Saline aerosol bolus dispersion. I. The effect of acinar airway alteration

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IN RECENT YEARS, AEROSOLS have been introduced as a promising new diagnostic tool allowing noninvasive monitoring of lung structural changes in lung disease (3). When a small aerosol volume (bolus) is inhaled to a given volumetric lung depth (VLD), the dispersion of the recovered bolus depends, among other things, on the structure it has encountered on the way in and out of the lungs. The aerosol bolus dispersion technique is particularly attractive because it can potentially reflect lung structural changes at different levels of the lung, depending on whether the aerosol bolus was inhaled to peripheral (high VLD) or shallow lung depths (low VLD). The drawbacks of the existing bolus dispersion technique are that it generally requires the inhalation of oil droplets or latex aerosol and that, even in the case of these monodisperse nonhygroscopic aerosols, the experimental bolus dispersion data in normal subjects are as yet not fully understood. This is partly because of the lack of quantitative information on 1) the effect of the orolaryngeal pathway, which is thought to also introduce a gender-related contribution to bolus dispersion in humans (6, 2) the potential differential contribution from left and right lungs (2, 25), and 3) the actual effect of asynchronous emptying and filling of lung units (8, 17).

Despite these drawbacks, the bolus dispersion test seems to pick up even subtle lung structural changes (1, 5, 15, 22), and we therefore further explored the possibilities of the technique in the case of mild lung alteration. We have used an aerosol bolus dispersion test similar to the one previously employed by others (1, 5, 22) but substituted nebulized saline for latex microsphere (1) or oil droplet (5, 22) aerosols. The rationale for using saline was that, in the VLD range (200–800 ml) and particle range (0.5–1 μm) of interest for detection of lung structural change (1, 5, 22), bolus dispersion appears to be only poorly sensitive to aerosol particle size (9, 19, 21) and bears no causal relationship to deposition (1, 18, 22). As a consequence, dispersion of the exhaled bolus is still expected to be similar to that obtained with monodisperse nonhygroscopic aerosols in a similar size range. Even if the absolute bolus dispersion value obtained with saline aerosol varied somewhat from that obtained with, e.g., 1-μm latex aerosol, its ability to detect lung structural alteration may persist as long as dispersion is evaluated by consistently using the same aerosol. The use of saline for bolus dispersion measurements could make this test more attractive for application in patients.

Because the potential of the aerosol bolus dispersion technique lies in the ability to detect structural change at different lung depths, we complemented the proposed saline bolus dispersion technique with a test of ventilation distribution that can also distinguish between alterations at the level of proximal and peripheral air spaces, i.e., a multiple-breath N2 washout test. Indeed, the normalized phase III slope analysis of the N2 washout (28) yields two independent measures of conductive and acinar ventilation heterogeneity (S_{cond} and S_{acin}).

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AEROSOL DISPERSION IN SMOKERS

and $S_{acini}$, respectively). We have previously used this technique in a number of clinical settings, such as during bronchoprovocation in hyperresponsive subjects (29), in stable chronic obstructive pulmonary disease (COPD) patients (28), and in asthmatic patients before and after bronchodilatation (27). In these populations, the observed increases in $S_{cond}$ and $S_{acini}$ were substantial with respect to normal, and it could therefore be expected that these indexes would also pick up more subtle lung alterations and, more importantly, locate them in the conductive or acinar zone of the airway tree.

We applied the saline bolus dispersion and the N$_2$ washout techniques in two groups of subjects who were expected to show distinct structural alterations at different lung depths. A first group consisted of asymptomatic smokers, in which we expected a priori a combination of conductive and acinar airway alteration on the basis of previous N$_2$ washout results in COPD patients (28). A second group involved nonsmoking normal subjects before and after 2-mg histamine provocation, for which we have previously shown only a conductive airway response (29). The smoker study will be presented here, and the provocation study will be outlined and discussed in the companion paper (26).

MATERIALS AND METHODS

All subjects participating in this study were recruited on a voluntary basis among hospital and laboratory personnel and had never undergone pulmonary function testing before. None of the subjects took any medication. Smokers (S) with at least 10 pack years (py) and never-smokers (NS) were recruited until the following criteria were fulfilled: 1) at least 10 subjects per group, 2) similar age, 3) similar proportion of female and male subjects, and 4) similar functional residual capacity (FRC). This yielded 12 subjects in the S group (5 women/7 men; 39 ± 6 yr; all values given as means ± SD) with a smoking history of 28 ± 9 py and 12 subjects in the NS group (4 women/8 men; 36 ± 6 yr). Smokers were asked to refrain from smoking in the 4-h period preceding the test procedure. Within the time span of ~1 h, each subject performed a set of lung function tests, three N$_2$ washout tests, and a series of ~15 aerosol dispersion tests.

Lung function parameters were obtained by means of standardized lung function laboratory equipment (Sensor-Medics Model 2200, Bilkhaven, The Netherlands). They included three forced expiration maneuvers [forced expired volume in 1 s (FEV$_1$), forced vital capacity (FVC), and forced expiratory flow after exhalation of 75% FVC] and a single-breath carbon monoxide diffusing capacity test [for $DlCO$] and for $DL_{CO}$ divided by alveolar volume ($K_{CO}$)]. For the multiple-breath N$_2$ washout and aerosol bolus tests, the subjects were aided by a computer-controlled breathing assembly, in which data acquisition, pneumatic valve control, and visual feedback to the subjects are handled by Labview software (National Instruments, Austin, TX). The schematic representation in Fig. 1 essentially shows the setup in its configuration for the aerosol bolus tests, and only part of it is used for the N$_2$ washout tests. Previous work reports the detailed functioning of the equipment to adequately perform the aerosol bolus experiments (23) and N$_2$ washout experiments (29). Both procedures can be briefly summarized as follows.

Saline bolus and N$_2$ washout tests: experimental setup and procedure. For the saline bolus procedure, all valves depicted in Fig. 1 were operational, but the inspiratory bag was blocked. The black box in front of the subject’s mouth represents a laser photometer (PARI, Starnberg, Germany) to measure the aerosol, and the pneumotachograph in the wall of the bag-in-box was used to monitor all respiratory volume changes. Photometer and volume data acquisition frequency were 100 Hz. The aerosol reservoir, i.e., the 60-ml tubing between valves 2 and 3, was placed at a right angle with respect to the mouthpiece and photometer to achieve a blunt aerosol profile of the bolus on its passage through the photometer (23). The aerosol to be fed into the 60-ml reservoir tube between valves 3 and 2 was obtained by nebulization of normal saline (0.9% NaCl) using an Acorn II (Marquest Medical Products, Englewood, CO) with a driving pressure of 1.5 bar. When measuring the wet aerosol sampled from the 60-ml reservoir by means of a particle counter/sizer (PCS2000, PALAS, Karlsruhe, Germany), we obtained an aerosol number concentration of ~10$^6$ droplets/cm$^3$ with 52% of the aerosol volume in the respirable range (1–5 μm) comparable to the 45% reported by others for this particular nebulizer (14). Of more relevance to the present experiments, however, 87% of the total number of droplets were seen to be sized under 1 μm.

The saline bolus test started with some tidal clean air breaths, i.e., inhalation from the box (via valves 5, 4, nonreturn (NR), and I) and exhalation into the expiratory bag (via...
valves 1 and NR). Simultaneously, saline was nebulized into an open circuit through the 60-ml aerosol tube between valves 3 and 2. The actual bolus test then started at FRC with clean air inhalation from the box (via valves 5, 4, NR, and 1) until a predefined volume above FRC was reached, and valves 1, 2, and 3 were reversed so that the aerosol bolus volume between valves 2 and 3 could be inhaled, followed by clean air beyond valve 3 from the box via valve 5. Inhalation continued until volume reached the target inspiration volume of 1.1 liter above FRC. At end-inspiration, further inspiration was prevented by switching valves 1 and 4 to the inspiratory bag pathway, which was blocked in the aerosol setup configuration. The subject then exhaled via valves 1 and NR into the exhalation bag to residual volume.

The VLD to which each bolus was delivered corresponded to the actual air volume after the aerosol bolus until end-inspiration. One bolus test sequence consisted of having each subject perform a series of ~15 bolus tests with VLD targeted between 200 and 800 ml. In this test sequence, the subject was not given a visual feedback but was coached by the operator. A few dry runs enabled the subject to practice a typical aerosol test, with particular attention to a steady inspiratory flow rate and a prompt reaction at end-inspiration while avoiding exaggerated expiratory flow in the initial phase of expiration. Bolus tests were considered acceptable when inspiratory and expiratory flow rates were between 250 and 350 ml/s and end-inspiratory breath hold was <1 s.

One subject from the NS group performed additional bolus tests using either saline or 1.07 ± 0.01 μm latex particles (Duke Scientific, Palo Alto, CA) as the inspired bolus aerosol. The 1-μm latex aerosols were prepared from a 10% solid aqueous suspension and diluted in pure water (aqua ad injectabilia, Braun, Melsungen, Germany) for nebulization (Acorn II, Marquest Medical Products). Finally, the nebulizer output was dried in a silica gel tunnel before being delivered to the aerosol reservoir between valves 2 and 3. In this NS subject, ~45 saline and latex bolus dispersion tests were accumulated in the VLD range 200–800 ml.

For the N2 washout procedure, valves 2 and 3 were removed, and the disconnected sides of valves 5 and 1 were blocked. The black box in front of the subject’s mouth now represents the N2 analyzer (P. K. Morgan, Kent, UK), and volume was again obtained from the pneumotachograph in the wall of the bag-in-box. N2 concentration and volume were now acquired at 25 Hz. The washout procedure started with tidal air breathing, with inhalation from the box via valves 5, 4, NR, and 1, and exhalation via valves 1 and NR into the expiratory bag. During a given exhalation back to FRC, valve 4 was switched to substitute the air from the box by the pure O2 in the inspiratory bag for all subsequent inhalations. The subjects were given a visual feedback of inspiratory volume to target 1-liter inspirations, whereas expiration back to FRC occurred spontaneously. The N2 washout test continued until the number of 1-liter O2 breaths yielded at least six lung turnovers, where one lung turnover (TO) is defined as tidal volume divided by FRC (for a subject with an FRC = 3.5 liters, the N2 washout test will consist of at least 21 breaths). At the end of the washout test, the subject is instructed to exhale to residual volume.

Saline bolus analysis. Photometer signals were normalized to inspiratory peak concentration and plotted against cumulative inspired and expired volume as in Fig. 2, representing typical dispersion traces for saline and 1-μm latex boluses inhaled to similar VLD (i.e., the volume difference between inspiratory peak and end of inspiration). With an inspiration of 1,100 ml and peak inspiration ~650 ml, the boluses in Fig. 2 corresponded approximately to VLD = 450 ml. Aerosol bolus dispersion traces such as these were analyzed in terms of half-width (H), standard deviation (σ) and skew (sk), using the standard formulas as specified in Brand et al. (4). Briefly, H is given by the square root of the difference H2 − H1, where H1 and H2 are inhaled and exhaled bolus half-width, determined as the volumetric width at half-inspiratory or half-expiratory peak height (see the H1 indications in Fig. 2). In analogy to H, σ is given by the square root of the difference σ2 − σ1, i.e., of the second moment of exhaled and inhaled boluses. sk was computed as the third moment of the exhaled bolus divided by σ3. The moment analyses for σ and sk computation are usually done by using a cutoff concentration for the integrations involved (1, 4). Brand et al. (4) recommended that integration of the inhaled and exhaled boluses should only involve aerosol concentrations >15% of the exhaled bolus peak concentration. We computed σ and sk, using 5, 15 and 25% cutoffs that will be specified in the subscript (e.g., σ25%, σ15%, or σ5%, respectively). All dispersion indexes were set out against VLD, fitted with a third-order polynomial, and interpolated to obtain values for VLD = 200, 400, 600, and 800 ml as was done in Anderson et al. (1).

N2 washout analysis. The N2 washout tests were first used to determine the subject’s FRC and the Fowler dead space of the first breath (Vd0). The actual N2 washout analysis and theory at the basis of the indexes of S(25%) and S(15%) have been extensively described elsewhere (24, 29). We only reiterate here the computation method, which started by plotting N2 concentration as a function of volume in each expiration, determining its N2 phase III slope, and normalizing each consecutive N2 phase III slope by the corresponding mean expired N2 concentration. If normalized slope is then plotted as a function of TO, this results in curves such as those shown in Fig. 3, that is, progressively increasing normalized slopes as a function of TO. The open and closed triangles in Fig. 3 correspond to the pooled, normalized slope curves of the NS and S in this study. Figure 3 also illustrates how
extracting from the slope of the first breath the part that is due to noncond. In the VLD range considered, saline and 1-

indexes $S_{cond}$ and $S_{acin}$ are derived; note, however, that actual $S_{cond}$ and $S_{acin}$ computations were done on the average of three normalized slope curves obtained in each subject. $S_{cond}$ is actually defined as the normalized slope difference per unit TO in the part of the $N_2$ washout in which only conductive airways are known to contribute to an increase normalized slope, i.e., between TO = 1.5 and TO = 6. This conductive airway contribution to the increasing normalized slopes should extrapolate to a zero slope for TO = 0. This is not the case (see also Fig. 3), and in fact the normalized slope of the first exhalation mainly originates in the more peripheral acinar airways. Therefore, $S_{acin}$ is determined by subtracting from the slope of the first breath the part that is attributed to the conductive airways, i.e., $S_{cond}$ multiplied by the TO value of the first breath.

**RESULTS**

Table 1 summarizes lung function and $N_2$ washout indexes obtained in the NS and S groups. The S group had significantly lower values on 75% FVC end-expiratory flow and on $D_{1CO}$ (but not on $K_{CO}$) with respect to the NS group. By contrast, the slightly lower FEV₁ and FEV₁/FVC values in the S group did not reach significance. There was a significantly larger $S_{acin}$ in the S vs. NS groups, whereas the slightly larger average $S_{cond}$ value in the S group was not significantly different from that obtained in the NS group. Fowler dead space was also similar between both groups. Finally, Table 1 shows comparable FRC values in both groups, discarding the possibility that FRC differences could have been at the origin of the presence or absence of differences in $N_2$ washout or bolus dispersion indexes among groups.

Figure 4 represents extensive sets of 1-$\mu$m latex and saline bolus dispersion data obtained from a NS subject. In the VLD range considered, saline and 1-$\mu$m latex showed similar trend lines on all depicted bolus dispersion indexes as a function of VLD. This was true for bolus dispersion in terms of $H$ (Fig. 4A) or $\sigma_{25\%}$ or $\sigma_{5\%}$ (Fig. 4B) and also $sk_{25\%}$ or $sk_{5\%}$ (Fig. 4C) despite the larger variability of the latter index; $\sigma_{15\%}$ and $sk_{15\%}$ are not depicted in Fig. 4, B and C, for clarity.

By interpolation of the third-order polynomial trend lines, such as those obtained from the saline data in Fig. 4, a set of $H$, $\sigma$, and $sk$ values was obtained for VLD = 200, 400, 600, and 800 ml for each subject under study. Comparison of $H$, $\sigma$, and $sk$ values from NS and S groups could then be done for each VLD level (1). The resulting average $H$ (Fig. 5) and average $\sigma_{25\%}$, $\sigma_{15\%}$, and $\sigma_{5\%}$ (Fig. 6) or $sk_{25\%}$, $sk_{15\%}$, or $sk_{5\%}$ (Fig. 7) per VLD level obtained from the NS and S groups are represented by the open and closed symbols, respectively. $H$ was significantly different between NS and S groups only for the most peripherally inhaled boluses (VLD = 800 ml; Fig. 5). Although $\sigma$ (Fig. 6) mimicked $H$ behavior, actual significance of the $\sigma$ difference between NS and S groups depended on the cutoff that was used, with the 5% cutoff obtaining a significant difference for VLD = 800 ml. The sk was also significantly greater in smokers in the higher VLD range, with a significant difference for VLD = 600 ml and 800 ml or only for VLD = 800 ml, depending also on the cutoff that was used for sk computation.

On the basis of previous observations by others (1, 5, 22) in which smokers were essentially characterized by a steeper increase of dispersion between shallow and deep VLD levels, we also computed for each subject the increase of the dispersion indexes $H$ and $\sigma$ between VLD = 200 ml and VLD = 800 ml [e.g., $\Delta H = H(800 \text{ ml}) - H(200 \text{ ml})$]. The resulting $\Delta H$ and $\Delta \sigma$ are summarized in Table 2, in which smokers showed a significantly larger dispersion increment between 200 and 800 ml for $\Delta H$ as well as for $\Delta \sigma$ (for cutoffs 5 and 15%).

| Table 1. Subject characteristics in terms of lung function and $N_2$ washout |
|-----------------------------|-----------------------------|-----------------------------|
|                            | NS ($n = 12$) | S ($n = 12$) | $P$ Value |
|-----------------------------|-----------------------------|-----------------------------|
| **Lung function**           |                            |                            |            |
| FEV₁, %pred                | 115 ± 14                   | 101 ± 20                   |            |
| FEV₁/FVC, %                 | 79 ± 5                     | 76 ± 9                     |            |
| FEF₂₅, %pred               | 82 ± 22                    | 64 ± 29*                   | 0.04       |
| $D_{1CO}$, %pred           | 100 ± 16                   | 83 ± 14*                   | 0.01       |
| $K_{CO}$, %pred            | 81 ± 9                     | 73 ± 14                    |            |
| Multiple-breath washout     |                            |                            |            |
| VD₆₃, ml                   | 152 ± 33                   | 152 ± 23                   |            |
| FRC, liters                | 3.4 ± 0.8                  | 3.6 ± 0.7                  |            |
| $S_{acin}$, liters⁻¹       | 0.064 ± 0.026              | 0.110 ± 0.039*             | 0.007      |
| $S_{cond}$, liters⁻¹       | 0.030 ± 0.014              | 0.035 ± 0.014              |            |

Values are means ± SD. NS, never-smokers; S, smokers; %pred, percent predicted; FEV₁, forced expired volume in 1s; FEV₁, FVC; FEV₁ divided by forced vital capacity (FVC); FEF₂₅, forced expired flow after 75% FVC is exhaled; $D_{1CO}$, diffusing capacity; $K_{CO}$, $D_{1CO}$ divided by alveolar volume; VD₆₃ and FRC, Fowler dead space and functional residual capacity, respectively; $S_{acin}$, $S_{cond}$, indexes of acinar and conductive ventilation inhomogeneity, respectively. *Significantly different from NS group (Mann-Whitney U-test).
DISCUSSION

The smokers under study present the particular feature that with respect to age- and sex-matched non-smokers, they show abnormal acinar ventilation distribution (significantly greater $S_{acin}$) in the absence of conductive ventilation distribution abnormality (no significant $S_{cond}$ change). The possibility that the absence of $S_{cond}$ change in the present study could have been due to a lack of $S_{cond}$ sensitivity is contradicted by the fact that twofold $S_{cond}$ increases are seen during histamine provocation for a FEV$_1$ decrease of only 11% (29), a result that was reproduced in the companion study (26). The severity of the acinar ventilation impairment observed in the S group with an average 28-py smoking history ($S_{acin} = 0.11 \pm 0.04$ liter$^{-1}$) is largely inferior to that previously reported in patients with overt COPD and a 16-py longer smoking history ($S_{acin} = 0.43 \pm 0.18$ liter$^{-1}$) (28). In fact, previous studies in COPD and asthmatic patients (27, 28) had always shown a combination of impaired ventilation distribution at the level of both conductive and acinar airways (abnormal $S_{acin}$ and $S_{cond}$ values). The observation that the structural alterations in our smokers are confined to the acinar lung zone (only $S_{acin}$ increased) makes the S group even more attractive for the study of aerosol bolus dispersion at different lung depths.

With respect to aerosol behavior in smokers, our points of comparison are the early work by McCawley and Lippmann (15) and more recent studies by Siekmeier et al. (22), Anderson et al. (1), and Brand et al. (5). These bolus dispersion studies made use of 0.5 μm triphenyl phosphate (15), 1-μm latex particles (1), or...
0.8-μm oil droplets (5, 22) with a variety of maneuvers (e.g., starting bolus inhalation from residual volume or FRC) and different analyses of bolus dispersion (using σ with different cutoffs and/or H). Despite these methodological differences, all three studies indicated an increased bolus dispersion in smokers vs. nonsmokers for the more peripherally inhaled boluses. In the present study, the same conclusion is reached by using a saline aerosol. Indeed, the saline bolus shows a pattern of increased H (Fig. 5) and increased σ (Fig. 6) in the S compared with the NS group as VLD increases. In fact, the rate of H increase with VLD (Table 2) was significantly larger in the S group [ΔH = 502 (S) vs. 377 ml (NS); P = 0.03], and σ showed statistically significant differences between NS and S only if Δσ15% (P = 0.03) and Δσ5% (P = 0.01) were used (although the mean differences in Δσ25%, Δσ15%, or Δσ5% between NS and S groups were all of the order of 30 ml; Table 2). Given these subtle differences between the various measures of bolus dispersion in the S group, they all indicated structural change in the lung periphery consistent with the abnormal Sacin values obtained in this group (Table 1).

The results in our S group and the results in Brand et al. (5) diverge somewhat from those obtained by Siekmieier et al. (22) and Anderson et al. (1) in that separation between smokers and nonsmokers in the latter two studies occurred for boluses inhaled as shallow as VLD = 400 ml in terms of H or even VLD = 200 ml in terms of σ. Neither Brand et al. (5) nor the present study (Figs. 5–6) revealed any significant differences in bolus dispersion (H or σ) between NS and S groups at shallow lung depth (VLD = 200 ml). We suspect that the increased dispersion at shallow lung depths of the smokers in the studies of Anderson et al. and Siekmieier et al. may be due to additional presence of conductive airway alterations. Their smoker populations had average smoking histories of 41 (1) and 44 (22) py, respectively, in contrast to the ~20 py estimated from the report of Brand et al. and 28 py in our S group. Table 1 indicates that our S group showed a tendency to increase Scond, but this increase failed to reach significance. Anderson et al. also found an increased N2 phase III slope in their smoker group, but because it derived from a vital capacity single-breath washout maneuver it is difficult to distinguish conductive from acinar lung zone contributions, probably because of the influence of airway closure on phase III slope (10).

Finally, Fig. 7 shows that sk of the peripheral boluses (VLD = 800 and/or 600 ml, depending on cutoff) was increased in S vs. NS groups. This is again consistent with a smoke-induced acinar lung structure alteration, but it also strongly suggests that the structural alterations were probably heterogeneously distributed in the lung periphery. Such structural heterogeneity could indeed lead to nonreversible first-in last-out bolus distribution patterns, which would increase sk (17). The smokers in the Siekmieier et al. (22) study also showed a consistent increase in sk. However, this occurred at all VLD levels between 200 and 800 ml, again consistent with the presence of conductive airway al-
terations in Siekmeier et al’s smoker group (22). Unfortunately, the other bolus dispersion studies in smokers (1, 5, 15) did not report sk.

Saline bolus- and N₂ washout-derived indexes of ventilation nonuniformity. It is interesting to explore the underlying theory of N₂ washout and bolus dispersion analysis to understand how gas and aerosol transport mechanisms operating in the same lung structure can be held responsible for the increased $S_{\text{acin}}$ and increased bolus dispersion in the deep lung (high VLD). On the basis of N₂ washout theory (11, 24) and experiments (29, 28), $S_{\text{acin}}$ originates from convection-diffusion interaction at the acinar level, where gas convective and gas diffusive transport are of the same order of magnitude, and $S_{\text{acin}}$ may be considered independent of gas-mixing events occurring proximal to the acinar lung level. Also, $S_{\text{acin}}$ heavily depends on the asymmetry of the acinar lung structure, and a perturbation in the volumetric or cross-sectional asymmetry of parallel intra-acinar units can modify $S_{\text{acin}}$. In Fig. 3, this is reflected by an upward shift of the entire normalized slope curve of the S group with respect to the NS group. Recent simulation work has shown that $S_{\text{acin}}$ is sensitive not only to structural asymmetry at any given intra-acinar branch point but even more so to heterogeneity in asymmetry among parallel intra-acinar branch points (11).

The parallel heterogeneity of lung structure (and alteration thereof in mild lung disease) was also the effect that was thought to allow efficacious detection of lung structural change by means of aerosol bolus dispersion (1, 15). In the context of studies in smokers, McCawley and Lippmann (15) and Anderson et al. (1) pointed to the crucial role of heterogeneity in structural alterations that would introduce different time constants when aerosol boluses distribute over and recombine from peripheral lung units. In mild lung disease such as shown here, in which FEV₁ is not significantly affected (Table 1), bolus dispersion was indeed expected to increase at any given lung depth.

Table 2. Increase of saline bolus dispersion indexes between 200-ml and 800-ml VLD in NS and S

<table>
<thead>
<tr>
<th></th>
<th>NS ($n = 12$)</th>
<th>S ($n = 12$)</th>
<th>$P$ value</th>
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</thead>
<tbody>
<tr>
<td>$H_{\text{VLD}}$</td>
<td>$377 \pm 93$</td>
<td>$502 \pm 145^*$</td>
<td>0.03</td>
</tr>
<tr>
<td>$\sigma_{\text{25%}}$</td>
<td>$136 \pm 26$</td>
<td>$163 \pm 44$</td>
<td>0.03</td>
</tr>
<tr>
<td>$\sigma_{\text{15%}}$</td>
<td>$142 \pm 24$</td>
<td>$177 \pm 44^*$</td>
<td>0.03</td>
</tr>
<tr>
<td>$\sigma_{\text{5%}}$</td>
<td>$134 \pm 23$</td>
<td>$169 \pm 36^*$</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD. VLD, volumetric lung depth; $H$, half-width, $\sigma_{\text{25%}}, \sigma_{\text{15%}}, \sigma_{\text{5%}}$, standard deviation using 5, 15, or 25% of the expiratory bolus maximum as a cutoff (see text for details); $\Delta H$ and $\Delta \sigma$, $H$ or $\sigma$ difference, respectively, between VLD = 200 ml and 800 ml. *Significantly different from NS group (Mann-Whitney $U$-test).
owing to an increased heterogeneity in airway structure rather than to a gross overall structure alteration. The combined information from increased $S_{a\text{cin}}$ and increased dispersion of VLD = 800 ml boluses strongly suggests that parallel heterogeneities in the peripheral lung structure constituted the link between the performance of saline bolus and N₂ washout-derived indexes of ventilation nonuniformity in the smokers under study.

**Aerosol bolus dispersion tests with saline.** Previous aerosol bolus dispersion studies in smokers made use of 0.5-μm triphenyl phosphate (15), 1-μm latex particles (1) or 0.8-μm oil droplets (5, 22). We used a nebulized saline aerosol that is generally referred to as unstable. However, in a recent editorial, Finlay and Smaldone (12) suggested that this assumption of aerosol instability is essentially based on studies using dried salt particles and may be exaggerated. These authors argued that, in the case of a wet aerosol cloud as used here, a two-way coupled hygroscopic effect, which is expected to stabilize hygroscopic aerosols against size changes, needs to be considered. In addition, isotonic (as opposed to hypotonic or hypertonic) aerosols are supposedly least subject to change in the airway tree (13, 16). Persons et al. (16) suggested the possibility of a relatively rapid growth in the mouth and trachea after the shrinkage of the saline aerosol in the breathing assembly through evaporation. Finally, Schmehl et al. (20) even used saline aerosols for single-breath deposition measurements by introducing a growth correction factor that was considered constant beyond the dead space. Although such a correction is not readily applicable to a bolus experiment, the study of Schmehl et al. points to the fact that saline aerosols can represent effects that are directly related to those observed with a nonhygroscopic aerosol.

For the saline bolus in Fig. 2, the area under the expiratory bolus curve exceeds that under the inspiratory bolus curve, in contrast to what is observed for the 1-μm latex bolus. This illustrates that in our saline bolus experiments some degree of hygroscopic growth occurred between saline bolus in- and exhalation. Considering, however, that photometer signal amplitude varies approximately with the square of particle diameter, this effect does not appear to be dramatic. On the other hand, not all particles contained in the inspiratory bolus contribute to the exhaled bolus signal and, in fact, the inspiratory bolus has very little impact on the exhaled H values (23). For all these reasons, saline bolus dispersion was expected to reflect comparable behavior to that usually observed with particles in the 0.5–1-μm range. Besides the general agreement in an exhaustive test sequence using either saline or 1-μm latex on a subject from the NS group (Fig. 4), the H values obtained for different VLD levels in our NS group (Fig. 5) were actually consistent with the ranges of values encountered in the literature using nonhygroscopic aerosols. Combing data from various previous reports (4, 5, 7, 18) using nonhygroscopic aerosols in the 0.5–1-μm size range, we found that H values for healthy subjects ranged 180–300 ml for VLD = 200 ml and 400–600 ml for VLD = 800 ml.

In summary, we presented N₂ washout and saline bolus dispersion results that are consistent with a pattern of smoking-induced structural alteration in the lung periphery. Both gas- and aerosol-related tests were obtained by having the subjects breathe in the same volume ranges (between FRC and 1 liter above FRC), thereby avoiding airway closure effects. The analyses of N₂ washout and saline aerosol bolus tests rely on totally different theoretical concepts of how gas or aerosol ventilation heterogeneities are identified at different lung levels. In the particular case of acinar structure changes, the present paper indicated the potential of saline dispersion as a strictly noninvasive probe for early detection of lung structural alterations.

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