

Ultrafine Particle Deposition and Clearance in the Healthy and Obstructed Lung

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Numerous epidemiologic studies have shown associations between exposure to particulate air pollution and acute increases in morbidity and mortality, particularly in persons with chronic obstructive pulmonary disease. The dosimetry of ultrafine particles in the human lung is poorly characterized. We studied the deposition and clearance of an ultrafine technetium-99m-labeled aerosol in 10 patients with chronic obstructive pulmonary disease and in 9 healthy subjects. Particle retention was followed for 2 hours after inhalation and again at 24 hours by γ scintigraphy. Central-to-peripheral ratios indexed airway deposition. Particle accumulation in the liver was examined by quantifying activity below the right lung. The dose rate for an aerosol exposure of 10 $\mu\text{g}/\text{m}^3$ was calculated. Patients had a significantly greater dose rate than healthy subjects (2.9 ± 1.0 versus 1.9 ± 0.4 $\mu\text{g}/\text{h}$, $p = 0.02$). Central-to-peripheral ratios were slightly greater in patients than in healthy subjects (1.11 ± 0.10 versus 1.01 ± 0.11 , $p = 0.05$). Clearance did not statistically differ between health and disease. On average, 24-hour retention was $85 \pm 8\%$ (corrected for isotope dissolution). No accumulation in the liver's vicinity was observed. Data suggest that relative to healthy subjects, patients with moderate-to-severe airways obstruction receive an increased dose from ultrafine particle exposure.

Keywords: aerosols; mucociliary clearance; chronic obstructive pulmonary disease

Epidemiologic studies have shown consistent associations between the exposure to particulate air pollution and acute increases in morbidity and mortality rates, especially for persons with obstructive lung and cardiovascular diseases (1, 2). Some toxicologic studies have implicated ultrafine particles (less than 0.1 μm in diameter), which by count constitute the majority of urban particulate air pollution, as having increased toxicity relative to larger particles composed of the same materials (3, 4). Epithelial uptake of ultrafine particles may occur in as little as 1 hour after exposure and may cause damage to epithelial cells, pulmonary edema, and eventually fibrosis (4, 5). An increased probability of death for persons with cardiovascular diseases has been proposed as a result of an inflammatory response caused by ultrafine particle overloading and subsequently increased blood coagulability (6).

Increased plasma viscosity has been found in association with elevated sulfur dioxide and particulate levels (7). A recent study has also shown an association between ultrafine particulate exposure and decreased peak expiratory flows in patients with asthma (8). Ultrafine aerosol exposure may contribute to the increase in morbidity and mortality observed by epidemiologic studies.

A fair amount of data on the deposition of ultrafine particles in healthy volunteers is available (9–13). However, ultrafine deposition data for the diseased lung are limited to five chronic obstructive pulmonary disease (COPD) patients and three restrictive lung disease patients studied by Anderson and colleagues (11). Data for ultrafine particle clearance from the human lung are far more limited. Roth and colleagues (14) reported a total lung clearance of 7% over a 24-hour period for an 18-nm (count modal diameter) indium oxide aerosol in three healthy subjects. To the best of our knowledge, no other ultrafine particle clearance data in humans are reported in the literature.

A number of studies have been published on the deposition and/or clearance of agglomeration-mode carbon particles, Technegas. The primary particles that compose Technegas are in the range of 5–20 nm (15, 16). However, before inhalation, these primary particles coagulate into aggregates with a median diameter in the range of 100 to 160 nm (15–17). Technegas particles can be as large as 225 nm if the time between aerosol generation and inhalation increases to 8.5 minutes (16). Although Technegas is frequently referred to as an ultrafine aerosol (18–20), inferences based on these studies with regard to the behavior of ultrafine particles in the respiratory tract should be made with caution.

From a dosimetric viewpoint, the distinction between an inhaled aerosol with a median diameter of 20 nm versus one of 100 or 200 nm is very important. Particle size affects the site and amount of particle deposition as well as subsequent clearance. Considering a sedentary breathing pattern (tidal volume [V_T] = 500 mL, frequency [f] = 15 min^{-1}), the total deposition of 20-nm particles is 2.7 times greater than predicted for 100-nm particles and is 4.3 times greater than predicted for 200-nm particles (21). Moreover, the site of deposition in the lung shifts proximally with decreasing particles size below 100 nm (13). This proximal shift in deposition affects not only the tissue dose to the airways but also the subsequent mucociliary clearance rate (i.e., a shorter path length for clearance from the lung).

The purpose of our study was to characterize the deposition and clearance of a technetium-99m-labeled ultrafine aerosol in patients with COPD and healthy age-matched volunteers. Ten patients with moderate to severe COPD (six men and four women; 45–70 years old) and nine healthy nonsmokers (three men and six women; 40–67 years old) participated in this study. Lung function, that is, spirometry, plethysmography, and diffusing capacity (MedGraphics, St. Paul, MN, and SensorMedics, Yorba Linda, CA) were measured in each subject. Single-breath nitrogen washout phase III slope and multibreath xenon whole-lung washout rate

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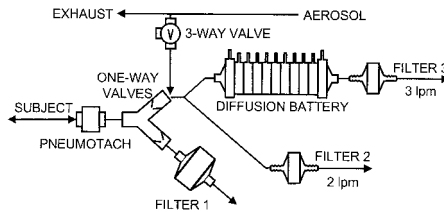


Figure 1. The aerosol delivery system. Once the subject began breathing through the pneumotach, a three-way valve was switched to supply aerosol rather than room air. One-way valves routed aerosol to and from the subject. Exhaled aerosol was captured on Filter 1, a low-resistance filter (BB-50T; Pall Biomedical). The aerosol's specific activity and *in vitro* isotope leaching from particles were based on analysis of Filter 2, a glass fiber filter (Type A/E; Gelman Sciences, Ann Arbor, MI). The aerosol's activity median diameter was determined using a diffusion battery (25) and a glass fiber filter, Filter 3.

were also determined (22). This study was approved by the Committee on the Protection of the Rights of Human Subjects (School of Medicine, University of North Carolina at Chapel Hill, NC). Before participating in this study, all subjects were informed of the study's associated risks and signed a statement of informed consent.

METHODS

Aerosol Inhalation

We have previously described the generation and characterization of the experimental technetium-99m-labeled ultrafine carbon aerosol (23). To control coagulation, aerosol was continuously generated and diluted with filtered air.

The subjects inhaled aerosol at their natural breathing pattern (24) until approximately 25 μCi was deposited in the lung. Activity was monitored by a single crystal (NaI) scintillation detector (ND detector, Model LMS-44; Nuclear Data Inc., Smyrna, GA) against the back. Figure 1 illustrates the aerosol delivery system and sampling locations. The aerosol's count median diameter (CMD) was measured by a Scanning Mobility Particle Sizer (TSI Incorporated, St. Paul, MN) for 10 subjects. The aerosol's activity median diameter (AMD), and leaching characteristics were determined for all subjects (23, 25).

Before aerosol inhalation, a xenon-133 equilibrium scan was acquired by a large field of view γ camera (Elsinc Apex 415, Haifa, Israel) (26). After aerosol inhalation, retention scans (2 minutes per scan) were recorded for 2 hours by the γ camera. At approximately 24 hours after inhalation, subjects returned for a 30-minute retention scan.

Data Analysis

The deposition fraction (DF) of aerosol in the lung was defined as

$$DF = 1 - \frac{A_{\text{Ex}}}{A_{\text{In}}}$$

where A_{Ex} and A_{In} are exhaled and inhaled activity, respectively. A_{Ex} was the ND detector measured counts on Filter 1 (Figure 1). The filter was centered at 1 foot from the detector face and counts accumulated for 30 seconds. This method was also used for diffusion battery stages, other filters, and isotope leaching measurements. A_{In} was the product of total inspired volume during aerosol inhalation and the aerosol's specific activity. Total inspired volume was computed as the product of average inspiratory flow, aerosol inhalation time, and fraction of breathing devoted to inhalation. Aerosol specific activity was the counts on Filter 2 (Figure 1) divided by volume sampled through this filter. DF data were corrected for a V_t -dependent bias (APPENDIX). The dose rate (D_{rate}) for sedentary breathing was calculated for each subject:

$$D_{\text{rate}} = DF \cdot V_{\text{min}} \cdot C$$

where V_{min} is minute ventilation and C is an assumed exposure concen-

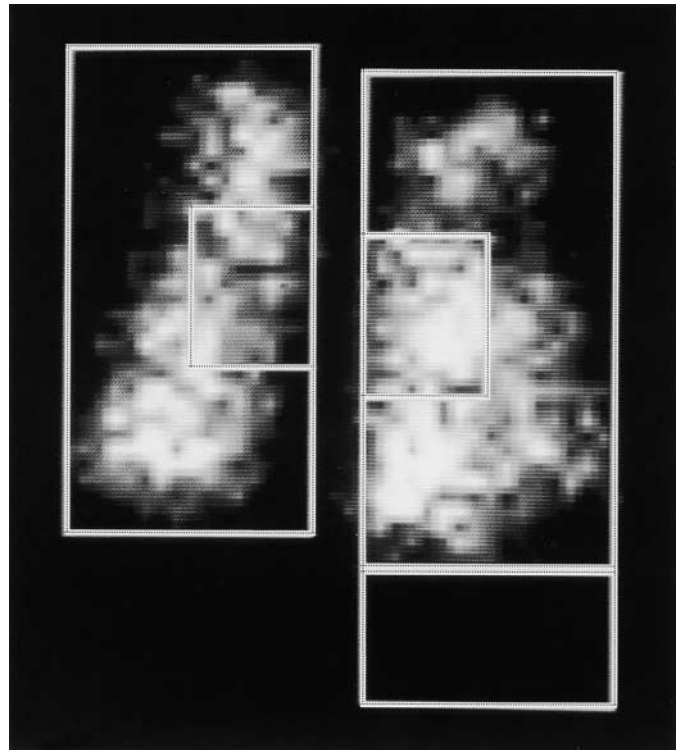


Figure 2. An initial deposition scan for a COPD patient (61-year-old female, 31% predicted FEV_1) illustrating whole lung, central, and the liver ROI. The whole-lung ROIs for each lung were set at 20% of the peak xenon equilibrium counts. Central ROIs, with dimensions equal to half the lung's width and one-third its height, were positioned on the interior boundary of the lung, centered by height. A liver ROI was established immediately below the right lung with dimensions of one third the lung's height and its full width. Despite severe COPD, qualitatively, the pattern of deposition appears fairly uniform and quantitatively, the patient's C/P_0 ratio is still only 1.17.

tration of $10 \mu\text{g}/\text{m}^3$. This exposure concentration was used because epidemiologic studies commonly express health risk from ambient particulate exposure in $10 \mu\text{g}/\text{m}^3$ increments (2).

Scintigraphic Analysis

Figure 2 illustrates regions of interest (ROIs). Pulmonary particle retention was determined from combined counts of the left and right whole-lung ROIs (background/decay corrected). Twenty-four-hour retention was "corrected" for isotope dissolution by dividing by one minus the fraction of activity leached from particles. Central-to-peripheral (C/P) ratios index large airways deposition (27–29) and were determined for the initial retention scan (C/P_0) and 24-hour retention scan (C/P_{24}). C/P ratios were computed as the fraction of aerosol deposited in central regions relative to peripheral regions and normalized to volume using the same regions with an xenon equilibrium scan (26).

Two statistical methods tested differences in clearance rates between healthy subjects and COPD patients. At 1, 2, and 24 hours after inhalation, differences were tested using a two-tailed t test. Differences in retention curves through 2 hours after inhalation were tested by a linear mixed-effects model (30, 31). The logarithm of retention was used in this model fit by S-plus 2000 Professional (MathSoft Inc., Seattle, WA).

Activity in the liver was quantified through 2 hours after inhalation for comparison with the results of Nemmar and colleagues (20). Liver ROI counts (background/decay corrected) were normalized by the sum of counts (background corrected) in the whole-lung ROIs and liver ROI from the initial retention scan.

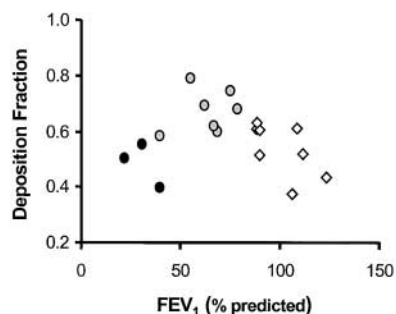


Figure 3. The particle DF in each subject versus pulmonary function. The DF in seven bronchitic patients (gray circles) was significantly ($p < 0.04$) greater than in the nine healthy subjects (open diamonds) and the three emphysemic patients (black circles) (see Table 2).

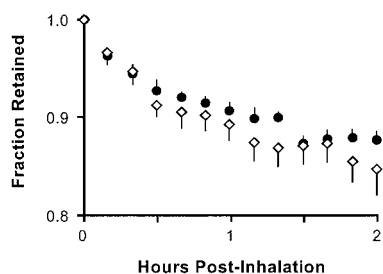


Figure 5. Pulmonary particle retention over the 2-hour period immediately after inhalation in 10 COPD patients (closed circles) and 9 healthy subjects (open diamonds). Values are mean data, and bars reflect SEs. Note that the ordinate begins at 80% retention. Although not

statistically significant, the COPD patients tended to have slower clearance than the healthy subjects.

RESULTS

Unless otherwise stated, data provided in the text are mean \pm SD, and data illustrated in Figures 5 and 6 are mean \pm SEM. Between-group differences were tested using Student's *t* test (two-tailed) with the exception of the mixed-effects model used to test for differences in particle retention. Pearson correlation coefficients were used to determine associations between variables. The experimental aerosol had an AMD of 61 ± 4 nm and a geometric standard deviation (σ_g) of 2.1 ± 0.1 . The aerosol's CMD was 33 ± 2 nm with a σ_g of 1.7 ± 0.03 . Primary particles that aggregated to form the experimental aerosol had a CMD of approximately 22 nm (23). There were no statistically significant differences in aerosol characteristics between groups. Anthropometric and baseline pulmonary function data are given in Table 1. Single-breath nitrogen washout was not conducted on one COPD patient because of equipment failure. The COPD patients were subdivided into a bronchitic and an emphysemic group, with the latter having less than 60% predicted carbon monoxide diffusing capacity and diffusing capacity normalized to lung volume. Significant differences between the COPD patient subgroups were observed for pulmonary function data (Table 1), DF data (Table 2), and spontaneous breathing patterns (Table 2).

Inhaled aerosol deposition (total and regional) and breathing patterns during the aerosol inhalations are provided in Table 2. The DF datum for one healthy subject was discarded because of valve leaks. The aerosol DF in the bronchitic patients was significantly greater than the DF in either the emphysemic patients or the healthy subjects. During aerosol inhalations, the bronchitic group had a significantly longer breathing period than both the emphysemic group and the healthy subjects. These bronchitic patients also breathed aerosol at a V_T that was significantly greater than the healthy subjects and tended to be greater than the emphysemic group. The V_T -dependent DF correction (described in the METHODS) increased with decreasing V_T ; hence, the effect of the DF correction should have been to bias the observed increase in DF for the bronchitic patients toward the

null. No significant correlations were observed between DF and breathing pattern within any group (e.g., bronchitic subjects) nor across groups (e.g., combined data for bronchitic and healthy subjects). DF data as a function of forced expiratory volumes are illustrated in Figure 3. In healthy subjects ($n = 8$), DF was significantly correlated with FEF_{25-75} ($r = -0.73$, $p = 0.04$). In the COPD patients ($n = 10$), because of the emphysemic data, there was a positive correlation between DF and both percentage predicted FEV_1 ($r = 0.65$, $p = 0.04$) and percentage predicted diffusing capacity normalized to lung volume ($r = 0.80$, $p = 0.005$). Significant negative correlations were again observed for the combined dataset ($n = 15$) of the bronchitic group and healthy subjects between DF and both FEF_{25-75} ($r = -0.77$, $p = 0.001$) and percentage predicted FEV_1 ($r = -0.65$, $p = 0.009$). Across all subjects, DF was not correlated with these variables. To better elucidate the driving factors affecting DF, a forward stepwise regression was performed for all subjects ($n = 18$), which included all pulmonary function data in Table 1, aerosol breathing pattern (Table 2), and lung volumes (FRC, RV, TLC, FRC/TLC, RV/TLC). In Table 3, the results of this analysis show that DF increases with the percentage predicted diffusing capacity normalized to lung volume ($p = 0.001$) and decreases with FEF_{25-75} ($p < 0.001$).

The D_{rate} of the aerosol (Table 2), assuming an exposure concentration of $10 \mu\text{g}/\text{m}^3$, was significantly increased in the COPD patients ($p = 0.02$) and the bronchitic patients ($p = 0.002$) relative to healthy subjects. The D_{rate} for the emphysemic patients was not significantly different from that of the bronchitic patients. Figure 4 illustrates D_{rate} as a function of percentage predicted FEV_1 . For combined dataset ($n = 15$) of the bronchitic group and healthy subjects, D_{rate} was correlated with percentage predicted FEV_1 ($r = -0.74$, $p = 0.002$) and to a lesser extent with FEF_{25-75} ($r = -0.61$, $p = 0.02$). Similarly, across all subjects ($n = 18$), D_{rate} was correlated with both percentage predicted FEV_1 ($r = -0.64$, $p = 0.004$) and FEF_{25-75} ($r = -0.48$, $p = 0.04$). A forward stepwise regression was performed to elucidate

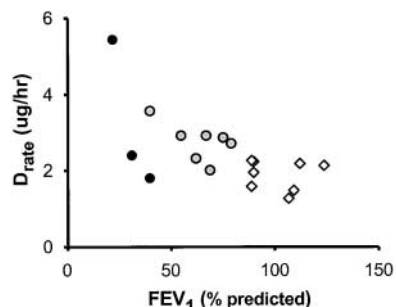


Figure 4. D_{rate} in each subject versus pulmonary function. Across all subjects ($n = 18$), D_{rate} and lung function were significantly correlated ($r = -0.64$, $p = 0.004$). Healthy subjects = open diamonds; emphysemic patients = black circles; bronchitic patients = gray circles.

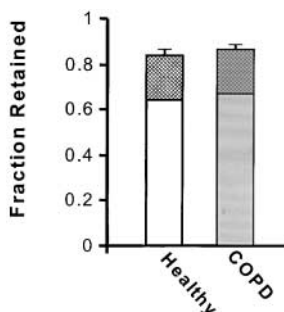


Figure 6. Pulmonary particle retention by disease status at 24 hours after inhalation in 10 COPD patients and 9 healthy subjects. Values are mean data, and bars reflect SEs. The hatched area indicates a correction for $23 \pm 4\%$ isotope leaching from particles determined by *in vitro* measurements.

TABLE 1. GROUP MEAN AGE AND PULMONARY FUNCTION DATA

| | Healthy Subjects (3 M, 6 F) | COPD Patients | | |
|---|--------------------------------|----------------------------|-----------------------------------|-----------------------------------|
| | | All Patients (7 M, 3 F) | Bronchitic Patients (5 M, 2 F) | Emphysemic Patients (2 M, 1 F) |
| Age, yr | 53 ± 11 | 61 ± 6.9* | 60 ± 7.5 | 64 ± 5.2 |
| FEF ₂₅₋₇₅ , lps | 2.82 ± 0.90 | 0.50 ± 0.23* | 0.57 ± 0.24* | 0.34 ± 0.02* |
| FEV ₁ , % predicted | 104 ± 16 | 54 ± 20* | 64 ± 13* | 31 ± 9.0* |
| FEV ₁ /FVC, % | 79 ± 3.1 | 44 ± 13* | 50 ± 10* | 29 ± 6.4* |
| Raw, cm H ₂ O/lps | 1.26 ± 0.42 | 2.52 ± 0.91* | 2.16 ± 0.63* | 3.36 ± 1.04* |
| sRaw, cm H ₂ O/s | 4.83 ± 1.69 | 16.8 ± 14.8* | 10.3 ± 3.65* | 32.0 ± 21.1* |
| D _{LCO} , ml CO/min/mm Hg | 23 ± 3.7 | 17 ± 7.0* | 20 ± 5.6 | 9.6 ± 1.8* |
| D _{LCO} , % predicted | 112 ± 9.1 | 72 ± 23* | 84 ± 13* | 43 ± 4.5* |
| D _{LCO} /VA, ml CO/min/mm Hg/L | 4.47 ± 0.81 | 3.03 ± 1.12* | 3.60 ± 0.68* | 1.68 ± 0.56* |
| D _{LCO} /VA, % predicted | 109 ± 17 | 78 ± 27* | 92 ± 15† | 44 ± 14* |
| ΔN ₂ , %/L | 1.85 ± 0.82 | 8.88 ± 5.26* | 5.69 ± 2.60* | 15.3 ± 1.5* |
| Xe _w , min ⁻¹ | -1.00 ± 0.21 | -0.75 ± 0.28* | -0.88 ± 0.19 | -0.42 ± 0.10* |

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; D_{LCO} = carbon monoxide diffusing capacity; D_{LCO}/VA = diffusing capacity normalized to lung volume; ΔN₂ = single breath nitrogen washout's phase III slope; Raw = airway resistance; sRaw = specific airway resistance; Xe_w = multibreath xenon washout rate.

* Significantly different from healthy subjects ($p < 0.05$).

† Significantly different from bronchitic subjects ($p < 0.01$).

‡ Marginally significant difference from healthy subjects ($p = 0.058$).

Values are means ± SD. Predictive equations for FEV₁ are from Knudson and colleagues (32). Predictive equations for D_{LCO} and D_{LCO}/VA are from Burrows and associates (33).

factors affecting D_{rate} in the same manner as described for DF. In Table 3, the results of this analysis show that D_{rate} increases with both V_T ($p < 0.001$) and specific airway resistance ($p < 0.001$).

An index of airway deposition, C/P₀ ratios were slightly increased in the COPD patients relative to the healthy subjects (COPD, 1.11 ± 0.10; healthy, 1.01 ± 0.11, $p = 0.05$). In COPD patients, but not in healthy subjects, C/P₂₄ ratios were significantly lower than C/P₀ ($p = 0.003$). The C/P₂₄ ratios do not differ significantly between the healthy subjects and COPD subjects. The C/P₀ in COPD patients was the only C/P ratio that was significantly different from one ($p = 0.007$). C/P ratios were not correlated with lung function either within or across groups.

Pulmonary particle retention through 2 hours after inhalation for the COPD patients and healthy subjects is illustrated in Figure 5. Statistically, there was no difference in the 2-hour

clearance curves between the COPD patients and the healthy subjects. However, a tendency for slower clearance in the COPD patients than healthy subjects could be observed. As illustrated in Figure 6, there was also an insignificant tendency for greater 24-hour retention (uncorrected and corrected for isotope leaching from particles) in the COPD patients relative to the healthy subjects. The 24-hour retention, measured at 23 ± 2 hours after inhalation, was 64 ± 5% in healthy subjects and 67 ± 4% in COPD patients ($p = NS$). Not differing between groups, the *in vitro* leaching of isotope from particles in saline was 23 ± 4% after 23 ± 2 hours in solution. The 24-hour retention after correction for this leaching was 84 ± 9% in healthy subjects and 87 ± 8% in COPD patients ($p = NS$). This leaching correction was applied only to the 24-hour retention data and not to the 2-hour clearance curves.

No accumulation of particles in the liver ROI was observed.

TABLE 2. AVERAGE DEPOSITION (AIRWAYS AND TOTAL) AND BREATHING PATTERNS GROUPED BY DISEASE STATUS

| | Healthy Subjects (3 M, 6 F) | COPD Patients | | |
|---------------------------|--------------------------------|----------------------------|-----------------------------------|-----------------------------------|
| | | All Patients (7 M, 3 F) | Bronchitic Patients (5 M, 2 F) | Emphysemic Patients (2 M, 1 F) |
| DF, % | 54 ± 9* | 62 ± 12 | 67 ± 8* | 48 ± 8† |
| D _{rate} , μg/hr | 1.88 ± 0.38 | 2.90 ± 1.02* | 2.77 ± 0.48* | 3.20 ± 1.94 |
| C/P ₀ | 1.01 ± 0.11 | 1.11 ± 0.10* | 1.13 ± 0.10* | 1.06 ± 0.11 |
| C/P ₂₄ | 0.92 ± 0.13 | 1.00 ± 0.13‡ | 1.04 ± 0.10‡ | 0.90 ± 0.14 |
| V _T , ml | 391 ± 92 | 621 ± 322 | 657 ± 333* | 537 ± 344 |
| T _{insp} , s | 2.02 ± 0.24 | 2.34 ± 0.87 | 2.73 ± 0.74* | 1.44 ± 0.17* |
| V _{min} , L/min | 5.83 ± 1.36 | 8.13 ± 3.74 | 6.93 ± 1.65 | 10.9 ± 6.2* |

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; C/P₀ and C/P₂₄ = central-to-peripheral ratio initially and at 24 hours, respectively; DF = particle deposition fraction in the lung; D_{rate} = deposition rate at a constant 10 μg/m³ exposure to the experimental aerosol; T_{insp} = average inspiratory time during aerosol inhalation; V_{min} = minute ventilation; V_T = tidal volume during aerosol inhalation.

* Significantly different from healthy subjects ($p \leq 0.05$).

† Significantly different from bronchitic subjects ($p < 0.02$).

‡ DF datum not available for one healthy male subject.

§ Significantly different from C/P₀ by paired *t* test ($p < 0.03$).

Values are means ± SD.

TABLE 3. BEST REGRESSION MODELS FOR PARTICLE DEPOSITION FRACTION IN THE LUNG AND DEPOSITION RATE IN ALL SUBJECTS

| Dependent Variable | Independent Variable | Coefficient | p Value |
|---------------------------|---|-------------|----------|
| DF, % | FEF ₂₅₋₇₅ , lps | -7.54 | 0.0003 |
| | D _{LCO} /V _A , % predicted | 30.9 | 0.0012 |
| | Constant, % | 40.6 | < 0.0001 |
| | (Overall regression, R ² = 0.62, p = 0.0007) | | |
| D _{rate} , µg/hr | V _T , L | 1.770 | 0.0008 |
| | sRaw, cm H ₂ O s | 0.044 | 0.0002 |
| | Constant, µg/hr | 1.015 | 0.0005 |
| | (Overall regression, R ² = 0.81, p < 0.0001) | | |

Definition of abbreviations: DF = particle deposition fraction in the lung; D_{rate} = deposition rate at a constant 10 µg/m³ exposure to the experimental aerosol; D_{LCO}/V_A = diffusing capacity normalized to lung volume, predictive equations Burrows and colleagues (33); sRaw = specific airway resistance; V_T = tidal volume during aerosol inhalation.

The fraction of activity in the liver ROI was analyzed at 10-minute increments throughout the 2-hour period after inhalation. On average across all subjects, the fraction of activity in the liver ROI was $1.3 \pm 1.2\%$ initially, $1.4 \pm 0.8\%$ at 1 hour, and $1.3 \pm 0.8\%$ at 2 hours after inhalation. No significant deviation in the fraction of activity in the liver ROI during the 2-hour period was observed in COPD patients, healthy subjects, nor across all subjects. There was no significant difference in the fraction of activity in the liver ROI between the COPD patients and healthy subjects.

DISCUSSION

We investigated the deposition and clearance of an ultrafine aerosol (CMD, 33 ± 2 nm; AMD, 61 ± 4 nm) in a group of 10 COPD patients (45–70 years old) with moderate to severe airways obstruction and 9 healthy volunteers (40–67 years old). We found no difference in particle clearance rates between the healthy subjects and COPD patients, nor were we able to detect the accumulation of particles in the vicinity of the liver. Based on diffusing capacity, the COPD patients were subdivided into groups of seven bronchitic patients and three emphysemic patients. We found an increased particle DF in the bronchitic patients relative to the healthy subjects and emphysemic patients (Table 2); however, when deposition was expressed as a D_{rate}, it was observed that the D_{rate} in emphysemic patients actually tended to be increased relative to the bronchitic patients. Moreover, larger relative increases in D_{rate} (54%) were observed than for DF (15%) in the COPD patients compared with healthy subjects. This is due to the fact that, at rest, the COPD patients breathe spontaneously with a higher minute ventilation than the healthy individuals (24).

A comparison of our results with prior studies is possible for the DF data in healthy subjects, and to a lesser extent, in the COPD patients. However, even comparisons of DF in healthy individuals are complicated because of differing methods. For example, decreasing particle size below 100 nm and increasing residence time will effect an increase in DF (9, 10, 12, 13). Theoretically, there is also an effect of polydisperse versus monodisperse aerosol inhalation on DF, such that increasing polydispersity below approximately 40 nm should decrease DF, whereas above this particle size, it should cause an increase in DF (34). Finally, age effects on ultrafine particle deposition are unknown. With these issues noted, the data of a few studies are discussed later here.

Jaques and Kim (12) conducted an extensive particle deposition study in 22 young healthy adults (11 females and 11 males)

with six breathing patterns and a CMD of 40, 60, 80, and 100 nm. For the breathing pattern of V_T = 500 ml and f = 15 min⁻¹ (the most comparable to that of our healthy subjects) and a CMD of 40, 60, 80, and 100 nm, they found the DF of $44 \pm 7\%$, $35 \pm 5\%$, $30 \pm 5\%$, and $26 \pm 4\%$, respectively. Schiller and colleagues (10) also provided a large dataset for a range of particle sizes (5 to 200 nm) and 11 different breathing patterns, but only in 4 healthy male subjects. These investigators found slightly lower DF than observed in male subjects by Jaques and Kim (12). For polydisperse carbon particle aggregates (CMD = 26 nm, $\sigma_g = 2.3$), Frampton and colleagues (35) reported a DF of $66 \pm 12\%$ by count and $58 \pm 14\%$ by mass in 12 healthy young adults (6 females and 6 males) spontaneously breathing on a mouthpiece. As a technical note, the DF data reported by Jaques and Kim (12) and by Schiller and associates (10) were for specific monodisperse particle sizes, as such there should be no differences in DF determined by count versus mass. Our DF data are for a polydisperse aerosol and are based on the amount of radioaerosol collected on filters making them similar to a DF determined by a change in mass. Although in the range of other studies, our methods and results are most similar to that of Frampton and colleagues (35), with a DF of $54 \pm 9\%$ for a polydisperse aerosol (CMD, 33 nm; $\sigma_g = 1.7$) inhaled with a V_T = 391 ml and f = 15 min⁻¹ by nine healthy subjects.

Only one prior study's data are available for comparison of DF results in patients. For the CMD of 20, 40, and 60 nm respired at V_T = 1000 ml and f = 12 min⁻¹, Anderson and colleagues (11) reported the DF (relative to CMD) of $47 \pm 9\%$, $38 \pm 6\%$, $33 \pm 9\%$ in 10 healthy subjects and $54 \pm 4\%$, $46 \pm 4\%$, $45 \pm 4\%$ in 5 patients with obstructive lung disease. These DF data are lower than we have observed. Anderson and colleagues' DF data for healthy subjects are also lower than reported by Jaques and Kim (12) and Schiller and colleagues (10). However, Anderson and colleagues' relative increases in DF due to obstructive lung disease are 15, 21, and 36% for the respective CMD of 20, 40, and 80 nm. Similarly, for our 33-nm CMD aerosol, we found DF increased by 15% in COPD as a whole and by 26% in the bronchitic patients relative to the healthy subjects. Anderson and colleagues (11) also reported that DF was not correlated with any spirometric or lung volume parameters in the five obstructive lung disease patients. Possibly due to our larger sample size, we found correlations between lung function and DF in the COPD patients.

Unlike most prior studies of particle deposition, the subjects in our study respired aerosol at their natural sedentary breathing pattern, whereas other studies typically have all subjects breathe at the same fixed conditions. Although this disparity in method complicates comparisons between subject groups and between studies, it is advantageous in that data may be derived that are applicable to real-world exposure-dose scenarios. Accordingly, D_{rate} were calculated and provide an estimate of the ultrafine particle mass that would be deposited per hour in the lungs of subjects while at rest. We found that the COPD patients had a 54% greater D_{rate} than did healthy subjects (p = 0.02). Based on DF alone, these patients had an increase of only 15% relative to healthy subjects (p = NS). Interestingly, the emphysemic patients appeared to have 11% less deposition than the healthy subjects based on their average DF, whereas their D_{rate} showed a 70% average increase in deposition relative to the healthy subjects. Again, this is due to the fact that, at rest, these patients tended to breathe spontaneously with a higher minute ventilation than the healthy individuals. These findings are consistent with Bennett and colleagues (24), who found greater increases in the D_{rate} than for the DF in COPD patients relative to healthy subjects for a fine 2-µm mass median aerodynamic diameter aerosol.

Particle deposition within the airways was also increased in COPD patients relative to the healthy subjects. An index of excess airways deposition, C/P_0 ratios were significantly ($p = 0.05$) increased in the COPD patients relative to healthy subjects (1.11 ± 0.10 versus 1.01 ± 0.11). The increased C/P_0 ratios in COPD patients could reflect enhanced airways deposition of ultrafine particles by turbulent diffusion caused by airway obstruction, although no significant correlations were observed between C/P_0 ratios and spirometry to support this supposition. In addition to their increased D_{rate} , the increased C/P_0 ratios show the potential for greater particle doses to the airways of patients relative to healthy individuals. It should be noted, however, that the "increased" C/P_0 ratios that we observed in COPD patients reflect far less airways deposition than is found for coarse particles, which deposit by inertial impaction. For example, Bennett and Zeman (36) found a C/P_0 of 2.2 in 12 COPD patients ($FEV_1/FVC = 55\%$) who respired a 5- μm mass median aerodynamic diameter aerosol at $V_T = 500$ ml and $f = 30$ min⁻¹. At 24 hours after inhalation, those patients had a mean retention of approximately 13%, suggesting that 87% of the particles were deposited within and subsequently removed from the airways via mucociliary clearance. Quite the opposite, we found a 24-hour retention of $87 \pm 8\%$ in COPD patients after correction of isotope leaching. Despite the statistically increased C/P_0 ratios in our COPD patients, comparison with the data for larger particles illustrates the small degree of ultrafine particle deposition in the bronchial airways.

Pulmonary particle clearance was similar between the COPD patients and healthy subjects. There was an insignificant tendency for greater retention in the COPD patients relative to the healthy subjects during the 2-hour period after deposition, which was also observed at 24 hours. Retention at 24 hours after deposition was corrected for 23% isotope leaching from particles in saline. The leaching characteristics of the isotope did not differ between the healthy subjects and COPD patients. On average, this leaching correction increased 24-hour retention from $66 \pm 5\%$ to $85 \pm 8\%$. This *in vitro* method has been used by others and provides a conservative estimate of leaching, that is, greater technetium-99m dissolution from particles, relative to *in vitro* leaching in simulated lung fluid (37) or *in vivo* leaching after radiolabeled aerosol inhalation (38). Hence, $85 \pm 8\%$ is likely an upper bound for the expected particle retention at 24 hours after deposition. It is not expected that isotope leaching would have caused differences between the healthy subjects and COPD patients. Smokers have been shown to have increased lung epithelial permeability as demonstrated by increased clearance rates of inhaled soluble compounds (39). Hence, an effect of leaching might have been to increase rapid (less than 20 minutes) clearance in the COPD patients relative to the healthy subjects. Referring to Figure 5, clearance rates were indistinguishable until 30 minutes after inhalation. The pulmonary clearance kinetics of the experimental aerosol appear quite similar for the COPD patients and healthy subjects.

Based on similar clearance kinetics and only modestly increased C/P_0 ratios, relative to healthy subjects, it is likely that the increased D_{rate} in COPD patients primarily affects the parenchymal lung. Assuming a uniform ventilation distribution in the lung, the parenchymal surface dose in COPD patients is 54% greater than in healthy subjects. However, in COPD patients, the assumption of uniform ventilation is questionable. In patients with COPD, the time constants of some diseased regions are thought to be in excess of 3 seconds, whereas healthy regions might be expected to have a time constant of the order of 0.1 seconds (40). This variability in regional time constants leads to the observation of slower and less uniform rates of air exchange in patients relative to healthy individuals (41), which may in

turn affect regional particle deposition. Ultrafine particles may penetrate into poorly ventilated regions and then have enhanced deposition due to the long time constant of these regions. Increased particle deposition in poorly ventilated lung regions has been reported for aerosols in the 0.5 to 1.0 μm size range and attributed to increased residence time in obstructed areas (42, 43). On the other hand, as ventilation is reduced to obstructed regions, healthier regions receive the balance of ventilation and associated particle exposure. To an extent, these phenomena have been observed in patients with mild-to-moderate asthma where large wedge-shaped regions of the lung were observed absent the deposition of fine particles (approximately 0.12 μm diameter) (44). Whether ultrafine particle deposition is enhanced in poorly ventilated regions or shifted to healthy regions, the pattern of deposition is in all likelihood far less uniform in COPD patients than in the healthy subjects. As a first approximation of this effect, Miller and colleagues (45) assumed that a quarter of the parenchymal lung in COPD patients might receive all the deposition normally distributed across the full parenchymal lung. Applying this approximation and the observed D_{rate} , the parenchymal lung tissues in COPD patients would receive a 600% greater dose than these tissues within a healthy individual. Such an increased surface dose in sensitive patients might be sufficient to elicit an inflammatory response with systemic effects such as those proposed by Seaton and colleagues (6).

Others have proposed that ultrafine particles elicit an effect by moving rapidly (within minutes after deposition) into circulation and accumulating in other organs such as the liver. We found no such accumulation of particles in the vicinity of the liver. On average across all subjects ($n = 19$), we observed $1.3 \pm 1.2\%$ of the deposited activity in the liver ROI on the initial retention scan. This activity may be due to scatter from the lung and/or overlap of lung parenchyma in the liver ROI. The relative amount of activity in the liver ROI did not significantly deviate over the 2-hour period after inhalation. In contrast, Nemmar and colleagues (20) reported that 8% ($n = 5$) of deposited activity accumulated in the liver by 5 minutes after inhalation. They also observed the accumulation of 25% of the deposited activity in the bladder by 45 minutes with large unquantified amounts of activity in the thyroid, salivary glands, and stomach. Although not reported, their pulmonary retention at 45 minutes after inhalation must be on average less than 67% after accounting for activity in the liver and bladder. This contrasts sharply with the 91% average retention that we observed at 45 minutes. Prior studies have reported pulmonary retention at 45 minutes for Technegas, the aerosol used by Nemmar and associates (20). In contrast to Nemmar and colleagues (20), the Technegas pulmonary retention reported by Roth and associates (17) was 95% ($n = 3$) and by Isawa and colleagues (46) was 98% ($n = 11$).

The disparity in results between Nemmar and colleagues (20) and the results of our present study and prior studies using Technegas may be due to Pertechnegas contamination. Unlike Technegas, which is considered relatively stable in the lung, Pertechnegas is thought to be rapidly ionized to pertechnetate (TcO_4^-) in the presence of moisture (39, 47, 48). In fact, the pulmonary clearance kinetics of inhaled pertechnetate and Pertechnegas are statistically indistinguishable (39, 47). Technegas and Pertechnegas are produced by the same proprietary generator under differing atmospheres (Technegas, 100% argon; Pertechnegas, 3% oxygen and 97% argon) (16, 39, 47, 49). The transition from Technegas to Pertechnegas occurs abruptly at an oxygen concentration of between 0.1% and 0.2% (49). Hence, minimal oxygen contamination within the generator can cause Pertechnegas contamination of Technegas. Even when produced under a 100% argon atmosphere, Technegas still has an immediately soluble fraction of approximately 20% (49). Xu and col-

leagues (48) reported Per technegas contamination of Technegas in their clearance study of patients ($n = 6$) and healthy volunteers ($n = 5$). These investigators noticed the contamination because of thyroid visualization and rapid clearance during the first 20 minutes after inhalation (9.3 ± 1.8 minute half-time; $n = 11$). The rapid clearance rates found by Xu and associates (48) are quite similar to the Per technegas half-times reported by Monaghan and colleagues (39) and Isawa and colleagues (47) in healthy subjects of 10.1 ± 2.4 minutes ($n = 22$) and 10.8 ± 1.8 minutes ($n = 5$), respectively. Interestingly, Monaghan and colleagues (39) provided a whole-body scan illustrating organ uptake of Per technegas, which appears remarkably similar to the whole-body scan from Nemmar and associates (20) with high amounts of activity evident in the bladder, stomach, thyroid, and salivary glands. Based on the organ uptake reported by Nemmar and colleagues (20) and rapid pulmonary clearance rates inferred from their findings, the bulk of the lung clearance that they observed was very likely that of per technetate ionized from Per technegas and not that of radiolabeled ultrafine particles.

We have found that patients with moderate to severe COPD have an increased D_{rate} of 54% relative to healthy subjects. Some of this increased deposition occurs in the airways, although most likely occurs in the parenchymal lung. Given that these patients have heterogeneous ventilation within their lungs, they may receive a tissue dose to the parenchymal lung that is many times that occurring in the healthy lung. We found no evidence of the rapid pulmonary clearance of these insoluble, carbon ultrafine particles into the circulation. The increased tissue dose received by COPD patients may contribute to systemic effects as a result of an exacerbation of their airway inflammation and cytokine release into circulation, but not the rapid movement of significant number of insoluble particles into the circulation.

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APPENDIX

The effect of apparatus dead space on DF data was investigated using filters (BB-5OT; Pall Biomedical, Fajardo, Puerto Rico) and a ventilator (Model 608; Harvard Apparatus, Holliston, MA). Referring to Figure 1, Filter 1 was placed as illustrated to capture exhaled aerosol. To simulate an individual with a DF of 100% breathing on the apparatus, a second filter (same type as Filter 1) was placed on the mouthpiece and attached to the ventilator. Twelve trials were conducted with V_T ranging from 330 to 1050 ml. These trials showed a tendency to underestimate DF with decreasing V_T . The DF from these simulated maneuvers (DF_{sim}), fit by linear regression as a function of V_T , showed $DF_{sim} = 0.73 + 0.20 V_T$ ($r = 0.93$, $p < 0.001$). Experimentally, a subject's DF was corrected for dead space by division of the predicted DF_{sim} at the subject's V_T during the aerosol inhalation. On average, the DF_{sim} applied as a correction factor to DF data was 0.83 ± 0.05 .