Free Rad. Res., Vol. 28(1), pp. 59–67
Reprints available directly from the publisher
Photocopying permitted by license only

Nasal Cavity Lining Fluid Ascorbic Acid Concentration Increases in Healthy Human Volunteers Following Short Term Exposure to Diesel Exhaust

A. BLOMBERG[a], C. SAINSBURY[b], B. RUDELL[c], A. J. FREW[d], S. T. HOLGATE[d], T. SANDSTRÖM[a] and F. J. KELLY[b,*]

[a]*Department of Pulmonary Medicine and Allergology, University Hospital of Northern Sweden and National Institute for Working Life, Medical Division, Umeå, Sweden;* [b]*Cardiovascular Research, The Rayne Institute, St. Thomas' Hospital, London, U.K. SE1 7EH;* [c]*Department of Occupational Medicine, University Hospital of Northern Sweden, Umeå, Sweden;* [d]*Air Pollution Group, University Medicine, University of Southampton, Southampton, U.K.*

Accepted by Prof. B. Halliwell

(Received 19 August 1997; In revised form 17 September 1997)

To determine if diesel exhaust (DE) exposure modifies the antioxidant defense network within the respiratory tract lining fluids, a randomized, single blinded, crossover control study using nasal lavage and flexible video bronchoscopy with bronchial and bronchoalveolar lavage was performed. Fifteen healthy, non-smoking, asymptomatic subjects were exposed to filtered air or diluted diesel exhaust (300mg m^{-3} particulates, 1.6ppm nitrogen dioxide) for one hour on 2 separate occasions, at least three weeks apart. To examine the kinetics of any DE-induced antioxidant reactions, nasal lavage fluid and blood samples were collected prior to, immediately after, and 5½ hours post exposure. Bronchoscopy was performed 6 hours after the end of DE exposure. Ascorbic acid, uric acid and reduced glutathione (GSH) concentrations were determined in nasal, bronchial, bronchoalveolar lavage and plasma samples. Malondialdehyde (MDA) and protein carbonyl concentrations were determined in plasma and bronchoalveolar lavage samples. Nasal lavage ascorbic acid concentration increased 10-fold during DE exposure [1.02 (0.26–2.09) Vs 7.13 (4.66–10.79) $\mu mol/L^{-1}$], but returned to basal levels 5.5 hours post-exposure [0.75 (0.26–1.51) $\mu mol/L^{-1}$]. There was no significant effect of DE exposure on nasal lavage uric acid or GSH concentration. DE exposure did not influence plasma, bronchial wash, or bronchoalveolar lavage antioxidant concentrations and no change in MDA or protein carbonyl concentrations were found. The physiological response to acute DE exposure is an increase in the level of ascorbic acid in the nasal cavity. This response appears to be sufficient to prevent further oxidant stress in the respiratory tract of normal individuals.

INTRODUCTION

Diesel exhaust (DE) is the major pollutant in urban areas and exposure to it results in increased airway resistance, increased closing volume (CV), reversible reductions in forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC).[1–3] Symptoms such as burning eyes, headache, nausea, difficult or laboured breathing,

* Corresponding author. Tel.: (44) 171 922 8155. Fax: (44) 171 928 0658. E-mail: f.kelly@umds.ac.uk.

coughing, excessive production of phlegm and wheezing have also been noted.[4,5] DE is responsible for much of the carbon based particulate matter and is a major source of gaseous pollutants such as oxides of nitrogen. Both particulate matter, especially particles <2.5 μm in diameter and nitrogen dioxide (NO_2) have been linked with Public Health concerns.[6] Increased ground level particle concentrations have been linked with deaths from cardiovascular and respiratory causes[7,8] as well as increases in hospital admissions, emergency department visits and exacerbations of asthma and COPD.[9] Exposure to NO_2 is associated with lung function impairment and respiratory illness as well as a predisposition to airway infections.[10–14] Although both particles and NO_2 have marked impacts on the respiratory system their biological mode of action(s) are still far from clear.

A likely common feature of NO_2 and DE particles is that they exert their effects through free radical mechanisms, either through the oxidation of biomolecules to give other radical species or by driving radical-dependent production of cytotoxic, non-radical species. These reactions are likely to take place within the respiratory tract lining fluid (RTLF) a thin layer of fluid which overlays the respiratory tract. Recently we demonstrated that NO_2, itself a gaseous free radical, reacts with, and depletes antioxidants present within RTLF.[15] Carbon based particles emitted by diesel vehicles may act as vectors, delivering the toxic substances such as hydrocarbons to the airways,[16] and it is therefore possible that they give rise to similar events, thereby acting synergistically with NO_2.

This study was designed to investigate the acute impact of controlled DE exposure on the human respiratory tract in terms of antioxidant status and markers of oxidative stress. Appreciating that the various components of DE may impact, and have effects, at different levels of the respiratory tract, we sampled the upper, middle and lower airways by lavage.

SUBJECTS AND METHODS

Chemicals

All chemicals used were obtained from Sigma (Poole, England) and h.p.l.c. grade solvents were obtained from Rathburn Chemicals (Walkerburn, Scotland).

Subjects

Fifteen healthy non-smoking, non-atopic volunteers (11 male, 4 female), median age 25, range 21–28, were recruited. Entrance criteria to this study excluded anyone with a prior history of asthma or other pulmonary disease or a history of airway infection during the preceding six weeks. Subjects presenting with a cold during the study were replaced and the use of anti-inflammatory drugs or vitamin supplements during the study was prohibited.

Study Design

Each subject was exposed in a single blinded crossover control fashion to either air or DE in a specially built diesel exposure chamber[17] on each of two visits separated by at least 3 weeks. The exposure duration was one hour, during which the subjects performed moderate exercise (V_E = 20L/min/m^2) on a bicycle ergonometer which was alternated with rest at 15 minute intervals. Nasal lavage and blood samples were collected immediately before (0 min), immediately after (60 min) and 330 min post exposure. Bronchoscopy with bronchial wash and bronchoalveolar lavage was carried out 360 min after the end of the exposure This approach allowed us to compare events occurring in the blood and upper airways compartment during the exposure period and in the blood, upper, proximal and distal airway compartments post exposure.

Exposure Protocol

DE was generated from a Volvo TD 45 B four cylinder, four stroke, turbocharged diesel engine, model 1991. Motor data: 87.5 kW at 2400 rpm, torque 450 nm at 1400 rpm, displacement 4.48 dm^3, bore 105.57 mm, stroke 128 mm, and compression ratio 15.6:1. The fuel used was, Diesel D 10 (OK Petroleum, Stockholm, Sweden): composition by weight 86.4% carbon, 13.5% hydrogen, <0.02% nitrogen, <0.1%, PAH, 0.06% sulphur. The engine was allowed to idle (680 r/min) on the test bench continuously throughout the exposure period. Approximately 10% of the exhaust was diluted with air before being fed into the exposure chamber. The air in the chamber was changed every 2.3 minutes with waste air being extracted via a tube in the ceiling. The conducting tubing and the dilution chamber were heated to 200°C to avoid condensation. The DE entering the environmental chamber was standardized to give a median steady state concentration of 1.6 ppm nitrogen dioxide and this resulted in a median particulate concentration of approximately 300μg m^{-3}. During the exposures, air was sampled in the breathing zone of the subjects and was continuously monitored for NO (4.5ppm), CO (7.5ppm), total hydrocarbons (4.3ppm), formaldehyde 0.26 mg/m^3 and suspended particles (4.3/cm^3). The temperature and humidity in the chamber were controlled at 20°C and 50% respectively.

Nasal Lavage

Nasal lavage samples were collected using a method according to Harder *et al.*[18] Ten × 0.1 ml saline were sprayed into one nostril and recovered into a sterile plastic cup. This procedure was repeated 5 times per nostril at each time point. The plastic cup was placed on ice and the recovered nasal lavage fluid was filtered through a 100 μm pore nylon filter before being centrifuged at 400g at 4°C for 15 minutes. The supernatant was recovered and frozen at –70°C prior to analysis. Previous studies have shown that antioxidants are stable for at least 6 months when samples are stored in this manner.[15]

Blood

Peripheral blood was collected into tubes containing the anticoagulant EDTA and immediately placed on ice. For GSH analysis 1 ml of blood was mixed with 5 mls of cold 5% sulphosalicylic acid, left on ice for 15 minutes, and centrifuged at 1500g at 4°C for 10 minutes. The supernatant was frozen at –70°C. The remainder of the blood was centrifuged at 4°C (400g) for 10 minutes to separate plasma, which was aliquoted and stored at –70°C for later analysis of antioxidants.

Bronchoscopy

Six hours after the end of each exposure, fibreoptic bronchoscopy was performed to obtain lavage samples using a standard procedure.[19] Thirty minutes before the bronchoscopy atropine was administered subcutaneously and lidocaine was used throughout for topical anaesthesia. The bronchoscope was inserted through the mouth via a mouthpiece with the subject in the supine position. Subjects were mildly sedated using propofol. A bronchial wash was performed by instilling 2 × 20 ml sterile phosphate buffered saline (PBS) at 37°C and bronchoalveolar lavage (BAL) was performed by instilling 3 × 60mL PBS. The fluid was instilled with the tip of the bronchoscope carefully wedged into either a lingula lobe bronchus or a middle lobe bronchus and gently suctioned back into a siliconised container on ice. The recovery from the first 20 ml instillation was used for cell differential counts and analyses of soluble components and is presented separately,[20] the recovered fluid from the second 20 ml instillation and the recovery from the 3 × 60 ml installations were separately filtered through a 100 μm pore nylon filter

before being centrifuged at 400g at 4°C for 15 minutes. The cell pellets were removed and the samples were aliquoted and frozen prior to analysis for antioxidants.

Analysis of Antioxidants

Determinations of ascorbic acid and uric acid were carried out by high performance liquid chromatography (HPLC) as described previously.[21] Glutathione (GSH) and glutathione disulphide (GSSG) concentrations were determined using the enzyme recycling method described by Teitze,[22] adapted for use on a microplate reader.[23]

Analysis of Markers of Oxidative Stress

MDA was determined in plasma samples by h.p.l.c. as described previously.[21] Determination of airway lining fluid MDA content by h.p.l.c. has been described recently.[15] Briefly, lavage samples (3 mls with 10 µl of 0.2% butylated hydroxytoluene, [BHT]) were concentrated by freeze-drying and reconstituted in 350 µl of water. To this, 50 µl of 25% H_3PO_4 were added, mixed, and allowed to stand at room temperature for 10 min. One hundred microliters of 0.67% thiobarbituric acid (TBA) were then added to the acidified sample which was mixed and then transferred to an 0.8-ml HPLC vial, sealed and incubated for 20 min at 95°C. After the immediate cooling of the sample, aliquots of 20 µl were injected for analysis using a Gilson model 234 autosampler. A 100 × 250 mm Spherisorb ODS2 5 µm column and a mobile phase of 50 mM KH_2PO_4 (pH6.8)/methanol at a ratio of 65:35 and a flow rate of 0.9 ml/min were used for elution of the TBA-MDA adduct. The model 980 fluorescence detector (ABI Applied Biosystems, Cheshire, UK) with an excitation set at 515 nm and emission filter of 550 nm was used for detection with the photomultiplier tube (pmt) signal set at 1.48.

Protein carbonyl determination was based upon the affinity of carbonyl groups for the compound 2,4-Dinitrophenylhydrazine (DNPH)[24] with the following modifications. Each sample was divided into two 100 ml aliquots which were ran in parallel throughout the assay. 400 ml of 10 mM 2,4-Dinitrophenylhydrazine in 2.5 M HCL were added to one of the 100 ml aliquots, '+ DNPH' sample, and 400 ml of 2.5 M HCL were added to the other '– DNPH' and samples incubated for 15 min at room temperature, with continual mixing. Post incubation protein was precipitated with 500 ml of 20% TCA (w/v), and samples centrifuged; 5 min. at 14,000 rcf, 4°C. Supernatants were then carefully decanted and discarded before the addition of 400 µl of 10% TCA (w/v) to both the '+' and '–' samples, followed by mechanical disruption of the protein pellet with a mild sonication, 14 microns for 10 sec., and centrifugation, conditions as above. Samples were washed three times in a 1:1 (v/v) absolute ethanol:ethyl acetate solution. On each occasion samples were mixed and allowed to stand for 5 min. in the wash solution before centrifugation and aspiration of the supernatant. The final pellet produced from these washings was redissolved in 1.0 ml of 6 M guanidine-HCL, in 20 mM potassium phosphate; pH adjusted to 2.3 using concentrated HCL, by warming at 37°C for 10 min followed by sonication at 14 microns for 10 sec. After centrifugation, the absorbance of each supernatant ('+' and '–' DNPH samples) were read against a blank of the Guanidine-HCL solution described above using a LKB Ultraspec II at a wavelength of 370 nm. The carbonyl content for each sample was calculated from the Beer Lambert's law, using the molar extinction coefficient of DNPH, 22,000 M cm^{-1}, and corrected for non-specific absorbance at 370 nm by subtraction of the absorbance associated with the '–' DNPH sample. In order to compare the carbonyl content of different samples, protein assays were performed on the '–' DNPH samples and the carbonyl content of each sample expressed as M mg^{-1} protein. Intra-assay variation associated with this assay was <5%. Inter-assay variation was corrected for by running aliquots of a positive control sample, a highly oxidised plasma

sample of known carbonyl concentration, in each assay. The detection limit of sensitivity was determined as 2.5×10^{-10} M/mg protein.

STATISTICS

Nasal lavage data were analyzed using Friedman's repeated measures analysis of variance on ranks test (Sigmastat for Windows version 1.0, Jandel Corp.) followed by Dunnett's all pairwise multiple comparison procedure. Results from blood, bronchial wash and bronchoalveolar lavage analyses were examined using Wilcoxon's signed rank test using SPSS for Windows version 6.1.3. Statistical significance was accepted at $p < 0.05$.

RESULTS

Nasal Lavage

Recovery was routinely between 47 and 64% for all lavages performed. All data presented are based on concentrations expressed per unit volume of recovered nasal lavage fluid. No significant differences in basal (i.e. time 0, air and diesel) nasal lavage ascorbic acid concentrations were seen in individuals, when measured on separate occasions three weeks apart, demonstrating the constancy of this antioxidant with time (Table I). Similarly, no differences were noted either for basal uric acid or GSH concentrations.

Loss (washout) of ascorbic acid and GSH were seen during air exposure (i.e. compare 60 min values relative to time zero values), although in the case of GSH, the concentration had returned to control levels by 330 min (Table I). Following exposure to DE for 60 min, nasal lavage ascorbic acid concentration increased 12-fold in comparison to that seen in the same individuals exposed to air (Table I). Neither, GSH or uric acid concentrations in nasal lavage were influenced by diesel exhaust.

Bronchial Wash and Bronchoalveolar Lavage Fluid

Of the 20 ml instilled for the second bronchial wash, approximately 16–17 ml were recovered in all subjects. Of the 180 ml installed for the bronchoalveolar lavage, recoveries were 127–142 ml and 129–145 ml following the air and DE exposures respectively. Ascorbic acid, uric acid and GSH were found in the bronchial wash and bron-

TABLE I Antioxidant responses of nasal lavage fluid following exposure to diesel exhaust

Lavage time (min)	Treatment	Ascorbic acid	Uric acid	GSH
0	Air	1.93 (0.39–3.18)	28.01 (23.67–43.33)	1.37 (0.66–2.51)
	Diesel	1.45 (0.71–2.93)	37.51 (17.20–53.71)	1.67 (0.66–2.38)
60	Air	0.58* (0.25–1.02)	22.21 (17.03–26.63)	0.62* (0.44–0.86)
	Diesel	7.13† (4.68–10.79)	23.15 (14.52–39.35)	0.87* (0.38–1.35)
330	Air	0.75* (0.44–1.12)	27.99 (23.33–33.69)	1.00 (0.59–1.28)
	Diesel	0.78 (0.26–1.51)	33.40 (16.04–59.06)	0.71 (0.34–1.07)

Median (interquartile range) values for antioxidant components of nasal lavage fluid. Results are expressed as μmol L^{-1}.
*$p < 0.05$ from time zero control † $p < 0.05$ from equivalent air control.

choalveolar lavage fluid of all volunteers. Following exposure to DE, no significant changes in antioxidant composition of either of these two samples of respiratory tract lining fluid were noted (Table II). Likewise, no change in MDA or protein carbonyls concentrations were seen in the bronchoalveolar lavage fluid following DE exposure (data not shown).

Blood

No significant changes in plasma ascorbic acid or uric acid or whole blood glutathione concentrations were seen following DE exposure in comparison to air exposure (data not shown). Likewise, exposure to DE did not alter MDA (421 [322, 734] versus 476 [375, 861] nmol/L or protein carbonyl levels (2.2 [0.5, 4.4] versus 2.1 [0.6, 3.8] nmol/mg protein, DE versus air exposure, respectively.

DISCUSSION

Breathing DE elicits a number of responses in the respiratory tract, including coughing, increased production of phlegm, wheezing and inflammation. The mechanisms underlying these responses are not well understood. Due to the oxidative capacity of DE, redox changes in the lung are a likely early event during DE exposure. On entering the respiratory tract, prior to any interactions with the respiratory epithelium, DE passes through the RTLF. As NO_2 is a free radical[25] and DE particles have free radical activity,[26] we hypothesised that DE would influence RTLF antioxidant activity. We tested this hypothesis in a human volunteer study exposing subjects to DE, containing PM_{10} at 300 ug/m^3 and NO_2 at 1.6 ppm. These pollution levels are encountered in most busy cities of the developing world, several occupational settings and during peak pollution episodes in the developed world.[27]

We were particularly interested in examining the antioxidant response to DE at all levels of the respiratory tract, hence we devised an approach to examine nasal cavity lining fluid, as well as proximal airways and distal airways lining fluid. As nasal lavage is a less invasive procedure than bronchial or bronchoalveolar lavage, we employed a sequential nasal lavage study design to help define the timing of particular responses in the nasal cavity. Repeated sampling of airway fluid in this manner was however found to have the drawback of washout. That is, even following air exposure, the concentration of ascorbic acid and GSH, but not uric acid, were significantly lower when nasal lavage was performed the second time i.e. at 60 min. Little is know about the source of airway lining fluid antioxidants, but it is likely that uric acid and ascorbic acid are derived from

TABLE II Antioxidant concentrations of bronchial and bronchoalveolar lavage fluid following exposure to diesel exhaust

Region	Treatment	Ascorbic acid	Uric acid	GSH
bronchial wash	Air	0.31 (0.24–0.42)	0.69 (0.43–0.95)	0.24 (0.10–0.44)
	Diesel	0.34 (0.24–0.38)	0.86 (0.52–0.99)	0.78 (0.20–1.02)
bronchoalveolar wash	Air	0.36 (0.31–0.51)	0.78 (0.51–1.26)	0.35 (0.16–0.41)
	Diesel	0.37 (0.34–0.39)	0.81 (0.54–1.05)	0.42 (0.25–0.65)

Median (interquartile range) values for bronchial and bronchoalveolar lavage fluid.
Results are expressed as μmol L^{-1}.

blood while GSH is synthesized and released locally by respiratory tract cells. Interestingly, washout of uric acid was not seen following the lavage procedure. This may be due to its much higher concentration in the nasal cavity,[28] which may be related to a higher rate of movement into this compartment.

Taking washout into account, nasal lining fluid ascorbic acid concentration increased markedly in 14 of the 15 subjects during the 60 min exposure to DE. Following exposure, the ascorbate concentration in the nasal cavity returned to control levels by 330 min. Wiester et al[29] have demonstrated a similar response in animals, albeit over a longer time period, during adaptation to ozone. They found airway ascorbic acid concentration increased, whereas other antioxidants such as uric acid and α-tocopherol were unaffected. In the present experiment, 60 min separated the first and second nasal lavage, hence it is not clear whether the increase in ascorbic acid, observed at 60 min in the nasal cavity, was in response to its prior depletion by DE.

No differences in proximal or distal airway antioxidant defences were seen 6 hours post DE exposure compared to following air exposure. DE consists of a combination of several pollutants including gaseous (oxides of nitrogen, carbon and sulphur) and particulate components. Previously we have shown that exposure to NO_2 alone depletes ascorbic acid and uric acid from the proximal and distal airways.[15] These losses however are resolved within 6 hours following the end of the exposure period, indicating that pathways can be brought into play to replace airways antioxidants. Interestingly, in the present study, the proximal airways had increased numbers of neutrophils 6 hours post DE exposure (data not shown). However, as no measure of neutrophil activation was made, it is not presently clear whether these cells were contributing to the oxidative stress in the airways at this time.

It is also presently unclear why there was such a marked increase in ascorbic acid in the nasal cavity in response to DE, especially as other airways antioxidants were unresponsive to DE. As mentioned above, DE exposure is associated with subsequent airway inflammation, hence the ascorbic acid response could be viewed as a protective one as increased levels of this antioxidant will help prevent neutrophil derived oxidant injury of the respiratory tract. Alternatively as DE particles contain metals, DE toxicity could paradoxically be increased in the presence of elevated ascorbic acid concentrations. Such an outcome would however be dependent not only on the ascorbic acid concentration present, but the nature of redox-active metals adsorbed on the surface of the DE particles and the duration of the exposure period.

The source of the ascorbic acid, which enters the nasal cavity post DE exposure, is unresolved. It may arise through increased transfer from the blood compartment. As membrane permeability was not determined in the present study this potential route can not be ruled out. Alternatively, the rise in ascorbate may be in response to the conversion of ascorbate to dehydroascorbate by iron contained within the soot particles in DE. Ascorbate, by reducing Fe^{3+} to Fe^{2+}, gives rise to the semidehydroascorbate radical which can undergo disproportionation to give ascorbate and dehydroascorbate from two molecules of semidehydroascorbate. Dehydroascorbate in turn, is rapidly taken up by neutrophils and reduced back to ascorbic acid.[30] We feel this mechanism to be less plausible explanation in the current setting as the number of neutrophils recovered in nasal lavage fluid did not increase following DE exposure and the ascorbic acid that neutrophils regenerate is not normally released back into the airways.

In summary, exposure of normal human volunteers to DE results in a marked increase in ascorbic acid concentration in the fluid that lines the nasal cavity. Although no evidence of overt oxidative stress was found, this ascorbic acid response suggests that free radical mechanisms underlie the impact of DE on the upper respiratory tract.

Acknowledgements

We are grateful to Kjell Holmgren and co-workers, The National Machinery Testing Institute, Umeå, Lennart Schönning, OK Petroleum, Stockholm and Jan-Olof Hjortsberg, Volvo Wheel Loaders AB, Eskilstuna, Sweden.

Supported in part by the Medical Research Council (UK), the Swedish Heart Lung Fund, the Swedish Work Environment Fund, The Swedish Association against Asthma & Allergy, and Umeå University.

References

[1] Ulfvarson, U., Alexanderson, R., Dahlquist, M., Ekholm, U. and Bergstrom, B. (1991). Pulmonary function in workers exposed to diesel exhausts: The effect of control measures. *American Journal of Industrial Medicine*, **19**, 282–289.

[2] Walters, S., Phupinyokul, M. and Ayres, J. G. (1995). Hospital admission rates for asthma and respiratory disease in the West Midlands: their relationship to air pollution levels. *Thorax*, **50**, 948–954.

[3] Rudell, B., Ledin, M. C., Hammarstrom, U., Stjernberg, N., Lundback, B. and Sandstrom, T. (1996). Effects on symptoms and lung functions in humans experimentally exposed to diesel exhaust. *Occupational & Environmental Medicine*, **53**, 658–662.

[4] Reger, R., Hancock, J., Hankinson, J., Hearl, F. and Merchant, J. (1982). Coal miners exposed to diesel exhaust emissions. *Annals of Occupational Hygiene*, **26**, 799–815.

[5] Gamble, J., Jones, W. and Minshall, S. (1987). Epidemiological-environmental study of diesel bus garage workers: acute effects of NO2 and respirable particulate on the respiratory system. *Environmental Research*, **42**(1), 201–214.

[6] Quality of Urban Air Review Group (1993). *Diesel Vehicle Emissions and Urban Air Quality*—Bradford: Department of the Environment.

[7] Schwartz, J. (1994). Air pollution and hospital admissions for the elderly in Birmingham, Alabama. *American Journal of Epidemiology*, **139**(6), 589–598.

[8] Dockery, D. W., Pope, C. A., Xu, X., Spengler, J. D., Ware, J. H., Fay, M. E., Ferris, B. G. and Speizer, F. E. (1993). An association between air pollution and mortality in six U.S. cities. *New England Journal of Medicine*, **329**(24), 1753–1759.

[9] Dockery, D. W. and Pope, C. A. (1994). Acute respiratory effects of particulate air pollution. *Annual Review of Public Health*, **15**, 107–132.

[10] Love, G. J., Lan, S. P., Shy, C. M. and Riggan, W. B. (1982). Acute respiratory illness in families exposed to nitrogen dioxide ambient air pollution in Chattanooga, Tennessee. *Archives of Environmental Health*, **37**(2), 75–80.

[11] Morrow, P. E. (1984). Toxicological data on NOx: an overview. *Journal of Toxicology & Environmental Health*, **13**(2–3), 205–227.

[12] Sandstrom, T., Andersson, S. M., Kolmodin-Hedman, B., Stjernberg, N. and Angstrom, T. (1990). Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time-kinetic study. *European Respiratory Journal*, **3**, 138–143.

[13] Helleday, R., Sandström, T. and Stjernberg, N. (1994). Smokers have a different bronchoalveolar cell response to nitrogen dioxide exposure compared to non-smokers. *European Respiratory Journal*, **7**, 1213–20.

[14] Sandstrom, T., Ledin, M., Thomasson, L., Helleday, R. and Stjernberg, N. (1992). Reductions in lymphocyte subpopulations after repeated exposure to 1.5 ppm nitrogen dioxide. *British Journal of Medicine*, **49**, 850–854.

[15] Kelly, F. J., Blomberg, A., Frew, A. J., Holgate, S. T. and Sandström, T. (1996). Antioxidant kinetics in lung lining fluid following exposure of humans to nitrogen dioxide. *American Journal of Respiratory & Critical Care Medicine*, **152**, 1700–1705.

[16] Nauss, K. M., Busby, W. J., Cohen, A. J. *et al.* (1995). Critical issues in assessing the carcinogenecity of diesel exhaust: A synthesis of current knowledge. In: Diesel exhaust—A critical analysis of emission, exposure and health effects. (A special report on the Institute's diesel Working group), Health Effects Institute, Cambridge, MA pp 13–18.

[17] Rudell, B., Sandstrom, T., Hammarstrom, U., Ledin, M. L., Horstedt, P. and Stjernberg, N. (1994). Evaluation of an exposure setup for studying effects of diesel exhaust in humans. *International Archives of Occupational Environmental Health*, **6**(2), 77–83.

[18] Harder, S., Peden, D., Koren, H. and Devlin, R. B. (1994). Comparison of nasal lavage techniques for analysis of nasal inflammation. *American Journal of Respiratory & Critical Care Medicine*, **151**(4), A565.

[19] Helleday, R., Sandström, T., and Stjenberg, N. (1994). Differences in bronchoalveolar cell response to nitrogen dioxide exposure between smokers and non smokers. *European Respiratory Journal*, **7**, 1213–1220.

[20] Salvi, S. S., Blomberg, A., Rudell, B., Kelly, F. J., Frew, A. J., Holgate, S. T. and Sandström, T. (1996). Acute inflammatory response in the airways and peripheral blood after exposure to diesel exhaust in healthy human volunteers. *European Respiratory Journal*, **9**, 415S.

[21] Brown, R. K. and Kelly, F. J. (1994). Evidence for increased oxidative damage in patients with Cystic Fibrosis. *Pediatric Research*, **36**, 1–7.

[22] Tietze, F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Annals of Biochemistry*, **27**, 502–522.

[23] Housley, D. G., Mudway, I., Kelly, F. J., Eccles, R. and Richards, R. J. (1995). Depletion of urate in human nasal lavage fluid following *in vitro* ozone exposure. *International Journal of Biochemistry and Cell Biology*, **27**, 1153–1159.

[24] Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S. and Stadman, E. R. (1990). The determination of carbonyl content in oxidatively modified proteins. In: Packer L, Glazer AN (eds) Methods in Enzymology. Academic Press, London, Vol 186: pp 464–477.

[25] Postlethwait, E. M. and Bidani, A. (1989). Pulmonary disposition of inhaled NO_2-nitrogen in isolated rat lungs. *Toxicology & Applied Pharmacology*, **98**, 303–312.

[26] Sagai, M., Saito, H., Ichinose, T., Kodama, M. and Mori, Y. (1993). Biological effects of diesel exhaust particles I in vitro production of superoxide and in vivo toxicity in mouse. *Free Radical Biology Medicine*, **14**, 37–47.

[27] Air Pollution in the world's megacities. (1994). A report from the UN Environment programme and WHO. *Environment*, **36**, 5–37.

[28] Peden, D. B., Hohman, R., Brown, M. E., Mason, R. T., Berkebile, C., Fales, H. M. and Kaliner, M. A. (1990). Uric acid is a major antioxidant in human nasal airway secretions. *Proceeding of the National Academy of Science*, **87**, 7638–7642.

[29] Weister, M. J., Tepper, J. S., Winsett, D. W., Crissman, K. M., Richards, J. H. and Costa, D. L. (1996). Adaptation to ozone in rats and its association with ascorbic acid in the lung. *Fundamental & Applied Tocicology*, **31**, 56–64.

[30] Washko, P. W., Wang, Y., M. and Levine, M. (1993). Ascorbic acid recycling in human neutrophils. *Journal of Biological Chemistry*, **268**, 15531–15535.