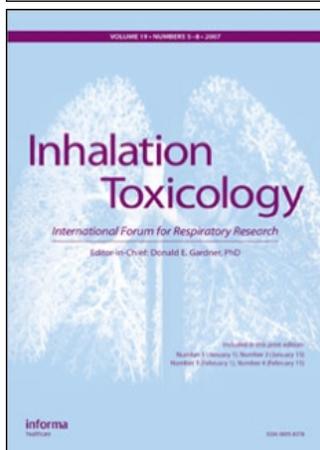


This article was downloaded by:[Rundell, Kenneth W.]
On: 31 January 2008
Access Details: [subscription number 790163969]
Publisher: Informa Healthcare
Informa Ltd Registered in England and Wales Registered Number: 1072954
Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Inhalation Toxicology

International Forum for Respiratory Research

Publication details, including instructions for authors and subscription information:
<http://www.informaworld.com/smpp/title~content=t713657711>

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Online Publication Date: 01 January 2008

To cite this Article: Rundell, Kenneth W., Slee, Josh B., Caviston, Renee and Hollenbach, Amanda M. (2008) 'Decreased Lung Function After Inhalation of

Ultrafine and Fine Particulate Matter During Exercise is Related to Decreased Total Nitrate in Exhaled Breath Condensate', *Inhalation Toxicology*, 20:1, 1 - 9

To link to this article: DOI: 10.1080/08958370701758593

URL: <http://dx.doi.org/10.1080/08958370701758593>

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Decreased Lung Function After Inhalation of Ultrafine and Fine Particulate Matter During Exercise is Related to Decreased Total Nitrate in Exhaled Breath Condensate

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This study was designed to investigate PM₁ inhalation during exercise on lung function, exhaled nitric oxide (eNO), and total nitrate (NO₃), S-nitrosoglutathione (GSNO), and malondialdehyde (MDA) in exhaled breath condensate (EBC). Inhalation of combustion-derived PM is associated with adverse respiratory health. A mechanistic action of PM on lung function is not defined; however, nitrosative/oxidative stress is likely. Prior to and after two 30-min exercise bouts 4–5 days apart, inhaling low (7382 ± 1727 particles cm⁻³) or high (252,290 ± 77,529 particles cm⁻³) PM₁, 12 nonasthmatic males performed spirometry and eNO and EBC collection. Normal resting lung function did not change after low PM₁ exercise. After high PM₁ exercise, FEV₁ and FEF₂₅₋₇₅ fell significantly ($p = .0005$, $p = .002$) and was related to [PM₁] ($r = -.55$, $p = .005$ and $r = -.61$, $p = .002$; respectively); 11- and 52-ml decreases were calculated for each 20,000 particles cm⁻³ increase for FEV₁ and FEF₂₅₋₇₅. NO₃ did not change after low PM₁ exercise (30.5% increase), but significantly decreased by 43.8% after high PM₁ exercise, and correlated with lung function changes ($r = .63$, and $r = .54$ for FEV₁ and FEF₂₅₋₇₅, respectively; $p = .001$ and $p = .007$). No change in GSNO was observed. Alveolar NO decreased after high PM₁ conditions ($p = .02$); eNO pre-to-post difference was related to changes in FEV₁ ($r = .60$, $p = .002$). MDA increased 40% after low PM exercise (NS) and increased 208% after high PM exercise ($p = .06$). Thus, high PM₁ inhalation during exercise caused a reduced alveolar contribution to eNO; NO₃ and eNO variables were decreased and were related to impaired lung function. Decreased NO₃ and eNO may be due to superoxide/NO formation of peroxynitrite, resulting in lipid peroxidation.

The link between air pollution and adverse respiratory health is substantial (Gauderman et al., 2004; Kim et al., 2004; Lin et al., 2005; McConnel et al., 2003; Pope, 1991; Romieu et al., 2002; Rundell, 2004; Rundell et al., 2004, 2005). Acute pulmonary responses to short-term particulate matter (PM) exposure (Atkinson et al., 2001; Oberdorster et al., 1995) and decreased resting lung function associated with chronic PM exposure have been reported (Gauderman et al., 2004; Mannix et al. 1996; Provost-Craig et al., 1996; Rundell, 2004; Rundell et al., 2004; Wilber et al., 2000).

Several authors (Chalupa et al., 2004; Daigle et al., 2003; Ferin et al., 1992; Frampton et al., 2004) suggest that peripheral airways are predisposed to high deposition of ultrafine and fine particles that are emitted in high concentrations from internal combustion engines and that fine particles are more closely associated than coarse particles with acute respiratory health effects in children (Schwartz & Neas, 2000). Chalupa et al. (2004) identified an increased deposition fraction of ultrafine PM during exercise, with the largest deposition fraction noted for smaller particles. In addition, the deposition fraction was greater for subjects with asthma than for healthy subjects (Chalupa et al., 2003). Fractional deposition of PM₁ during breathing is high, increasing 4.5-fold during mild (38 L/min) exercise with high deposition in airway generations 18 and above (Daigle et al., 2003). Elevated exercising V_E (often above 100 ml min⁻¹ during moderate intensity exercise) would be favorable for PM₁ deposition (Spiering et al., 2003).

Marginal associations between increased exhaled nitric oxide (eNO) and particle exposure have been shown (Koenig et al., 2005). Koenig et al. (2005) found an association between

Received 23 February 2007; accepted 19 September 2007.

This study was funded by the Murray Award for Research and Development. The authors thank Dan Warren, laboratory assistant, and Tina Evans, laboratory manager/technician, for their technical assistance.

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increased exhaled nitric oxide (eNO) and $PM_{2.5}$ exposure in asthmatic children, and Bai et al. (2001) showed in vitro upregulation of pulmonary artery endothelial cell nitric oxide synthase (eNOS) in response to diesel exhaust particles (DEP). In contrast, Yamawaki and Iwai (2006) showed decreased eNOS activity in vascular endothelial cells after diesel carbon black particle exposure, and Sun et al. (2006) demonstrated eNOS inhibition by redox-active quinones found in DEP in cultured endothelial cells. NO is an unstable free radical that is proposed to be important in vasodilation and is a marker of airway inflammation. NO readily reacts with oxygen to form oxides (nitrite and nitrate) and with superoxide to form the toxic oxidant peroxynitrite. Likewise, NO reacts with glutathione to form a potent airway bronchodilator, *S*-nitrosoglutathione (GSNO) (Henderson et al., 2005; Que et al., 2005). It has recently been proposed that GSNO is protective to the airway hyperresponsiveness characteristic of asthma (Que et al., 2005). Further, GSNO has been proposed to be a useful index of NO production (Liu et al., 2004).

Exercise has been shown to increase levels of eNOS mRNA and eNOS protein in rats; likewise, nitrites and nitrates (NO_x) in rat lung tissue have been shown to be elevated after exercise (Miyauchi et al., 2003). NO_x is a stable end product of NO and as such has been used as an estimate of NO production. The primary source of NO_x is NO₂, since NO₂ is easily converted to the more stable NO₃.

We evaluated effects of exercise in high ambient PM conditions on airways of healthy nonasthmatic males. We hypothesized that acute exercise in high PM would decrease lung function, lung eNO, NO₃, and GSNO and increase the lipid peroxidation marker malondialdehyde.

METHODS

Twelve physically fit, nonasthmatic, nonsmoking males (age, 20.5 ± 2.42 yr; weight, 78.4 ± 5.00 kg; height, 177.8 ± 4.25 cm; body mass index [BMI], 24.9 ± 2.10 ; mean \pm SD) served as subjects. All subjects signed a written informed consent. This study was approved by the Marywood University Institutional Review Board. Subjects were instructed to abstain from caffeine ingestion for at least 24 h prior to testing and were required to report to the Human Performance Laboratory for testing at midmorning, 3 h after a light low-fat breakfast.

Exercise Challenge

Subjects performed 2 random-order exercise bouts 4 to 5 days apart while breathing either low ambient particulate matter (PM_1) air, or high ambient PM_1 air. Exercise trials involved 30 min of running at between 85 and 90% of maximal heart rate; exercise intensity was verified using portable heart-rate monitors (Polar Vantage XL, Polar Electro, Finland). Exercise was performed in ambient conditions either on an inner campus loop free of auto/truck traffic with low ambient particulate matter (aerodynamic diameter 0.02–1 μ m, [PM_1]) or on a soccer field

and trail within 20–50 m of a major highway with high ambient [PM_1] from auto and truck emissions.

Air Pollutant Determination

Particulate matter (PM_1) was determined at each study site as previously done (Rundell, 2003; Rundell et al., 2006). Measurements were made at 1.5 m height using a calibrated condensation particle counter (CPC, P-Trak ultrafine particle counter, model 8525, TSI, Inc., St. Paul, MN) at a sampling frequency of 1 Hz and recorded as 10-s means of PM_1 per cubic centimeter. Multiple readings were taken for each measurement and averaged to provide the most representative particle count for a specific measurement site. The P-Trak CPC sensitivity size range is 0.02–1.0 μ m diameter; this range includes ultrafine and fine PM, defined as PM_1 in this study. PM_1 has been shown to account for >90% of total particle count and >95% of particle surface area (μ m²/cm³) for unit density mass concentration of combustion derived air samples (Oberdorster et al., 1992, 1995). The low [PM_1] exercise site had measurements of 7382 ± 1727 particles cm⁻³ and the high [PM_1] site had measurements of $252,290 \pm 77,529$ particles cm⁻³. We have previously measured PM_1 at this site over 62 days with daily mean concentrations of 115,000 to 134,000 particles cm⁻³ (Rundell et al., 2006). During all study trials, ambient CO was below 1.0 ppm, and ambient NO₂ was below 100 ppb; ambient O₃ was 0.041 ± 0.020 ppm (GrayWolf Direct Sense TOX, Trumbull CT) at both low [PM_1] and high [PM_1] sites.

Pulmonary Function Test Procedure

Pulmonary function was measured by spirometry using a calibrated computerized pneumotachograph spirometer (Jaeger Masterscope PC, Hoechberg, Germany; LAB Manager Software version 4.53.2, 2002). Standard measures of pulmonary function were collected, including forced vital capacity (FVC), forced expiratory volume in the first second of exhalation (FEV₁), and forced expiratory flow at 25% to 50% of vital capacity (FEF₂₅₋₇₅). FEV₁/FVC ratio was calculated. The procedure for all pulmonary function tests (PFTs) included (1) three “normal” tidal volume breaths, followed by (2) inhalation to total lung capacity and (3) forced maximal exhalation lasting at least 6 s, terminating with (4) a final maximal inhalation. Resting baseline pulmonary function was established prior to each challenge by selecting the best of three resting PFTs based on the highest sum of FVC and FEV₁. Postchallenge pulmonary function was measured at 5, 10, 15, and 20 min after each challenge. If any baseline or postchallenge time point measurement was technically unacceptable, the PFT maneuver was repeated immediately. The postchallenge time point with the lowest fall from baseline FEV₁ was used for analysis.

Exhaled Breath Condensate Collection

Exhaled breath condensate (EBC) was collected prior to and 30 min post challenge using a commercially available collection

device (Jaeger ECoScreen, Hoechberg, Germany). The noninvasive procedure involved an initial mouth rinsing followed by tidal breathing through a mouthpiece (containing a saliva trap) while wearing a nose clip for 15 min until 1–2 ml of condensate was obtained. The expired air was condensed and collected at -10°C and immediately stored at -80°C .

S-Nitrosoglutathione Determination in EBC

GSNO was analyzed spectrophotometrically using a commercially available kit (OXON BioAnalysis nitroso-thiol kit OX9). This kit utilized the Griess and Saville reactions, which cleave the S–N bond of nitroso-thiols. This results in the release of an equivalent of NO, which in turn gives an equivalent of nitrite. The nitrite is detected colorimetrically using the Griess reagent. Griess reagents were prepared by mixing 400 μl Griess activator with Griess A and cleavage reagent; 200 μl of sample was then mixed with 50 μl Griess activator, Griess A, and cleavage reagent mixture. This was followed immediately by the addition of 50 μl Griess B reagent. After 10 min, samples were analyzed spectrophotometrically at 540 nm (Shimadzu BioSpec, USA). EBC samples were quantified by calculations against standard curves.

Nitrate Determination in EBC

Analysis was done by conversion of total NO_3^- to NO, which was detected using chemiluminescence (NOA 280i nitric oxide analyzer, NO analysis software version 3.21, Sievers Instruments, Boulder, CO). Briefly, 10 μl EBC was injected in a purge vessel containing vanadium (III) chloride in 1 M hydrochloric acid in a heated water bath (95°C) to reduce NO_3^- to NO. Results were quantified by calculation against standard curves.

Malondialdehyde (MDA) Determination in EBC

Malondialdehyde (MDA) concentration was measured in EBC according to Araneda et al. (2005). Briefly, 500 μl EBC was mixed with 167 μl of 25 mM thiobarbituric acid, incubated for 1 h at 95°C , and cooled on ice for 5 min followed by 30 min at room temperature. Samples were then analyzed using high-performance liquid chromatography (HPLC) fluorescence detection (SC10A, RF-10A_{XL} Shimadzu, USA) with excitation and emission wavelengths at 532 and 553 nm, respectively. The mobile phase was acetonitrile in 20 mM potassium phosphate buffer (pH 6.8), 20:80 (v/v) at a flow rate of 1 ml/min. Detection limit was at the femtomole level.

Exhaled Nitric Oxide Determination

Online visual measurement of eNO utilizing a restricted exhaled breath (REB) protocol (NOA 280i nitric oxide analyzer, Accurate NO Breath Kit, thermal mass flow meter, NO analysis software version 3.21, Sievers Instruments, Boulder, CO) was applied. Prior to and 45 min post challenge, measurement techniques were employed as outlined by the American Thoracic Society (1999). Three exhalations were performed without nose clips, with at least 30 s elapsing between exhalations. The pro-

cedure was (1) maximal inhalation (through the inhalation NO scrubber fitted mouthpiece) to total lung capacity over 2–3 s and, (2) immediate exhalation against increased expiratory resistance for at least 6 s to obtain an NO plateau lasting at least 3 s. During exhalation, subjects were instructed to monitor a visual computer display to maintain a flow rate of 50 ml s^{-1} . Exhaled NO was determined at four additional flow rates to calculate NO flux (nl s^{-1}) and fractional alveolar contribution to eNO (CaNO, ppb). A plot of NO output (nl s^{-1}) versus expiratory flow rate (ml s^{-1}) was used to calculate CaNO and NO flux. The volume of eNO (VeNO) was calculated by multiplying FeNO plateau values by the expiratory flow rates. Linear least squares was used to determine the best fit line through a plot of VeNO versus flow rate. NO flux was indicated as the Y intercept and CaNO was indicated as the slope of the line.

Statistical Analysis

Comparisons between conditions were made by analysis of variance (ANOVA), followed by paired *t*-tests (SPSS). Pearson product moment correlations were used to identify relationships between particle number count and lung function change, and lung function change and NO_3^- , eNO, and lung function change. Significance was considered at $p < .05$ and .02 using Bonferroni correction for EBC mediators.

RESULTS

Resting lung function measured by spirometry for the 12 nonasthmatic subjects is presented in Table 1. Values were normal and above 100% of age and height predicted values. Lung function was maintained after low PM exercise. Although not clinically significant, the decrease in lung function after high PM exercise was highly statistically significant (Figure 1; FEV_1 , $p = .0005$; FEF_{25-75} , $p = .002$). The change in FEV_1 was significantly correlated to the change in FEF_{25-75} ($r = .66$, $p = .001$). Postexercise change in lung function was significantly related to PM_1 concentration (Figure 2; FEV_1 , $y = -1.09x - 0.344$, $R = -0.55$, $p = .005$; FEF_{25-75} , $y = -4.28x + 4.72$, $R = -0.61$, $p = .002$). For every increase of 20,000 particles cm^{-3} , there was a decrease of 11.1 ml in FEV_1 and a decrease of 52 ml in FEF_{25-75} after 30 min of exercise.

Exhaled nitrate (NO_3^-) collected in EBC was not different between pre- and postexercise in low PM conditions. Pre-high PM exercise NO_3^- was not different from pre- or postexercise low PM values. After exercise in high PM conditions, a significant decrease in EBC NO_3^- was observed (Figure 3, $p = .008$).

TABLE 1
Resting lung function

	FEV_1 (L)	FEV_1/FVC (L)	FEF_{25-75} ($\text{L}\cdot\text{s}^{-1}$)
	5.8 ± 0.51	5.1 ± 0.53	87.6 ± 5.42
	$112 \pm 11.2\%$	$117 \pm 13.2\%$	5.7 ± 1.24 $116 \pm 24.6\%$

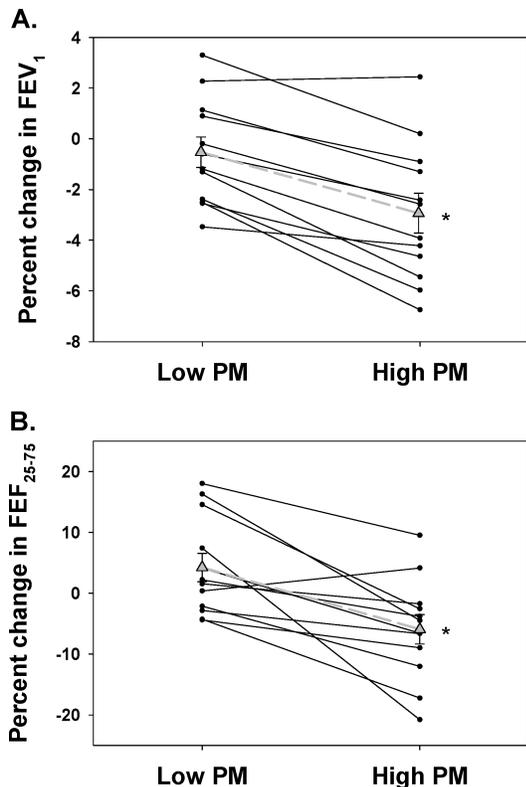


FIG. 1. Significant change from prechallenge values in FEV₁ and FEF₂₅₋₇₅ after 30 min of high PM₁ exercise, but not low PM₁ exercise, was identified ($p = .0005$ for FEV₁ and $p = .002$ for FEF₂₅₋₇₅).

Likewise, post-high PM exercise NO₃ was statistically lower than pre- or post-low PM exercise values ($p = .005$ and $p = .003$, respectively). The percent change in NO₃ between pre- and postexercise for low and high PM conditions was significantly different between the two conditions (30.5% increase for low PM exercise, 43.8% decrease for high PM exercise, $p = .05$; Figure 3A insert).

S-Nitrosoglutathione (μM) in EBC was not significantly different between or within exposure treatments: 4.36 ± 1.05 vs. 4.77 ± 1.11 and 5.36 ± 1.30 vs. 5.06 ± 1.12 (for pre- and postexposure and for low and high [PM], respectively). No significant relationships were identified between GSNO and lung function or [NO₃] or eNO.

Exhaled NO at 50 ml s^{-1} was not different between pre- and post-exercise values or between low or high PM conditions (pre 20.5 ± 17.3 , post 21.2 ± 20.1 , and pre 21.5 ± 12.7 , post 20.9 ± 16.5 , for low and high PM conditions, respectively). A trend ($p = .1$) for decreased eNO as pre-to-post percent change was shown for high PM exercise (Figure 4A). Figure 4B shows percent change in NO flux and alveolar NO concentration (CaNO) for low and high PM exercise. A significant difference was noted between the percent change for low PM and high PM in CaNO ($p = .02$), with no change in conducting airway NO flux.

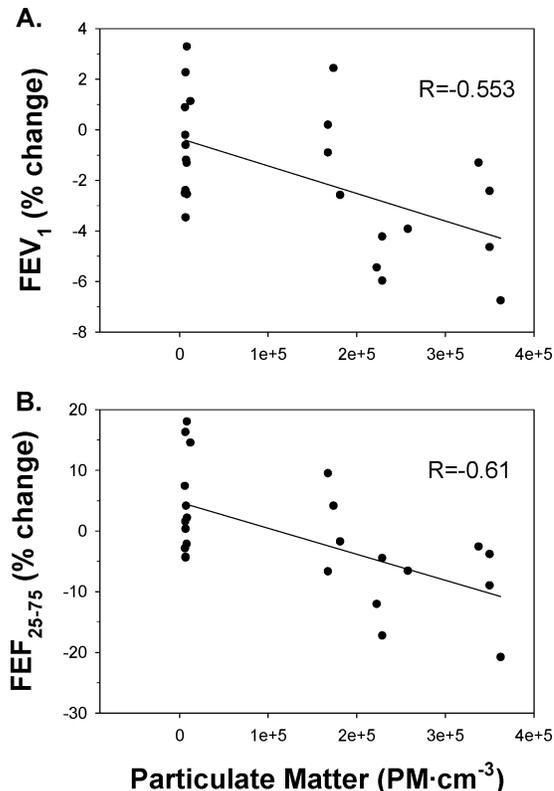


FIG. 2. Post-exercise decrease in lung function was significantly related to PM₁ concentration; for FEV₁, $y = -1.09x - 0.344$, $r = -.55$, $p = .005$; for FEF₂₅₋₇₅, $y = -4.28x + 4.72$, $r = -.61$, $p = .002$.

MDA was determined in EBC from 8 of 12 subjects. A non-significant increase in MDA of 40% was noted for low PM exercise, while MDA increased 208% after high PM exercise (Figure 5, $p = .06$). MDA did not correlate with lung function change, NO₃, or eNO variables.

Figure 6 shows Pearson product moment correlations between lung function and NO₃ (μM) collected in EBC and eNO collected at 50 ml s^{-1} . The changes in FEV₁ were significantly related to the changes in NO₃ ($p = .001$) and eNO ($p = .002$), expressed as either absolute change or percent change, suggesting the decrease in NO and NO metabolites are related to PM-induced bronchoconstriction. The relationship of FEF₂₅₋₇₅ to NO₃ and eNO changes was significant for the absolute difference in NO₃ (μM ; $R = .54$, $p = .007$) and in percent change in eNO ($R = .45$, $p = .03$).

DISCUSSION

The overall objective of this study was to evaluate the acute consequences of breathing high PM air during a 30-min bout of exercise by healthy subjects free of lung disease. Previous studies have documented adverse effects on resting lung function and airway hyperreactivity from chronic PM exposure. Moderate increases in long-term ambient air pollution have

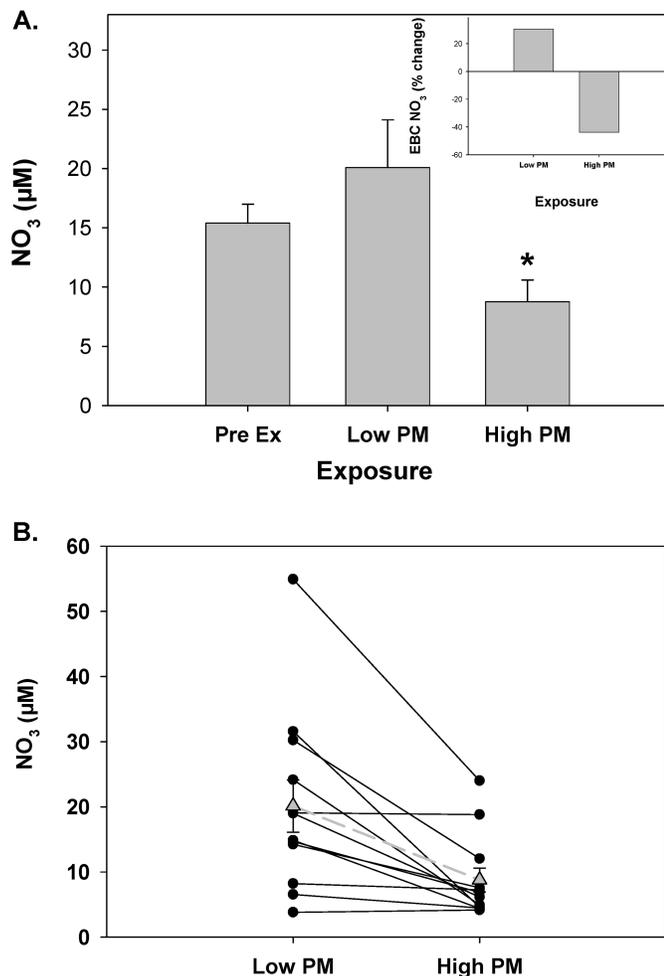


FIG. 3. (A) Total nitrate (NO₃, μM) from EBC was significantly lower after high PM₁ exercise than before or after low PM₁ exercise ($p = .005$, $p = .003$). (B) Individual levels of NO₃ after low PM₁ and high PM₁ exposure exercise.

been associated with increased respiratory and atopic indices in children (Penard-Morand et al., 2005), increases in respiratory symptoms (Kim et al., 2004; Hoppin et al., 2003), deficits in the growth of FEV₁ (Gauderman et al., 2004; Trenga et al., 2006), and increases in levels of inflammatory nasal markers and eNO (Steerenberg et al., 2001).

Acute particle exposure studies using healthy subjects have shown no change in lung function (Frampton et al., 2004; Salvi et al., 1999), small but significant change in maximal mid-expiratory flow rate (Gong et al., 2005; Pietropaoli et al., 2004), and modest but significant increases in airway resistance (Holgate et al., 2003). Gong et al. (2005) showed greater decrements in lung function in healthy normal subjects than in those with chronic obstructive pulmonary disease (COPD); however, others have suggested that asthmatics have enhanced susceptibility to adverse events from PM exposure; PM exposure is associated with increased emergency room visits by asthmatics (Atkinson

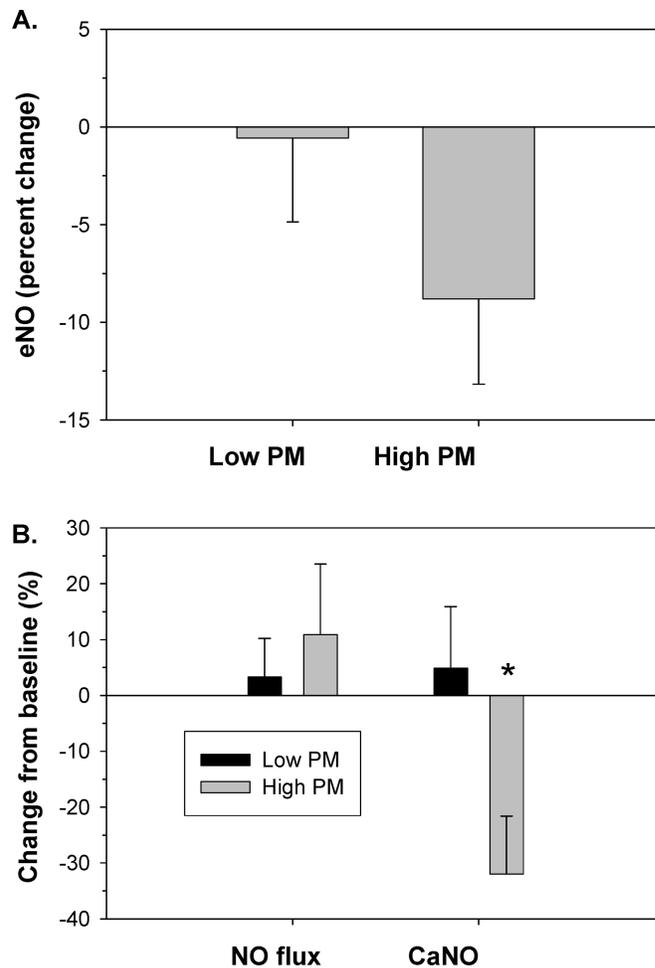


FIG. 4. The ~10% decrease in eNO after high PM₁ exercise was not significantly different from the less than 1% decrease in eNO after low PM₁ exercise. Conducting airway NO flux was not different between exposures, but CaNO decreased 32% after high PM₁ exercise ($p = .02$).

et al., 2001; Tolbert et al., 2000) and increased bronchodilator use (Hiltermann et al., 1998). We (Rundell et al., 2005) have shown that six of nine subjects with mild exercise-induced bronchospasm (EIB) under high PM exercise conditions had normal spirometry when exercising in low PM conditions. The present study confirms significant falls in lung function in healthy non-asthmatic subjects after acute PM exposure exercise. We identified significant decreases in EBC NO₃ and exhaled CaNO with no change in NO flux or GSNO after high PM exercise; no significant changes in NO₃, CaNO, NO flux, or GSNO were noted after low PM exercise. Postexercise change in lung function was significantly related to the changes in EBC NO₃ and eNO. We found evidence of lipid peroxidation under high PM exercise conditions by a >200% increase in EBC MDA. Significant correlations between acute changes in lung function and PM concentrations during exercise were identified.

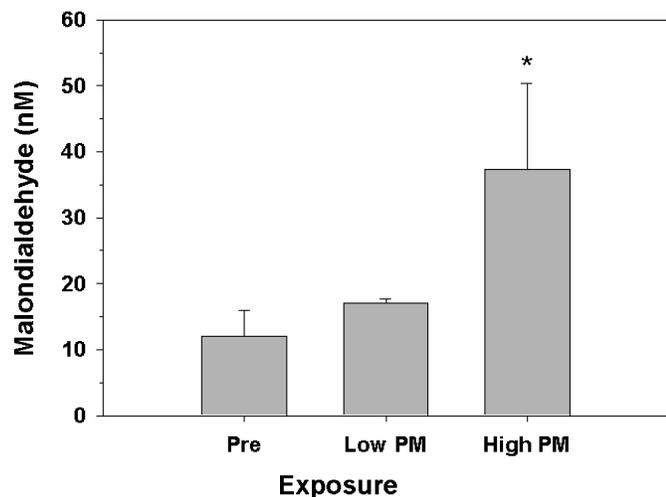


FIG. 5. MDA concentration in EBC doubled after high PM_{10} exercise ($p = .06$), but not after low PM_{10} exercise, suggesting increased lipid peroxidation from high PM_{10} exposure exercise.

Our exposure model was dependent upon ambient weather conditions and highway traffic volume; at the high PM exercise site, prevailing winds and traffic at this site are typically conducive to high $[PM]$ (Rundell et al., 2006). The high PM site

had nearly 30 times the mean PM number count of the low PM exercise site. Although not a pure exposure system, this model may be more relevant to “real-world” conditions of recreational and competitive exercise than the generated carbon particles (Frampton et al., 2004; Pietropaoli et al., 2004) or concentrated ambient particles (Gong et al., 2005) that have been used. Important to our model, levels of ambient CO , NO_2 , and O_3^- during all air quality measurements were below levels that have been shown to affect exercise or lung function. Additionally, the moderate variability in $[PM]$ allowed us to better estimate a relationship between particle number count and the effect on lung function.

The decrease in lung function in these nonasthmatic subjects during high PM exercise was not clinically significant, but was highly statistically significant ($p = .00005$ and $p = .002$; for FEV_1 and FEF_{25-75} , respectively), supporting the notion that PM inhalation during exercise acutely affects lung function. Given the inability to blind our low and high PM challenges, one cannot rule out a psychological impact on lung function tests; however, it seems unlikely that results from EBC or eNO would be affected by knowledge of exposure conditions. The significant correlations between particle number counts and lung function argue against a preconceived notion that the high PM exercise site would affect lung function (cf. Figure 2). From this study,

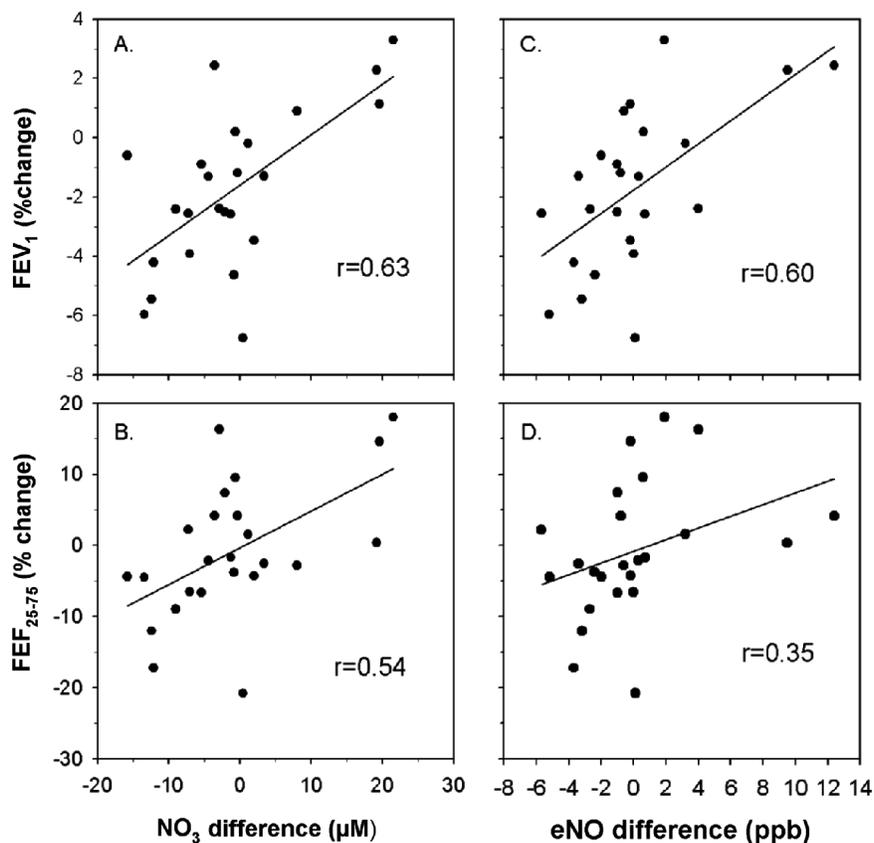


FIG. 6. Change in FEV_1 was significantly related to change in NO_3 ($p = .001$) and eNO ($p = .002$). Change in FEF_{25-75} was significantly related to NO_3 ($p = .007$).

we calculated estimates of lung function decrease of 11 ml and 52 ml (for FEV₁ and FEF₂₅₋₇₅, respectively) per 20,000 particles cm⁻³ increase. This modest decrease in lung function is in agreement with that found by others (Gong et al., 2005; Holgate et al., 2003; Pietropaoli et al. 2004).

There has been a recent interest in measuring compounds in condensed water vapor of exhaled breath. EBC is attractive because of the ease in which samples can be collected; however the methodology is not without pitfalls and limitations (Effros et al., 2006; Marteus et al. 2005)—sample dilution and low mediator levels are primary concerns. Attempts to control for dilution have been made by normalizing to air ventilated during collection, standardized collection times, and EBC cationic concentrations normalized to plasma concentrations, while low mediator concentrations have been addressed by lyophilization and solid-phase extraction procedures (Debley et al., 2007; Effros et al., 2003). In this study, we collected EBC over a specified time. The collection methods we employed could have resulted in type II error because of high variability between subjects; however, we have shown consistent results within subjects using a standardized EBC collection time of 15 min (unpublished data, Slee et al., 2007).

To our knowledge, this is the first study to measure NO₃, GSNO, or MDA in EBC after PM exposure exercise. Although ingested nitrate has been shown to increase plasma, pharyngeal, and respiratory nitrate concentration (Marteus et al. 2005), the subjects in the present study refrained from nitrate ingestion the morning of and prior to both exercise challenges. In the present study, low PM exercise showed a nonsignificant increase in NO₃ while high PM exercise showed a significant decrease in NO₃; the pre-to-post percent changes of 30.5% increase and 43.8% decrease for low and high PM exercise were significantly different and argue against dilution artifact.

No change in eNO after particle inhalation exercise has been reported by Frampton et al. (2004), or slight but significant reductions in CaNO have been shown by Pietropaoli et al. (2004). Although no significant decrease in eNO at 50 ml s⁻¹ flow was found in the present study, we observed an approximate 10% decrease in eNO and a significant 32% decrease in CaNO after high PM exercise. These findings are in agreement with Pietropaoli et al. (2004) and Morino et al. (2001), who note a decrease in NO production in rats exposed to ammonium sulfate particles. Since NO₃ is a stable end product of the short-lived NO, it has been suggested that NO₃ can be used as an estimate of NO production (Miyauchi et al., 2003). Sun et al. (2006) demonstrated nitric oxide synthase inhibition in endothelial cells by diesel particles, and Lei et al. (2005) showed a decrease in total nitrate (NO_x) in rats after fine particle exposure. The change in NO₃ was significantly related to the change in eNO when expressed as either absolute change or percent change ($r = .44$ and $r = .48$, $p = .03$ and $p = .02$; respectively). The percent change in eNO was significantly related to the percent change in CaNO ($r = .50$, $p = .01$) but not to NO flux, implying that deposition of the ultrafine PM occurred in the alveolar region. Fractional

deposition has been shown to increase 4.5-fold over rest during exercise, with high deposition occurring in airway generations 18 and above (Daigle et al., 2003).

Upregulated NOS and elevated NO_x after exercise in the rat lung (Miyauchi et al., 2003) are in agreement with results from our low PM exercise; however, the decrease in eNO and NO₃ after high PM exercise supports a particle-induced downregulation of NOS or the formation of peroxynitrite by the reaction between NO and super oxide anions (O₂⁻). Peroxynitrite (ONOO⁻) is a highly reactive oxidant species that is involved in lipid peroxidation. The formation of peroxynitrite results in decreased availability of nitric oxide, and diminishes the pro-vasodilatory effects of NO; in this study we were unable to measure 3-nitrotyrosine (a stable measurable product of peroxynitrite). However, the >200% increase in MDA supports peroxynitrite initiated lipid peroxidation (Radi et al., 1991).

The significant correlations between lung function and change in NO₃ and eNO support a mechanism where a decrease in NO availability causes a sympathetic-induced broncho- or vasoconstriction; this could provide a plausible explanation for the reduction in carbon monoxide diffusing capacity and decreased mid expiratory flow rates after ultrafine particle inhalation noted by Pietropaoli et al. (2004).

GSNO, formed by the reaction between NO and GSH, has been suggested to be a reaction to stabilize NO in a nontoxic form (Gaston et al., 1994). Like NO₃, GSNO has been proposed to be an index of NO production (Stamler & Slivka, 1996). Levels of GSNO in EBC in the present study showed no significant change, in spite of significant decreases in CaNO and NO₃. High physiological levels of GSNO have been shown to protect against methacholine-induced airway hyperresponsiveness in mice (Que et al., 2003,) and asthmatics undergoing challenge demonstrate accelerated breakdown of GSNO (Dweik et al., 2001). In the present study, GSNO levels in EBC were ~5 μM, and levels did not change after 30 min of exercise in either low or high PM.

In summary, we have demonstrated statistically significant PM dose-dependent falls in lung function in healthy nonasthmatic subjects after high PM exercise but not after low PM exercise. Decreases in NO₃ and eNO were significantly related to the decreases in lung function, and the decrease in eNO was attributed to CaNO but not to conducting airway NO flux. The decrease in NO₃ and eNO combined with the large increase in MDA provides a plausible pathway for peroxynitrite-mediated lipid peroxidation.

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