average age was 25.7 years, approximately one third of the subjects were female, and three quarters were non-Hispanic white.

**Diesel and B75 Emission Composition**

Respirable diesel particulate matter (rDPM) sampling performed during mucking demonstrated a median of 336.40 μg/m³ for diesel exposures and 267.80 μg/m³ for B75 exposures, a 20% reduction (Table 2). There were no statistical differences between the fuel types when comparing acetaldehyde, formaldehyde, or NO levels. Nitrogen dioxide (NO₂) and CO were significantly reduced after both exposures. Among all subjects, FEV₁ was significantly reduced after both diesel and B75 exposures compared with baseline (Table 3). Post-diesel FEV₁ was also significantly lower than post-B75 FEV₁. FVC was only significantly reduced after diesel exposure. In addition, post-diesel FVC was significantly reduced compared with post-B75. FEV₁/FVC was significantly reduced after both diesel and B75 exposures. Both %COHb and FENO significantly increased in subjects after both exposures (Table 4). There were no significant differences in the median levels of post-diesel %COHb or FENO compared with post-B75.

**Lung Inflammatory Cell Infiltration**

Sputum total white blood cell, neutrophil, and macrophage counts increased after emission exposures to both fuels, and lymphocytes increased after diesel, but not B75 exposures (Table 5). When comparing post-diesel with post-B75 cell counts, there were no significant differences in any cell type.

**Sputum and Plasma Inflammatory Mediators**

Sputum and plasma fluid-phase mediators selected a priori (IL-6, IL-8, MMP-9, MPO, and ET-1) increased from baseline after both exposures (Table 6). P-selectin decreased in the plasma after both exposures. There were no significant differences found in these levels when comparing post-diesel with post-B75.

| TABLE 1. Study Participants |
|-----------------------------|
| **Characteristic**          |
| Participants (n)            | 48 |
| Mean age (yrs) (min-max)    | 25.7 (19–53) |
| Sex                         |     |
| Male                        | 31 (64.6%) |
| Female                      | 17 (35.4%) |
| Race                        |     |
| White, non-Hispanic         | 36 (75.0%) |
| Hispanic                    | 5 (10.4%) |
| African                     | 4 (8.3%) |
| Asian                       | 2 (4.2%) |
| Other                       | 1 (2.1%) |
| Mean height (cm), SD        | 174.5 (9.2) |
| Mean weight (kg), SD        | 76.9 (17.2) |
| Mean body mass index (kg/m²), SD | 25.2 (5.2) |

SD, standard deviation.

**TABLE 2. Analyte TWAs Exposure Concentrations**

| Analyte                              | Diesel       | B75          | P      |
|--------------------------------------|--------------|--------------|--------|
| rDPM (μg/m³)b                        | 336.40 (226.13–432.00) | 267.80 (159.10–378.30) | 0.0780 |
| Acetaldehyde (ppm)c                  | 0.04 (0.03–0.04) | 0.03 (0.02–0.04) | 0.1922 |
| Formaldehyde (ppm)c                  | 0.10 (0.07–0.12) | 0.10 (0.08–0.12) | 0.7648 |
| NO (ppm)b                            | 10.27 (7.45–12.03) | 12.54 (6.32–15.15) | 0.1647 |
| NO₂ (ppm)b                           | 1.58 (1.16–1.89) | 1.19 (0.91–1.54)* | 0.0423 |
| CO (ppm)b                            | 13.39 (7.45–19.73) | 8.87 (6.88–15.55)* | 0.0182 |

*P < 0.05 for comparison by the Wilcoxon signed-rank test.

b Data are presented as median (interquartile range). The differences in n resulted from equipment malfunction.

b n = 47.

b n = 40.

b n = 44.

CO, carbon monoxide; NO, nitric oxide; NO₂, nitrogen dioxide; rDPM, respirable diesel particulate matter; TWAs, time-weighted over an 8-hour exposure period.
TABLE 3. Changes in Lung Function (n = 48)a

| Parameter      | Baseline       | Post-Diesel | Post-B75       | p  |
|----------------|----------------|-------------|----------------|----|
| FEV1 (L)       | 4.05 ± 0.65    | 3.85 ± 0.64*** | 3.96 ± 0.66*** | 0.0051 |
| % Predicted FEV1 | 98.46 ± 11.02  | 93.65 ± 10.59*** | 96.10 ± 11.14*** | 0.0047 |
| FVC (L)        | 4.86 ± 0.83    | 4.74 ± 0.81**  | 4.83 ± 0.83     | 0.0263 |
| % Predicted FVC | 99.17 ± 11.42  | 96.67 ± 9.76**  | 98.48 ± 10.88   | 0.0148 |
| FEV1/FVC (%)   | 83.77 ± 5.60   | 81.60 ± 6.16*** | 82.32 ± 6.10**  | 0.2006 |

**P < 0.01; ***P < 0.001 compared with baseline using the paired t test.
aData are presented as mean ± SD.
 bThe paired t test comparing post-diesel with post-B75.
 FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

TABLE 4. Carboxyhemoglobin (n = 33) and Fraction of Exhaled Nitric Oxide (n = 45)a

| Parameter      | Diesel         | Baseline       | Post-Diesel | Post-B75 | p  |
|----------------|----------------|----------------|-------------|----------|----|
| % COHb         | 0.5 (0.3–0.5)  | 0.6 (0.6–1.0)*** | 0.3 (0.3–0.6) | 0.6 (0.5–1.0)*** | 0.1421 |
| FENO (ppb)     | 19.0 (13.0–29.0)| 23.0 (18.0–33.0)*** | 18.0 (13.0–31.0) | 22.0 (17.0–32.0)* | 0.4659 |

*P < 0.05; **P < 0.01; ***P < 0.001 for comparison with baseline by the Wilcoxon signed-rank test.
aData are presented as median (interquartile range). The differences in n resulted from equipment malfunction.
bComparing post-diesel with post-B75 using the Wilcoxon signed-rank test.
COHb, carboxyhemoglobin; FENO, fraction of exhaled nitric oxide.

TABLE 5. Sputum Total and Differential Cell Counts (n = 48)a

| Cell (10⁶/mL) | Baseline       | Post-Diesel | Post-B75       | p  |
|---------------|----------------|-------------|----------------|----|
| Total WBC     | 1.06 (0.50–1.78)| 2.59 (0.97–4.91)*** | 2.59 (1.46–4.53)*** | 0.6729 |
| Neutrophil    | 0.40 (0.15–0.65)| 1.31 (0.45–2.63)*** | 1.32 (0.72–2.30)*** | 0.6731 |
| Macrophages   | 0.58 (0.26–1.16)| 1.12 (0.47–2.16)*** | 1.15 (0.57–2.11)*** | 0.6880 |
| Lymphocytes   | 0.01 (0.00–0.02)| 0.02 (0.00–0.04)*  | 0.01 (0.00–0.03)     | 0.1993 |

*P < 0.05; ***P < 0.001 compared with baseline by the Wilcoxon signed-rank test.
aData are presented as median (interquartile range).
bComparing post-diesel with post-B75 using the Wilcoxon signed-rank test.
WBC, white blood cells.

TABLE 6. Sputum and Plasma Inflammatory Mediators (n = 48)a

| Analyte         | Baseline       | Post-Diesel | Post-B75       | p  |
|-----------------|----------------|-------------|----------------|----|
| Sputum          |                |             |                |    |
| IL-6 (pg/μg)    | 0.1 (0.0–0.1)  | 0.3 (0.1–0.7)*** | 0.3 (0.1–0.8)*** | 0.8350 |
| IL-8 (pg/μg)    | 3.7 (2.9–6.3)  | 5.8 (3.4–9.7)*** | 7.5 (4.3–10.5)*** | 0.0840 |
| MMP-9 (ng/μg)   | 0.7 (0.4–1.2)  | 1.7 (0.9–3.4)*** | 1.8 (1.2–2.9)*** | 0.9878 |
| MPO (ng/μg)     | 0.8 (0.5–1.5)  | 1.7 (0.6–3.4)*** | 1.6 (0.9–2.5)*** | 0.6219 |
| MMP-8c (ng/μg)  | 0.5 (0.3–0.8)  | 1.2 (0.6–2.2)*** | 1.1 (0.8–2.1)*** | 0.7030 |
| GRO-αc (pg/μg)  | 18.3 (9.4–45.8)| 19.7 (9.2–48.3) | 23.8 (13.4–76.3)** | 0.0859 |
| Plasma          |                |             |                |    |
| ET-1 (pg/mL)    | 1.5 (1.3–1.8)  | 1.6 (1.3–2.2)** | 1.6 (1.4–1.9)  | 0.6350 |
| P-selectin (ng/mL) | 39.5 (32.4–49.7)| 33.6 (29.2–40.4)*** | 32.4 (25.7–40.5)*** | 0.4301 |
| TN-Cc (ng/mL)   | 64.3 (45.0–82.7)| 67.6 (46.6–94.6)*** | 71.8 (50.4–88.3) | 0.3356 |

*P < 0.05; **P < 0.01; ***P < 0.001 for comparison with baseline using the Wilcoxon signed-rank test.
aData are presented as median (interquartile range). The sputum protein levels were normalized to total protein.
bComparing post-diesel with post-B75 using the Wilcoxon signed-rank test.
cProtein was identified as a candidate biomarker using proteomic strategies.
IL-6, interleukin-6; IL-8, interleukin-8; MMP-8, matrix metalloproteinase-8; MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; GRO-α, growth-regulated alpha protein; ET-1, endothelin-1; TN-C, tenascin-C.
Sputum levels of each analyte, not adjusted for total protein, are included in Supplemental Digital Content Table S1 (available at http://links.lww.com/JOM/A200).

Novel Biomarker Discovery

After proteomic analysis, a total of 848 sputum and 407 plasma proteins were identified. Using label-free spectral counting for relative quantitation, 42 and 32 candidate protein markers in the sputum and plasma, respectively, were identified that met the previously defined criteria (see Supplemental Material Methods [available at http://links.lww.com/JOM/A199] and Supplemental Digital Content Table S2 [available at http://links.lww.com/JOM/A201] and Digital Content Table S3 [available at http://links.lww.com/JOM/A202]). Two sputum (MMP-8 and GRO-α) and one plasma (TN-C) candidate markers were further validated in all samples using ELISA (Table 6). Sputum MMP-8 significantly increased after both exposures. Sputum GRO-α was only significantly elevated in post-B75 exposures, although there were no significant differences when comparing post-diesel to post-B75 levels. Plasma TN-C was significantly increased after diesel exposure, and, albeit not quite significantly, in the post-B75 exposures. There were no significant differences in post-diesel and post-B75 TN-C levels.

Urinary 8-OHdG

8-hydroxydeoxyguanosine levels normalized to creatinine (ng 8-OHdG/mg creatinine), or SG did not significantly increase or decrease from baseline after exposures to diesel or B75. There were no significant changes in 8-OHdG when comparing post-diesel and post-B75 levels (Table 7).

Additional Analyses

A subanalysis limited to subjects without any reported medical conditions or medication use (n = 42) had similar outcomes to the analyses using all subjects, with the following exceptions. Sputum IL-8 increased significantly higher post-B75 compared with post-diesel exposures (P = 0.041, median values 6.7 and 5.5 pg/μg, respectively). Plasma ET-1 lost statistical significance comparing baseline and post-B75 (P = 0.093, median values 1.4 and 1.6 pg/ml, respectively) but remained significant when comparing baseline with post-diesel (P = 0.028, median values 1.4 and 1.5 pg/ml, respectively). When normalizing urinary 8-OHdG to SG, the level decreased significantly after diesel exposures compared with baseline levels (P = 0.045, median values 9.0 and 11.1 ng, respectively).

Discussion

In this study, switching to B75 from diesel fuel led to a 20% reduction in rDPM exposure. This was consistent with previous reports demonstrating a reduction in DPM using biodiesel blends, although at least one study reported no difference in DPM.6,8,17 The mixed results can likely be attributed to the fuel blend, engine operating conditions, and pollution control devices.7,19 This study did not find significant increases in formaldehyde when switching from diesel to a biodiesel blend, which is inconsistent with a previous report; however, CO levels were reduced significantly, which has been reported.20 In addition, there were no statistical differences in acetaldehyde or oxides of nitrogen (sum of NO and NO2) in this study when switching to a biodiesel blend from diesel, consistent with what has been previously reported.17,20

The respirable particulate concentrations reported in this study are at the high end of reported ambient exposures, including those found most commonly in occupational settings.13,21 Nevertheless, the particulate concentrations are in the maximum range of those reported for highly polluted cities, such as Beijing.21,22 At these elevated exposure concentrations, significant reductions in lung function as well as increases in both respiratory and systemic inflammation were observed. The marked inflammation is a likely pathway for lung cancer, other adverse respiratory effects, and cardiovascular disease; all of which are known sequelae of chronic DPM exposure.1,4,23 The increased concentration seen postexposure for most of the inflammatory biomarkers did not differ significantly between the two fuels, despite the 20% reduction in rDPM observed with B75 use. These findings bring into question the assumption that reductions in DPM concentrations from the use of alternative fuels will necessarily lead to decreased chronic toxicity.

Lung function was one of the health endpoints for which a significant difference was found comparing post-diesel and post-B75 values. Although acute changes in spirometry are not typically reported in studies where subjects are exposed to diluted diesel exhaust using controlled exposure chambers,12,17 at least one previous study demonstrated exposure to diesel exhaust decreased peak expiratory flow.28 In general, previous studies had a lower number of subjects, different exposure durations and environments, and lower particulate concentrations; all of which are plausible reasons for the observed variability.

Exhaled CO (expressed as %COHb) increased to a small extent with exposure to emissions from both fuel types. Percentage COHb data were missing for 15 subjects due to equipment malfunction. Comparing subjects with and without full %COHb measurements, sex, but not age or ethnicity, varied significantly between the two groups. It is possible that the missing values in the study could have had a small effect on the %COHb levels that were reported, but given the relatively minor increase with both fuel types, the effect of the missing data is not likely to be biologically important.

In addition, this study revealed that FENO, a marker of airway inflammation, increased significantly and to the same levels after emission exposures to both fuel types, comparable with previous findings of FENO increasing after acute diesel exhaust exposures.24 FENO has also been shown to increase in asthmatic children from urban air pollution with positive correlations between the concentration of pollutants and the level of airway inflammation.29

Diesel exhaust has been shown to increase inflammatory cell recruitment into the airways.6,8,25 These increases were typically measured 6 to 24 hours after initial diesel exposure, consistent with the approximately 8.5 hours after initial exposure to each fuel type in this study. We observed similar increases in cell counts for neutrophils and macrophages after exposures to diesel and B75, although lymphocytes increased significantly after diesel, but not B75, exposures.

| Table 7. Urine 8-Hydroxydeoxyguanosine (n = 47) |
|-----------------------------------------------|
| **Analyte** | **Diesel Baseline** | **Post-Diesel** | **B75 Baseline** | **Post-B75** |
|-----------|-------------------|---------------|-----------------|-------------|
| 8-OHdG/creatinine | 6.4 (4.8–8.3) | 7.1 (5.0–8.4) | 7.0 (6.1–8.2) | 6.5 (5.1–8.9) |
| 8-OHdG/SG | 9.5 (5.0–16.7) | 8.7 (3.6–13.4) | 12.2 (8.4–18.4) | 9.9 (3.5–17.3) |

*Data are presented as median (interquartile range). The 8-OHdG levels were normalized to creatinine levels (ng/mg) and specific gravity (SG). None of the baseline to postexposure comparisons are significant (P < 0.05) using the Wilcoxon signed-rank test.

*Comparing post-diesel with post-B75 using Wilcoxon signed-rank test.

8-OHdG, 8-hydroxydeoxyguanosine; SG, specific gravity.
Interleukin-6 and IL-8 are cytokines released by several cell types during an inflammatory response. The mRNA and protein expression of these two cytokines has been studied in vitro and in vivo to measure the level of inflammation from exposures to diesel particulates and air pollution. In addition, the increased airway release of IL-6 and IL-8 has been seen in human studies analyzing acute diesel exhaust exposures. Accordingly, we observed a significant increased release of both IL-6 and IL-8 in the sputum after diesel and B75 exposures. The extent of IL-6 release was similar for both fuel types, and IL-8 was slightly (but not significantly) higher for B75 exposures.

Matrix metalloproteinase-8 (secreted exclusively by neutrophils) and MMP-9 (secreted by many cell types) are involved in the remodeling of the extracellular matrix under normal physiological processes and also inflammation and metastasis. Matrix metalloproteinase-8 has been used as a biomarker of inflammation and cardiovascular disease. Nevertheless, the acute elevation of MMP-8 observed in response to diesel and B75 emission exposures is a novel finding. The increased levels measured in this study are likely related to the neutrophil infiltration observed in the lung. Induced sputum MMP-9 levels have been shown to be correlated with lung function and airway inflammation, displaying an inverse relationship with FEV₁ and a significant correlation with total white cells, neutrophils, and IL-8. This suggests that MMP-9 may be a promising marker of airway effects for DPM exposure.

Myeloperoxidase is an enzyme that has potent pro-oxidative and proinflammatory properties and is typically released from activated neutrophils; accordingly, its levels have been used as a marker of inflammation and cardiovascular disease. In a recent study analyzing mouse lung and liver toxicity after equivalent doses of diesel or biodiesel emissions, MPO levels displayed a greater dose-related increase after biodiesel, compared with diesel, emission exposures. In this study, MPO levels in the sputum increased to similar levels after diesel and B75 exposures, despite the lower rDPM exposure with B75 use.

Growth-regulated alpha protein-α preferentially chemotacts and activates neutrophils. This protein is inducible by tumor necrosis factor-α and interleukin-1. Previous human studies have shown a nonsignificant increase of GRO-α after acute diesel exhaust exposures. In this study, GRO-α was identified in the sputum via proteomic strategies as a candidate biomarker being elevated after B75 exposures. When ELISA analysis was performed on all sputum samples, GRO-α was significantly elevated after only B75 emission exposures, also a novel finding.

Endothelin-1 is an endothelin-derived vasoconstrictor peptide that is an inflammatory mediator. Increased levels of ET-1 have been reported in cardiovascular and inflammatory lung diseases. A previous human study showed increases in plasma ET-1 levels after acute exposures to diesel exhaust, suggesting an early endothelial response and vascular constriction. This study demonstrated similar significant increases in plasma ET-1 levels after acute exhaust exposure. Suggesting that the level of vascular dysfunction is similar from exposures to both fuel types.

P-selectin is responsible for mediating the rolling of leukocytes over vascular surfaces during early stages of inflammation. In chamber studies of acute human exposure to filtered diesel exhaust (300 µg/m³ for 1 hour), plasma P-selectin levels were not significantly altered at 2 or 6 hours postexposure but increased significantly at 24 hours postexposure. These results are inconsistent with this study findings, where P-selectin decreased 5.5 hours after initial exposure to both fuel types. Different exposure environments, engine, and engine operating conditions may be plausible explanations for the inconsistencies between the studies. It has also been shown that exercise training can reduce plasma inflammatory mediators such as P-selectin. Although participation in this study required moderate physical exertion during mucking, it is unclear if this level of exertion caused the decrease in plasma P-selectin.

Tenascin-C is an extracellular matrix protein important not only in tissue injury and repair but also in disease states such as chronic inflammation and tumorigenesis. In addition, TN-C is capable of inducing proinflammatory cytokines. High circulating concentrations of plasma TN-C have been reported in association with mortality and cardiovascular disease in chronic kidney patients. In this study, TN-C was identified in the plasma via proteomic strategies as a novel biomarker candidate that increased after exposures to diesel and B75 emissions. These increased TN-C plasma levels may be due to lung and/or endothelial damage.

This study is the first human study to use proteomic strategies to reveal novel biomarkers of emission exposures to both diesel and B75. The candidate biomarkers in the sputum and plasma (MMP-8, GRO-α, and TN-C) were validated by ELISA. The most comparable previous study was performed on rat bronchoalveolar lavage fluid after different exposure concentrations and durations to diesel exhaust particles; this study revealed a total of 65 proteins using liquid chromatography–mass spectrometry analysis on whole and weak cation exchange extracted bronchoalveolar lavage fluid, with two distinct proteins (anaphytoxin C3a and calgranulin A) appearing post-exposure at all diesel exhaust particles doses.

The accumulation of rDPM in the lung leads to a large inflammatory response, as seen in this study and many others. It is well established that inflammatory events lead to an increase in reactive oxygen species that can damage proteins and DNA. A classic biomarker of oxidative stress to DNA is urinary 8-OHdG. Myeloperoxidase is an oxidized DNA nucleoside that is eliminated in the urine after excision by DNA repair enzymes. Increased levels of 8-OHdG have been measured after a minimum of 3 month’s occupational exposures to ambient PM₂.₅ in Taiwanese traffic conductors. In this study, urine 8-OHdG did not increase significantly 8 to 10 hours after initial diesel or B75 exhaust exposure.

This study has several important limitations. It measured health effects reflecting the use of diesel and an alternative (B75) fuel while operating the same vehicle, producing different rDPM concentrations, and therefore did not compare the toxicity of the same concentration of rDPM from each fuel type. Multiple health parameters of plasma were assessed, but each at a single time point after a single 200-minute exposure. More prolonged exposure periods, or the measurements of health parameters at other time points, would potentially produce different results. In addition, only one blend of biodiesel (B75) and type (soy methyl ester) was evaluated in this study, whereas there are various biodiesel blends in use. Also, the use of different pollution control devices and engine operating conditions will affect the overall toxicity of the exhaust produced, so it is important not to generalize the study findings beyond the use of diesel engines with only a diesel oxidation catalyst. A cross-over study design was used, rather than randomizing by fuel type for each day of testing, which may have introduced bias. Finally, this study concentrated on markedly elevated exposure concentrations, and the effects of lower concentrations would be expected to result in less marked health effects, which could potentially vary to a greater or lesser degree by fuel type.

CONCLUSIONS

In this study, we evaluated multiple health parameters after acute exposure to diesel and B75 emissions. Although lung function was affected to a greater degree by diesel emissions, many biomarkers of effect including measures of inflammation and oxidative stress were similar when comparing the two fuel types, despite the 20% reduction in rDPM achieved by the use of B75. Additional studies are needed to evaluate the potential differential health effects from emissions of these fuels at both lower concentrations and for more chronic
exposure periods, as well as for a larger selection of biodiesel fuel sources, blend concentrations, and pollution control devices. This study highlights the need to further evaluate the health effects associated with alternative fuels, and not assume that reduction in rDPM alone will lead to reduced health effects.

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