Ultrafine Particle Deposition in Subjects with Asthma

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Epidemiologic studies have shown links between mass concentrations of ambient particulate matter and increased morbidity and mortality in compromised individuals (Pope et al. 2004). People with asthma appear to be at increased risk for the adverse effects of particulate air pollution (Frampton et al. 2000; Karol 2002; Gavett and Koren 2001). Increased levels of particulate air pollution are associated with asthma exacerbations, increased respiratory symptoms, decreased lung function, increased medication use, and increased hospital admissions (Utell and Frampton 2000; Devlin et al. 2004).

Particles < 100 nm (0.1 μm) in diameter [ultrafine particles (UFPs)] are ubiquitous in ambient particulate pollution and dominate ambient particle number and surface area concentrations, both indoors and outdoors, because of their small size (Oberdörster et al. 1995; Frampton 2001). UFPs may contribute to the health effects of particulate matter because of their high surface area, oxidant capacity, ability to evade macrophage phagocytosis, and propensity for inducing pulmonary inflammation. Although few studies have assessed the health effects of exposure to UFPs, ambient UFP concentrations have been associated with mortality (Wright et al. 1998). A panel study of subjects with asthma (Peters et al. 1997) found that peak flow varied more closely with the 5-day mean of UFP number than with fine particle mass concentration, suggesting that the UFP component of fine particle pollution contributes to airway effects in asthmatics. Penttinen et al. (2001) noted that UFP number concentrations tended to be inversely but nonsignificantly associated with measures of lung function.

Various epidemiologic studies have shown that ultrafine particles are associated with increased airway responsiveness in asthmatics. Penttinen et al. (2001) noted that UFP number concentrations tended to be inversely but nonsignificantly associated with measures of lung function. However, some epidemiologic studies have not found associations between UFP exposure and health effects (de Hartog et al. 2003).

Inhaled UFPs have a high predicted deposition efficiency in the pulmonary region [International Commission on Radiological Protection (ICRP) 1994]. Thus, the expected number of particles retained in the lung with each breath is greater for UFPs than for larger particles. We and others have confirmed the relatively high predicted deposition of UFPs in healthy people breathing at rest (Anderson et al. 1990; Brown et al. 2002; Daigle et al. 2003; Jaques and Kim 2000; Roth et al. 1994; Schiller et al. 1988; Wilson et al. 1985). We recently demonstrated that UFP fractional deposition increases significantly with breathing during exercise in healthy subjects (Daigle et al. 2003). Brown et al. (2002) observed an increased total deposition, expressed as dose rate, for patients with chronic obstructive lung disease, compared with healthy subjects, with exposure to a 33-nm (count median diameter) ultrafine technetium-99m-labeled aerosol. We are unaware of any previous studies measuring UFP deposition in people with asthma.

Asthma is characterized by airway obstruction, with air trapping and increases in lung residual volume. Increases in alveolar volume would be expected to enhance diffusional deposition, the primary mechanism of deposition for UFPs, although impaired alveolar ventilation would counter this increase. We hypothesized that the fractional deposition of UFPs is greater in subjects with mild asthma than in healthy subjects without asthma, and that UFP deposition increases further with exercise.

Materials and Methods

Subjects and experimental design. The Research Subjects Review Board of the University of Rochester approved the study, and informed written consent was obtained. Subjects were 16 men and women with mild asthma who had never smoked, were 18–55 years of age, and were without a recent respiratory infection. Subjects were considered to have asthma if they had a history of repetitive symptoms characteristic of intermittent bronchoconstriction (wheezing, shortness of breath) and either a) improvement in forced expiratory volume in 1 sec (FEV1) ≥ 12% with the administration of inhaled albuterol if normally low values were obtained for airway conductance, FEV1, or FEV1/forced vital capacity (FVC) (Morris et al. 1971); or b) airway hyperresponsiveness with methacholine challenge. At the time of screening, subjects exercised on a bicycle ergometer for 15 min to determine the intensity necessary to achieve a target minute ventilation of 20 L/min/m2. Subjects with FEV1 < 70% of predicted at baseline screening, or with > 20% reduction in FEV1 after the screening exercise, were excluded.

For the methacholine challenge, increasing concentrations of methacholine (0.00, 0.08, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00, 10.00 mg/mL) in normal saline were administered at 4-min intervals using a nebulizer (model 646; Devilbiss Company, Summerset, PA) with a dosimeter (Rosenthal-French model D-2A; Laboratory for Applied Immunology Inc., Fairfax, VA) calibrated to deliver 0.01 mL/breath. Subjects were instructed to take five breaths each lasting
6 sec, and FEV₁ was measured 30 sec after the last breath. The concentration of methacholine that produced a partial (20%) decrease in FEV₁ (PD20) was determined by interpolation using the regression line of the methacholine dose response. Subjects with a PD20 > 10 mg/mL were excluded from the study.

All exposures were by mouthpiece with a nose clip for 2 hr, with a 10-min break off the mouthpiece after the first hour. In the exposure, subjects (n = 16) were exposed to a target mass concentration of 10 µg/m³, which corresponded to an empirically determined number concentration of 2 x 10⁶ particles/cm³. Exposures lasted 2 hr and included four alternating 15-min rest and exercise (target minute ventilation, 25 L/min/m² body surface area) periods.

**Exposure system.** The exposures were undertaken within an environmental chamber in the General Clinical Research Center at the University of Rochester Medical Center. A mouthpiece exposure system was chosen in order to facilitate accurate and relevant measurements of respiratory deposition. Details of particle generation and the mouthpiece exposure system have been described elsewhere (Chalupa et al. 2002; Duigle et al. 2003).

Briefly, the design is a one-pass, dynamic-flow exposure system. Particles were generated from pure graphite electrodes by spark discharge in anhydrous argon, using a commercial generator (Palas Co., Karlsruhe, Germany). The generator settings were adjusted to provide a nominal particle count median diameter (CMD) of 23 nm with a geometric standard deviation of 1.6. Particles were passed through a charge neutralizer after generation, in order to achieve Boltzmann’s equilibrium, and were delivered continuously into diluting air in a mixing chamber. The dilution air was passed through charcoal and high-efficiency particle filters and supplied into the mixing chamber at 120 L/min. The intake air flow rate was monitored with a Manahelic pressure gauge (Dwyer Instruments, Inc., Michigan City, IN), which was calibrated using a dry test meter (Singer American Meter Company Division, Wellesley, MA). All tubing was electrically conductive with lengths minimized to avoid particle loss.

Subjects wore a nose clip, inhaled through a mouthpiece connected to the exposure system via one-way rebreathing valves (Hans Rudolph Inc., Kansas City, MO), and exhaled into a dedicated exhaust line. Particles in the reservoir entered the circuitry to the mouthpiece according to the demands of the subject. A resilient reservoir was placed on the expiratory side of the subject, loosely coupled to a dedicated filter and exhaust system. The system was designed to keep both sides of the non-rebreathing valves at atmospheric pressure, unaffected by the subject’s respiration. Tubing on the expiratory side was heated to approximately 37°C to avoid condensation.

Measurements of both inhaled and exhaled air included particle number (condensation particle counters, model 3220a; TSI, Inc., St. Paul MN) and particle size distribution (Scanning Mobility Particle Sizer, model 3071; TSI, Inc.). Particle mass concentration was continuously measured on the inhaled aerosol [tapered element oscillating microbalance (TEOM); Rupprecht and Patanchick, Albany, NY]. The target exposure mass concentration was 10 µg/m³.

Electronic integration (HPChem Integrating Software, Hewlett Packard, Wilmington, DE) of a pneumotachographic airflow transducer (E for M Co., White Plains, NY) on the expiratory limb provided continuous measurements of tidal volume (Vₜ), respiratory rate, and minute ventilation.

To determine particle losses, a reciprocal pump was used to simulate respiration. A resting minute ventilation of 10 L/min was simulated using a volume of 800 mL at 12.5 cycles/min. Mild exercise (22 L/min) was simulated using a volume of 1,200 mL at 18.3 cycles/min. Continuous upstream and downstream measurements of particle number and volume were determined for the whole system and for a respiratory valve alone. Mass losses were calculated using particle volume determined by the electrostatic classifier. During exercise simulation, losses were 0% for particles ≥ 23.7 nm midpoint diameter; maximum losses were 3.9% for 7.5 nm particles. At resting conditions, maximum losses were 13.2% for 7.5 nm particles.

**UFP deposition.** The total respiratory deposition fraction (DF) was calculated for both particle number and mass concentrations, with correction for system losses (Chalupa et al. 2002). Inspiratory and expiratory UFP number concentrations were measured continuously and recorded every 5 sec during the exposure. Particle number concentration was then averaged for the periods at rest and exercise. Particle size distribution from the inspiratory circuit was determined before each exposure and just after the exposure was completed. Particle size distribution from the expiratory circuit was measured during one rest and one exercise period each hour. For computational simplicity, data on particle size distribution from the scanning mobility particle sizer were grouped into 12 particle size bins. Four size bins each contained less than 1% of the total expired particle number (midpoint diameters < 8.7 and > 64.9 nm), and these were excluded, leaving a total of eight size bins with midpoint CMD from 8.7 to 64.9 nm (particle CMD ranging from 7.5 to 75.0 nm), which included ≥ 98% of the particles. The mean size-specific inspiratory particle concentration was determined by multiplying the average inspiratory number concentration by the percentage of particles in each size bin in the inspiratory circuit.

The mean size-specific inspiratory particle concentration was determined by multiplying the average expiratory number concentration by the percentage of particles in each size bin in the expiratory circuit. The correction factors for system losses were subtracted from the measured inspired concentrations and added to the measured expired concentrations. The number DF was then calculated by subtracting the corrected expiratory number concentration from the corrected inspiratory number concentration and dividing the difference by the corrected inspiratory number concentration.

The particulate mass DF was calculated as follows: Inspired and expired particle volume (mass) concentrations were determined for each size bin from the scanning mobility particle sizer data. The percentages of inspired and expired particles by volume per bin were determined by dividing each bin volume concentration by the total volume concentration (sum of individual bins). The mean expired mass concentration was calculated by multiplying the ratio of the total expired volume concentration to the total inspired volume concentration, times the measured (TEOM) inspired mass concentration. The inspired mass concentration for each bin was calculated as the product of the inspired volume percentage of particles in each bin and the mean inspired mass concentration from the TEOM. The expired mass concentration for each bin was the product of the expired volume percentage for each bin and the calculated overall expired mass concentration. This mass data was corrected for system losses by multiplying each bin by the loss correction factor for that bin and then subtracting that product from the inspired data and adding to the expired data. Finally, a loss-corrected DF was calculated as the loss-corrected inspired mass concentration minus the loss-corrected expired mass concentration.

| Table 1. Subject demographics and lung function. |
|----------------|-----------------|
| Characteristics | Mean ± SD       |
| Age (years)    | 23 ± 2.7        |
| M/F            | 9/8             |
| Height (cm)    | 170 ± 8         |
| Weight (kg)    | 80 ± 15         |
| FEV₁ (L/% predicted) | 3.71 ± 0.91/97.6 ± 5.0 |
| FVC (L/% predicted) | 4.77 ± 1.05/106.2 ± 14.5 |
| FEF₂₅–₇₅ (%)   | 77.8 ± 6.9      |
| FEF₂₅–₇₅ (L/% predicted) | 3.32 ± 1.34/77.6 ± 29.7 |
| DCO (mL/min/mmHg/% predicted) | 31.35 ± 7.15/97.9 ± 12.5 |

Abbreviations: DCO, diffusing capacity for carbon monoxide; FEF₂₅–₇₅, forced expiratory flow rate at 25–75% of vital capacity.

| Table 2. Breathing parameters (mean ± SD, n = 16). |
|----------------|-----------------|
| Tidal volume (L) | Respiratory frequency (breaths/min) | Minute ventilation (L/min) |
| Rest            | 0.78 ± 0.14     | 18 ± 2.5 | 13.3 ± 2.0 |
| Exercise        | 1.71 ± 0.46     | 25 ± 3.8 | 41.3 ± 9.0 |
mass concentration, divided by the loss-corrected inspired mass concentration.

The data were then compared with theoretical total respiratory DFs calculated using three models: a) ICRP (1994), b) National Council on Radiological Protection and Measurements (NCRP 1997), and c) the Multiple Particle Deposition (MPPD) model (version 1.0; Chemical Industry Institute of Toxicology, Research Triangle Park, NC). We found generally good agreement among the three models, and only the data from the MPPD predictions are shown. This model was chosen in part because it allowed predictions to be calculated using each subject’s functional residual capacity, mean respiratory frequency, and mean VT during rest and exercise. Default values entered for all subjects were mouth breathing, upper respiratory tract volume 50 mL, inspiratory:expiratory ratio 1:2, and nominal particle density 1.5 g/cm³. Model predictions for 23 nm particles were not affected by changes in particle density or inspiratory:expiratory ratio.

Data means were compared using the two-tailed Student’s t-test (Brown 1980), with \( p < 0.05 \) denoting significance.

### Results

The sex, mean age, and spirometric values of the subjects are shown in Table 1. Spirometry was within normal limits for most subjects; for five subjects the FEV\(_1\) was < 80% of predicted. Table 2 shows the mean VT, respiratory frequency, and minute ventilation during the exposures, at rest, and during exercise.

The particle size distributions for the inhaled and exhaled aerosols were nearly identical, indicating little particle agglomeration or hygroscopic growth. Technical difficulties precluded the measurement of the number DF at rest in one subject, and the mass DF during exercise in another subject. Table 3 lists the DF for each particle size bin at rest and during exercise, as well as the total particle DF by number and mass. The total respiratory number deposition was high at 0.76 and increased further to 0.86 with exercise. The DF increased with exercise in all size bins. The mass DF for the smallest particles in the size distribution (< ~15 nm) was > 0.9. No significant sex differences were found.

Figure 1 compares these experimental data with predicted deposition according to the MPPD model. Overall, the model predicted little increase in DF with exercise, and the experimental data exceeded model predictions during exercise (Figure 1B).

Table 4 and Figure 2 provide comparisons of particle deposition in the present study with our previous findings in healthy subjects. The number DF during breathing at rest was significantly increased in subjects with asthma compared with healthy subjects. Deposition was similar in healthy and asthmatic subjects during exercise. In both healthy and asthmatic subjects, exercise produced an approximate 4-fold increase in particle deposition rate, as a consequence of both increased minute ventilation and DF. The calculated total number (and mass) of deposited particles during the 2-hr exposures was 74% (and 80%) higher during rest, and 43% (and 47%) higher during exercise, for the asthmatic subjects than for the healthy subjects. This was the result of both the higher DF and increased minute ventilation in the asthmatic subjects. When both the healthy subjects from Daigle et al. (2003), and the present subjects with asthma were considered together, there was no significant relationship between FEV\(_1\) and the DF of UFPs (Figure 3). However, the DF increased with increases in VT (Figure 4).

### Discussion

The dose of particles that reaches the lung is a determinant of the pulmonary response to...
inhalation. If the lung dose of UFPs is higher for people with asthma than for healthy people given the same exposure, the risk for health effects may also be increased. Thus, determining particle deposition is important in understanding susceptibility.

These studies confirm our previous observation of high total respiratory deposition of UFPs in humans (Daigle et al. 2003) and indicate that subjects with asthma, when breathing on a mouthpiece, have increased UFP fractional deposition compared with healthy subjects. Previous studies (Anderson et al. 1990; Bennett et al. 1997; Brown et al. 2002; Svartengren et al. 1991) have shown that patients with chronic obstructive lung disease have enhanced deposition of fine particles and UFPs. Fine particle deposition is increased in people with asthma. For example, Kim and Kang (1997) studied healthy and asthmatic subjects inhaling an aerosol of 1 μm sebacate particles. Fractional deposition was 0.14 ± 0.02 and 0.22 ± 0.02 for the healthy and asthmatic subjects, respectively, and the DF correlated inversely with the severity of airway obstruction. The present study is the first effort to measure UFP deposition in subjects with asthma.

In our previous study of healthy subjects (Daigle et al. 2003), UFP deposition increased significantly with exercise, exceeding model predictions. In the present study, deposition also increased with exercise in the subjects with asthma, but the increase was of a smaller magnitude and was not statistically significant, perhaps because possible factors that increase UFP deposition during exercise, such as increased alveolar volume and airway turbulence, are already present in the asthmatic lung at rest. Patients with obstructive lung disease have a higher minute ventilation than do healthy people, because of increased dead-space ventilation (Tobin et al. 1983). In comparison with our previous study of healthy individuals, breathing on the mouthpiece at rest, the breathing frequency was 10% higher (17.8 vs. 16.0 breaths/min), and V\textsubscript{T} was 25% higher (564 vs. 749 mL), giving a minute ventilation for asthmatics that was 32% higher (9.0 vs. 13.3 L/min). We speculate that the increased minute ventilation and hyperinflation that are characteristic of even mild asthma enhance diffusional deposition of UFPs in the distal airways and alveoli.

We did not find a significant relationship between FEV\textsubscript{1} and DF (Figure 3), perhaps partly because this study was limited to subjects with mild airway obstruction, and FEV\textsubscript{1} was > 70% of predicted for all subjects. It is also possible that the degree of airway obstruction is a less important determinant of UFP deposition than of fine particle deposition, where impaction and sedimentation play more important roles. Additional studies are needed in people with asthma with greater impairment in lung function to determine this relationship. We did observe a significant correlation between V\textsubscript{T} and DF. Indeed, increases in V\textsubscript{T} would be expected to increase diffusional deposition because of longer residence time for particles in the distal lung; this has been confirmed experimentally in subjects inhaling UFPs under controlled breathing conditions (Jaques and Kim 2000).

Breathing on a mouthpiece tends to alter respiratory patterns, with larger V\textsubscript{T} and minute ventilation than during unencumbered breathing (Paek and McCool 1992). It is possible that mouthpiece breathing induced greater increases in V\textsubscript{T} and minute ventilation in the subjects with asthma than in healthy subjects, and that this contributed to the observed increase in UFP deposition. Nasal deposition would also be expected to contribute to the deposition values, further increasing the total value. The demarcation between upper respiratory tract and lower respiratory tract deposition would be different but mainly in that the former is larger. Thus, the conclusions reached in this study would be expected to apply to nasal breathing studies as well. Further studies using controlled breathing patterns, or face mask exposures, in healthy and asthmatic subjects would help to address this possibility.

These studies indicate that UFP deposition is greater in people with asthma than in healthy people. When both the increased DF and minute ventilation were considered, the total number of particles retained in the lung was 74% greater in subjects with asthma than in healthy subjects. Thus, people with asthma have a higher total respiratory dose of UFPs for a given exposure, which may contribute to their increased susceptibility to the health effects of air pollution.

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