Saline aerosol bolus dispersion. II. The effect of conductive airway alteration

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IN MOST OF ITS CLINICAL APPLICATIONS, the aerosol bolus dispersion technique has been promoted for its sensitivity to small airway structural change, essentially on the basis of the fact that the bolus test is performed in the tidal volume range and that aerosol boluses can be volumetrically pushed into and recovered from the peripheral lung spaces. Although several papers (reviewed in Refs. 2 and 4) reported the behavior of aerosol boluses delivered to a range of volumetric lung depths (VLD, typically 200–800 ml), other studies only included the measurement of aerosol boluses that were targeted to one given VLD of choice (11–13). In these latter studies, high sensitivity of the aerosol bolus to lung structural change was indeed obtained and was generally attributed to the sensitivity of selected bolus dispersion indexes to heterogeneity of ventilation. Yet, irrespective of whether the boluses were delivered to VLD ~400 (11, 12) or VLD ~800 ml (13), it was invariably suggested that this heterogeneity originated mainly in the small airways. Although boluses targeted to such VLD do travel the peripheral spaces, they must negotiate the extrathoracic and conductive airways, both of which have a considerable impact on the outcome of the aerosol bolus dispersion test (7, 15).

In fact, the identification of small or large airway structural alteration on basis of aerosol bolus tests can only be done if the measurements span a considerable VLD range. For purely small airway alterations, the relative effect on shallow (VLD = 200 ml) and deep (VLD = 800 ml) bolus dispersion is straightforward: dispersion of shallow boluses will remain essentially unchanged because these boluses spend most of their time proximal to the acinar space, and boluses will get gradually more dispersed as they are sent deeper into the lungs. Thus, when bolus dispersion becomes abnormally large with increasing VLD, this suggests small airway structural alteration, as was shown to be the case for acinar structure alterations in a group of asymptomatic smokers (22). In contrast to acinar structure alterations, which can only affect the deep boluses that travel well within the acinar space, conductive airway alterations are bound to affect both shallow and deep boluses to some extent. Conductive airway alterations should be reflected in a marked dispersion increase of the shallow boluses, an effect that gets attenuated as VLD increases (8). The primary purpose of the present study was to verify experimentally whether this pattern can be obtained with the saline bolus dispersion technique.

The previous saline bolus study of acinar lung alteration, which will be further referred to as the smoker study (22), involved two groups of different subjects. In the present study, it is possible to induce conductive airway alteration in one and the same group of subjects by a histamine challenge procedure (21). This has the advantage that possible interindividual bolus dispersion differences owing to extrathoracic air space geometry cannot interfere with the actual effect of the struct-

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tural change we are aiming to detect. As in the smoker study (22), we also independently assessed the location of structural change by using a conceptually different technique [N₂ washout; indexes of conductive (S_{cond}) and acinar (S_{acin}) ventilation inhomogeneity] that distinguishes between events occurring proximal to and peripheral to the diffusion front. Structural alterations at the level of conductive and acinar airways are expected to be associated with an independent increase of either S_{cond} or S_{acin}, respectively. We chose to study never-smoker subjects who are categorized as nonresponders according to standard lung function criteria but who are nevertheless expected to show a marked S_{cond} increase in the absence of S_{acin} change (21).

**MATERIALS AND METHODS**

**Experimental procedure.** Normal never-smoker test subjects were recruited on a voluntary basis, and none of the subjects had ever undergone pulmonary function testing before. Each subject performed baseline spirometry by means of standardized lung function laboratory equipment (Sensor-Medics Model 2200, Bihlthoven, The Netherlands), including three forced expiration maneuvers (forced expired volume in 1 s (FEV₁), forced vital capacity (FVC), and forced expiratory flow after exhalation of 75% FVC). In addition, three N₂ washout tests and a sequence of 15 saline bolus tests were performed in exactly the same manner as described in the smoker study (22). Subsequently, all subjects underwent a histamine challenge procedure using the dosimeter technique (MEFAR dosimeter MB3; vital capacity breath).

Histamine was administered in four steps (0.16-, 0.48-, 1.08-, and 2-mg cumulative doses) during which spirometry was monitored. In the final step, the subjects who had not decreased FEV₁ by ≥20% predicted were actually included in this study (n = 10). These subjects then continued with a sequence of 15 saline bolus tests and two N₂ washout tests, followed by a final spirometry. The latter spirometry was included to account for possible time-dependent histamine effects occurring over the course of the saline and N₂ washout test sequences. The average FEV₁ decrease in each subject was computed from the FEV₁ obtained immediately after the final saline dose and final FEV₁ measurement (i.e., after saline bolus and N₂ washout tests).

**Data analysis.** Baseline and histamine N₂ washout and aerosol bolus tests were analyzed in an identical fashion to that employed in the smoker study (22). The most relevant N₂ washout-derived indexes and curves are summarized in Fig. 1. Open and closed triangles correspond to the pooled baseline normalized slope curve (S) obtained on the 10 subjects in baseline condition and after histamine challenge, respectively. S_{cond} and S_{acin} are depicted with respect to the baseline curve (open triangles). Fig. 1 also illustrates how the increase in the rate of rise of S represents a function of lung turnover (TO) due to histamine challenge (closed triangles) will be reflected in an increased S_{cond}. Actual S_{cond} and S_{acin} values were obtained on each subject individually, i.e., computed from the average of three (baseline) or two (posthistamine) S vs. TO curves obtained in each subject. The saline bolus dispersion-derived indexes half-width (H), standard deviation (σ₂₅%, σ₁₅%, or σ₅%), and skew (sk₂₅%, sk₁₅%, or sk₅%) using cutoffs of 25, 15, and 5%, respectively, were set out against VLD and fitted with third-order polynomials to obtain interpolated values for VLD = 200, 400, 600, and 800 ml.

A set of saline bolus indexes was obtained for each subject before and after histamine.

**RESULTS**

Table 1 summarizes lung function and N₂ washout indexes obtained before and after histamine in the 10 subjects under study (30 ± 11 yr, means ± SD). All spirometric parameters in Table 1 (FEV₁, FEV₁/FVC, and forced expiratory flow after 75% FVC) were significantly decreased after 2 mg histamine. On the part of N₂ washout, S_{acin} and functional residual capacity remained unaffected by histamine, and the average 13-ml decrease in Fowler dead space (VDp) did not reach significance (P = 0.06). S_{cond} was significantly increased, from 0.033 to 0.068 liter⁻¹, consistent with Fig. 1, in which the rate of S increase with TO (beyond TO = 1.5) is seen to be doubled after histamine. The corresponding saline bolus results are graphically represented in Figs. 2–4, depicting H, σ, and skew values for VLD = 200, 400, 600, and 800 ml before (open triangles) and after (solid triangles) histamine. As in the smoker study (22), σ and skew values are presented in various panels according to the cutoff that was used for σ or skew computation (25, 15, or 5%). The present study involved the same subjects before and after histamine, requiring pairwise comparison of H, σ, and skew values for each VLD level (Wilcoxon signed-rank test). The resulting significant (P < 0.05) differences are indicated by asterisks on the VLD axes of Figs. 2–4.

H was significantly different before and after histamine for VLD = 600 and 800 ml (Fig. 2). Although σ became significantly different after histamine at all
Table 1. Spirometry and N₂ washout results before and after histamine challenge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Histamine</th>
<th>P value</th>
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<tbody>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FEV₁, %pred</td>
<td>107 ± 10</td>
<td>97 ± 11*</td>
<td>0.009</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>85 ± 5</td>
<td>77 ± 6*</td>
<td>0.007</td>
</tr>
<tr>
<td>FEF₂⁵₋₇⁵, %pred</td>
<td>90 ± 23</td>
<td>64 ± 20*</td>
<td>0.009</td>
</tr>
<tr>
<td>Multiple-breath washout</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VDₑ, ml</td>
<td>130 ± 26</td>
<td>117 ± 24</td>
<td></td>
</tr>
<tr>
<td>FRC, liters</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>S_acin, liters</td>
<td>0.059 ± 0.019</td>
<td>0.059 ± 0.019</td>
<td>0.008</td>
</tr>
<tr>
<td>S_cond, liters</td>
<td>0.033 ± 0.015</td>
<td>0.068 ± 0.036*</td>
<td>0.008</td>
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</tbody>
</table>

Values are means ± SD. FEV₁, forced expired volume in 1 s; FEV₁/FVC, FEV₁ divided by forced vital capacity; FEF₂⁵₋₇⁵, forced expired flow after 75% FVC exhaled; VDₑ, Fowler dead space; FRC, functional residual capacity; S_acin and S_cond, indexes of acinar and conductive ventilation inhomogeneity, respectively. *Significantly different from baseline (Wilcoxon signed-rank test).

VLD levels (Fig. 3), there was a very distinct pattern of absolute σ differences at the different VLD levels, depending on the cutoff that was used. For instance, σ₂⁵% (Fig. 3A) showed an increase after histamine of only 11 ml for VLD = 200 ml, which amplified to 33 ml for VLD = 800 ml, thereby also mimicking the increasing H difference toward the more peripheral VLD levels (Fig. 2). By contrast, σ₅% (Fig. 3C) showed an increase after histamine of 62 ml for VLD = 200 ml, which attenuated to 29 ml for VLD = 800 ml. Skew (Fig. 4) was significantly increased after histamine to more or less the same extent at all VLD levels for any given cutoff (25, 15, or 5%). Finally, we also computed here, for each subject, the increase of the dispersion indexes H and σ between VLD = 200 ml and VLD = 800 ml [e.g., ΔH = H(800 ml) - H(200 ml)]. The resulting ΔH and Δσ are summarized in Table 2 and show that ΔH appeared to be significantly larger after histamine, an effect that was only reproduced by Δσ₂⁵%, consistent with the results displayed in Fig. 3.

To illustrate the paradoxical behavior of σ₂⁵% and σ₅% as a function of VLD as seen across Figs. 2–3, individual bolus dispersion curves were retrieved from a subject, with tests of comparable VLD before and after histamine in the low-VLD range (VLD = 196 and 185 ml; Fig. 5A) and in the high-VLD range (VLD = 715 and 736 ml; Fig. 5B). Both panels of Fig. 5 show expired curves that are normalized to their respective maximum and also indicate the 50, 25, and 5% levels that are used for H, σ₂⁵%, and σ₅% computation, respectively. The corresponding H, σ₂⁵%, and σ₅% values are also represented, showing a behavior in line with the overall findings of Figs. 2 and 3. After histamine, H and σ₂⁵% remain virtually unchanged at low VLD (Fig. 5A), and both H and σ₂⁵% increase at high VLD (Fig. 5B). By contrast, histamine induced a marked σ₅% increase at low VLD (Fig. 5A) and a smaller σ₅% increase at high VLD (Fig. 5B).

DISCUSSION

The present study shows that histamine challenge, which provokes considerable parallel heterogeneity in conductive airway constriction, i.e., doubling S_cond for an FEV₁ decrease of only 10%, can also be reflected in a characteristic pattern of saline bolus dispersion behavior at different lung depths. In particular, bolus dispersion in Fig. 3C shows a marked increase for shallow boluses (low VLD) and an attenuation of this increase for the deeper boluses (high VLD), i.e., a pattern consistent with the effect of alterations in the conductive airways. However, Fig. 2 and the other panels of Fig. 3 provide a dramatic demonstration of how a different conclusion can be reached as to whether acinar or conductive airways are involved in the histamine challenge process, depending on the dispersion index (or cutoff) used. This contrasts with the smoker study (22), in which acinar airway changes were reflected in an increased bolus dispersion at high VLD, irrespective of the dispersion index (or cutoff) of choice. We will show that this apparent paradox can be at least in part explained on the basis of a markedly increased bolus skew at all VLD levels in the case of histamine provocation (Fig. 4).

Saline bolus dispersion. The H data in Fig. 2 and the significantly increased dependence of H on VLD (P = 0.005; Table 2) show a very similar pattern to that observed in the smoker study (22). This could have suggested that histamine had also induced an acinar airway alteration, yet S_cond and S_acin behavior in Table 1 clearly indicate histamine-induced alteration of the conductive airways only. The reason that the dependence of H on VLD is more or less mimicked by σ₂⁵% (Fig. 3A) and not by σ₅% (Fig. 3C) can be grasped from the experimental bolus curves in Fig. 5. Figure 5A shows how, at low VLD, histamine produces a skewed bolus with a bolus tail that contains much of the dispersed aerosol bolus material that is missed out completely at the 50% and 25% levels (H and σ₂⁵%).
Figure 5B illustrates that at high VLD boluses are less skewed, and the increased dispersion after histamine becomes apparent, irrespective of whether dispersion is quantified at the 50%, 25%, or 5% level (H, $\sigma_{25\%}$, or $\sigma_{5\%}$). As a consequence, H and $\sigma_{25\%}$ underestimate actual bolus dispersion and to a different degree at different VLD, depending also on bolus skew. Hence, these dispersion indexes are unsuitable to distinguish between structural changes in the shallow and deep lung, and the use of $\sigma$ with a low cutoff is imperative.

Some aerosol bolus dispersion data previously obtained in the context of hyperresponsivity protocols, in which time is often limited for multiple measurements, are now reconsidered with respect to our observations. For instance, the monitoring of H for only one VLD level (380 ml) in a dose-response protocol to methacholine (11) could have been partly responsible for loss of sensitivity and loss of information on whether small or large airways are involved. The same holds for bolus studies after ozone exposure using VLD = 380 ml (12) or in hyperresponsive women using VLD = 800 ml (13), in which the resulting H measurements cannot be conclusive about whether the observed changes involve small airways or not. Yet the identification of large vs. small airway involvement in the diseased lung constitutes the main reason for using aerosol bolus dispersion technique in addition to traditional lung function, as pointed out by Schultz et al. (17). Interestingly, their study in asthmatic children showed exactly the same pattern of bolus dispersion and skew (using a 15% cutoff) as $\sigma_{15\%}$ and $sk_{15\%}$ in the present study (Figs. 3B and 4B), and it is tempting to also interpret this as a result of conductive airway alterations. In fact, these authors commented that the comparison of two subgroups (younger vs. older asthmatic children) actually revealed a different dependence on VLD, suggesting mainly conductive airway involvement in the younger asthmatic children and an additional peripheral alteration in the older ones. Such pathophysiological information on the contribution of large vs. small airways in asthma (20) certainly warrants further investigation.

In the clinical context, bolus dispersion has been most frequently expressed in terms of H for ease of computation and robustness (2, 4). When $\sigma$ is computed, a cutoff is considered to avoid noise from the bolus tail in the experimental curves, and typical cutoffs used in the past range from 5 to 20% (4). In a study on healthy subjects, Brand et al. (5) stated that their preliminary analysis had indicated that a 15% cutoff provided least dependency of $\sigma$ on the exhaled peak signal-to-noise ratio. Neither in the present study nor in the smoker study (22) did we observe consistent differences in variability between $\sigma_{20\%}$, $\sigma_{15\%}$, or $\sigma_{5\%}$. The good performance of $\sigma_{5\%}$ may have been due to the use of interpolation polynomials of $\sigma$ vs. VLD, as sug-
suggested by Anderson et al. (3), rather than the use of σ data pooling into VLD bins to obtain dispersion values for any given VLD level.

**Saline bolus skew.** It is generally accepted that ventilation heterogeneity can lead to increased bolus skew. According to Rosenthal (14), bolus dispersion and skew are expected to be affected by ventilation heterogeneity, although the study considered time constants and zero initial lung volumes, which are difficult to relate quantitatively to lung physiology. Darquenne and Paiva (8) introduced a typical flow sequence that was thought to exist between gravity-dependent lung regions, and they did not find a significant effect on exhaled bolus characteristics, probably because of the symmetry of flow sequencing between in- and exhalation. A three-dimensional treatment of how aerosol boluses split and recombine at bifurcations, instead of one-dimensional (8) or compartmental (14) approaches, could help us better understand bolus skew, even in the normal lung. However, in the absence of more extensive simulation and experimental studies on bolus skew in general, we interpret the observed skew behavior as follows.

Bolus skew of the normal lung is seen to decrease as a function of VLD (Fig. 4; open triangles) probably because the conductive air spaces induce some initial skew but the acinar air spaces, i.e., roughly beyond 200 ml, do not introduce an additional source of skew, whereas σ continues to increase (skew computation involves normalization by σ³). Histamine challenge appears to bring about an additional source of skew that is again confined mainly to the conductive airways (VLD = 200 ml in Fig. 4; solid triangles). Beyond VLD = 200 ml, skew curves before and after histamine provocation show a parallel decrease over the remaining VLD range, probably because the histamine boluses are subject to the attenuating effect of the acinar space, as in normal lungs. In the smoker study (22), in which the structural alteration introduced an addi-

### Table 2. Increase of saline bolus dispersion indexes between VLD = 200 ml and 800 ml before and after histamine challenge

<table>
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<tr>
<th></th>
<th>Baseline</th>
<th>Histamine</th>
<th>P value</th>
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<tr>
<td><strong>Half width H:</strong></td>
<td></td>
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<tr>
<td>ΔH, ml</td>
<td>346 ± 76</td>
<td>480 ± 107*</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Standard deviation, σ:</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Δσ25%, ml</td>
<td>116 ± 21</td>
<td>138 ± 12*</td>
<td>0.02</td>
</tr>
<tr>
<td>Δσ15%, ml</td>
<td>127 ± 24</td>
<td>135 ± 22</td>
<td></td>
</tr>
<tr>
<td>Δσ5%, ml</td>
<td>123 ± 31</td>
<td>94 ± 46</td>
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</table>

Values are means ± SD. VLD, volumetric lung depth; H, half-width; σ25%, 15%, 5%, standard deviation using 25, 15, or 5% of the expiratory bolus maximum as a cutoff (see text for details); ΔH or Δσ, H or σ difference between VLD = 200 ml and 800 ml. *Significantly different from baseline (Wilcoxon signed-rank test).

Fig. 4. Average ± SE values of skew (sk) for 200, 400, 600, and 800 ml VLD obtained before (•) and after (○) histamine challenge. A, B, and C: sk computed using 25, 15, or 5% cutoff, respectively (see text for details). *VLD level showing significantly different sk after histamine (P < 0.05; Wilcoxon signed-rank test).
tional source of heterogeneity in the acinar spaces, a small but significant sk increase was seen, but only for high VLD.

Rosenthal et al. (15) previously drew attention to the need for more detailed modeling of the shape of the entire exhaled bolus to better understand bolus behavior in the lungs. Skew may carry a crucial piece of information on whether possible first-in last-out patterns of aerosol bolus spreading over lung units during inhalation are symmetrically reversed on bolus recombination during exhalation. Yet experimental bolus dispersion reports rarely include skew. In normal adult subjects, Siekmeier et al. (19) obtained skew values that decreased from $\sim 1.4$ (VLD = 200 ml) to $\sim 0.7$ (VLD = 800 ml), whereas in Brand et al. (5) skew values remained more or less stable around 0.2 between VLD = 200 ml and 800 ml. Other observations in normal children (17) showed that skew decreased from 0.23 to 0 between most shallow and most peripheral boluses, and in a study on the effect of intrinsic particle properties on bolus characteristics in beagle dogs (18) skew actually varied from 1.5 to 0. In addition, the latter study showed no dependence of skew on particle size between 0.5 and 2 µm. The baseline data in Fig. 4 show that the decrease of skew with VLD heavily depends on the cutoff that is used for skew computation. However, Fig. 4 also shows that the overall pattern of skew increases as a function of VLD after histamine is very similar for all cutoffs used.

**Saline bolus and N₂ washout-derived indexes of ventilation nonuniformity.** It is interesting to note the difference between aerosol and gas behavior in the particular case in which structural alteration induces sequential filling and emptying of units subtended by the conductive lung zone. In the case of aerosols, asymmetry between inspiratory and expiratory flow patterns can increase aerosol bolus dispersion and skew (14). In the case of gases, $S_{\text{cond}}$ increase is brought about by a combination of specific ventilation differences and expiratory flow asynchrony between these units. Yet specific ventilation is only determined by average inspiratory flow and therefore is insensitive to possible inspiratory flow sequencing between units. Therefore symmetry of flow sequencing between inhalation and exhalation does not affect the outcome for $S_{\text{cond}}$ as it does for aerosol dispersion and skew. From

Fig. 5. Typical exhaled bolus traces before (○) and after (■) histamine for inhaled saline boluses delivered to comparable VLDs (A: VLD = 196 and 185 ml; B: VLD = 715 and 736 ml). All exhaled boluses are normalized to their respective maxima, and horizontal lines are drawn at 100, 50, 25, and 5% of the exhaled bolus maximum. $H$, $\sigma_{25\%}$, and $\sigma_{5\%}$ values (in ml; subscripts represent cutoffs used) are also represented. Corresponding $sk_{25\%}$ and $sk_{5\%}$ values were, respectively, 0.39 and 1.02 for VLD = 196 ml and 0.49 and 1.33 for VLD = 185 ml in A and 0.14 and 0.68 for VLD = 736 ml in B.
this viewpoint, the increased $S_{\text{cond}}$ and aerosol bolus skew after histamine are complementary in suggesting, respectively, the occurrence of asynchronous emptying and an asymmetrical pattern between emptying and filling. Also in an effort to relate aerosol- and gas-related measures of convective ventilation heterogeneity, Brown et al. (6) found an association between bolus dispersion and $^{133m}$Xe washout-derived indexes that are independent of breath-by-breath flow asymmetry between units.

Saline vs. latex aerosol bolus tests. In the case of lung disease, it may be of considerable advantage to use saline instead of oil droplets or latex aerosol. We chose to assess dispersion and skew indexes derived from the saline bolus because these indexes have been shown to be poorly sensitive to particle size (9, 16, 18). In the smoker study (22), we performed exhaustive testing on a healthy subject as an example to show overall consistency between dispersion of saline and a nonhygroscopic aerosol. Possibly, simultaneous measurement of nonhygroscopic 0.5-, 1-, and 2-$\mu$m as well as hypotonic, hypertonic, and isotonic aerosol boluses in the same laboratory animals (possibly with induced lung disease) could provide some new insights into the actual fate of saline aerosols that is still very much under debate today (10). The measurement of indexes such as mode shift or deposition that do depend on particle size could then possibly be included to obtain an “effective particle size” that droplets have during most of their residence time in the lungs. The present study at least indicates the potential of saline aerosol to provide consistent measurements reflective of lung structure alteration at different lung depths.

In summary, and taken together with the data from the smoker study (22), the present results demonstrate that the saline bolus dispersion test has the potential of monitoring lung structural change at different levels of the bronchial tree, provided that the bolus tests are performed spanning a considerable VLD range and the resulting aerosol traces are adequately analyzed. In the particular case in which structural alterations induce additional bolus skew, dispersion indexes must be used that include as much of the exhaled bolus tails as possible. Besides the important result from the smoker study (22), namely that the saline aerosol dispersion test can be a sensitive tool to monitor structural change in the silent zone of the lungs in which traditional lung function tests perform poorly, the ability of the saline bolus dispersion to distinguish lung structural alterations occurring in the proximal lung from those appearing in the peripheral lung could enhance quality of diagnosis as well as the therapeutic targeting of drugs for various lung diseases. These are most often a combination of both proximal and peripheral lung alterations, and in the two companion papers presented here we have tried to isolate the effect of mild acinar and conductive airway alterations.

REFERENCES