

Nonuniformity of diffusing capacity from small alveolar gas samples is increased in smokers

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BACKGROUND: Although centrilobular emphysema, and small airway, interstitial and alveoli inflammation can be detected pathologically in the lungs of smokers with relatively well preserved lung function, these changes are difficult to assess using available physiological tests. Because submaximal single breath washout (SBW_{SM}) manoeuvres improve the detection of abnormalities in ventilation inhomogeneity in the lung periphery in smokers compared with traditional vital capacity manoeuvres, SBW_{SM} manoeuvres were used in this study to measure temporal differences in diffusing capacity using a rapid response carbon monoxide analyzer.

OBJECTIVE: To determine whether abnormalities in the lung periphery can be detected in smokers with normal forced expired volumes in 1 s using the three-equation diffusing capacity (DLco^{SB}-3EQ) among small alveolar gas samples and whether the abnormalities correlate with increases in peripheral ventilation inhomogeneity.

PARTICIPANTS AND DESIGN: Cross-sectional study in 21 smokers and 21 nonsmokers all with normal forced exhaled flow rates.

METHODS: Both smokers and nonsmokers performed SBW_{SM} manoeuvres consisting of slow inhalation of test gas from functional residual capacity to one-half inspiratory capacity with either 0 or 10 s of breath holding and slow exhalation to residual volume (RV). They also performed conventional vital capacity single breath (SBW_{VC}) manoeuvres consisting of slow inhalation of test gas from

RV to total lung capacity and, without breath holding, slow exhalation to RV. DLco^{SB}-3EQ was calculated from the total alveolar gas sample. DLco^{SB}-3EQ was also calculated from four equal sequential, simulated aliquots of the total alveolar gas sample. DLco^{SB}-3EQ values from the four alveolar samples were normalized by expressing each as a percentage of DLco^{SB}-3EQ from the entire alveolar gas sample. An index of variation (D_I) among the small-sample DLco^{SB}-3EQ values was correlated with the normalized phase III helium slope (S_n) and the mixing efficiency (E_{mix}).

RESULTS: For SBW_{SM}, D_I was increased in smokers at 0 s of breath holding compared with nonsmokers, and correlated with age, smoking pack-years and S_n . The decrease in D_I with breath holding was greater in smokers and correlated with the change in S_n with breath holding. For SBW_{VC} manoeuvres, there were no differences due to smoking in S_n or E_{mix} , but D_I was increased in smokers and correlated with age and smoking pack-years, but not with S_n .

CONCLUSIONS: For SBW_{SM} manoeuvres the increase in D_I in smokers correlated with breath hold time-dependent increases in S_n , suggesting that the changes in D_I reflected the same structural alterations that caused increases in peripheral ventilation inhomogeneity. For SBW_{VC} manoeuvres, the increase in D_I in smokers was not associated with changes in ventilation inhomogeneity, suggesting that the effect of smoking on D_I during this manoeuvre was due to smoke-related changes in alveolar capillary diffusion, rather than due solely to alterations in the distribution of ventilation.

Key Words: Diffusion index, Inhomogeneity, Slope of phase III, Small airways, Three-equation diffusing capacity, Tobacco smoking

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L'hétérogénéité de la capacité de diffusion dans des petits échantillons de gaz alvéolaire augmente chez les fumeurs

HISTORIQUE : Bien que l'emphysème centrolobulaire et l'inflammation des petites voies aériennes, l'inflammation de l'interstitium et des alvéoles, puissent être décelés à l'aide d'un examen pathologique dans les poumons des fumeurs présentant par ailleurs une fonction pulmonaire relativement bien conservée, ces changements sont difficiles à évaluer au moyen des tests physiologiques disponibles. Parce que le test de rinçage en respiration unique sous-maximale (SBW_{SM}) permet de mieux déceler les anomalies dans l'hétérogénéité de la ventilation dans la périphérie du poumon chez les fumeurs comparativement à l'épreuve classique se basant sur la capacité vitale, le test de SBW_{SM} a été utilisé dans la présente étude pour mesurer les différences temporelles dans la capacité de diffusion à l'aide d'un analyseur rapide de monoxyde de carbone.

OBJECTIF : Déterminer si les anomalies de la ventilation dans la périphérie du poumon peuvent être décelées chez les fumeurs dont le volume expiratoire maximum/seconde (VEMS) est normal au moyen de la méthode de la capacité de diffusion à trois équations (DL_{co}^{SB-3EQ}) dans des petits échantillons de gaz alvéolaire, et si les anomalies corrèlent avec les augmentations de l'hétérogénéité de la ventilation périphérique.

PARTICIPANTS ET MODÈLE : Étude transversale auprès de 21 fumeurs et de 21 non-fumeurs présentant tous des débits expiratoires normaux.

MÉTHODES : Les fumeurs et les non-fumeurs ont été soumis au test de SBW_{SM} consistant en une inhalation lente du gaz test à partir de la capacité résiduelle fonctionnelle jusqu'à la première moitié de la capacité inspiratoire, et après 0 s ou 10 s en apnée, en expirant lentement

jusqu'au volume résiduel (VR). Ils ont aussi été soumis au test classique de rinçage basé sur la capacité vitale (SBW_{VC}) consistant en une inhalation lente du gaz test à partir du VR jusqu'à la capacité pulmonaire totale et, sans apnée, en expirant lentement jusqu'au VR. La DL_{co}^{SB-3EQ} a été calculée à partir de l'échantillon complet de gaz alvéolaire. La DL_{co}^{SB-3EQ} a aussi été calculée à partir de quatre parties aliquotes égales, simulées et en séquence de la totalité de l'échantillon de gaz alvéolaire. Les valeurs de la DL_{co}^{SB-3EQ} provenant des quatre échantillons alvéolaires ont été standardisées en exprimant chacune d'entre elles comme un pourcentage de la DL_{co}^{SB-3EQ} obtenue à partir de la totalité de l'échantillon de gaz alvéolaire. Un index de variation (D_I) parmi les valeurs de la DL_{co}^{SB-3EQ} provenant des petits échantillons a été corrélu avec la pente de la phase III normalisée de l'hélium (S_n) et l'efficacité de la mixique (E_{mix}).

RÉSULTATS : Dans le cas du SBW_{SM} , le D_I augmentait chez les fumeurs à 0 s d'apnée comparativement aux non-fumeurs, et corrélait avec l'âge, le nombre de paquets/année et la S_n . La diminution de D_I en apnée était plus importante chez les fumeurs et corrélait avec le changement dans la S_n en apnée. Pour ce qui est des épreuves du SBW_{VC} , il n'y avait aucune différence attribuable au fait de fumer dans la S_n ou dans le E_{mix} , cependant, le D_I augmentait chez les fumeurs et corrélait avec l'âge et le nombre de paquets/année, mais pas avec la S_n .

CONCLUSIONS : En ce qui concerne les épreuves de SBW_{SM} , l'augmentation de D_I chez les fumeurs corrélait avec les augmentations de la S_n dépendante du temps d'apnée ce qui laisse croire que les changements dans D_I reflétaient les mêmes altérations structurales qui causaient une augmentation de l'hétérogénéité de la ventilation périphérique. Dans le cas des épreuves de SBW_{VC} , l'augmentation de D_I chez les fumeurs n'était pas associée avec les changements dans l'hétérogénéité de la ventilation, ce qui permet de croire que l'effet de fumer sur D_I pendant cette épreuve était dû aux changements induits par la fumée sur la diffusion capillaire alvéolaire plutôt qu'aux seules altérations de la distribution de la ventilation.

Smoking is associated with inflammation and fibrosis in small airways and alveoli (1-3), and vascular deficiency emphysema (4,5). Using conventional vital capacity single breath washout (SBW_{VC}) manoeuvres without breath holding, abnormalities in the phase III slope of nitrogen were found in healthy smokers which correlated with pathological changes in the small airways (3). However, prospective studies showed that the abnormalities in SBW_{VC} manoeuvres did not specifically identify those at risk of developing chronic airflow limitation (6). Others have proposed the use of more complex physiological techniques that highlight abnormalities in ventilation inhomogeneity in the lung periphery (7) using multibreath washout techniques (8) and gases of differing diffusivities (9), but these approaches are more demanding to implement as screening tests in large populations.

A refinement of SBW_{VC} manoeuvres with potential application to epidemiological investigation was proposed because it might provide more information about peripheral ventilation inhomogeneity (10,11). This refinement (SBW_{SM}) consisted of reducing the inspired volume of the SBW manoeuvre to one-half inspiratory capacity (IC) and initiating the SBW manoeuvre at a preinspiratory lung volume of functional residual capacity (FRC). In smokers with normal forced exhaled flow rates, abnormalities in the normalized phase III slope for helium (S_n) were detected using SBW_{SM} that were not evident using SBW_{VC} manoeuvres (11).

The difference between the two types of manoeuvres was thought to be caused by differences in the relative contributions of several types of ventilation inhomogeneity. For

SBW_{VC} manoeuvres, a dominant mechanism of nonuniform gas distribution was convective dependent, topographical ventilation inhomogeneity due to asynchronous emptying of large regions with unequal specific ventilations (12-14). In contrast with SBW_{SM} manoeuvre, the major origin of the ventilation inhomogeneity was in the lung periphery due to either intraregional convective dependent gradients among closely adjacent regions or the interaction of diffusion and convection at peripheral branch points within the acinus (12,15). A characteristic feature of inhomogeneity due to the interaction of convection and diffusion at peripheral branch points, and to a lesser extent to inhomogeneity due to intraregional convective dependent gradients, was that the tracer gas concentration gradients caused by this type of inhomogeneity were markedly diminished with short periods of breath holding. This is as a result of cardiogenic and diffusive gas mixing within the lung periphery (7,12). Hence, the steeper S_n in smokers for SBW_{SM} manoeuvres at 0 s of breath holding and the greater change in S_n in smokers over short periods of breath holding indicated a superior ability of the SBW_{SM} manoeuvre to identify an abnormality in peripheral ventilation inhomogeneity in smokers compared with the SBW_{VC} manoeuvre (11).

In this study, we extended the analysis of SBW_{SM} manoeuvre in smokers by evaluating the simultaneously measured disappearance curve of carbon monoxide. The three-equation diffusing capacity for carbon monoxide ($DLCO^{SB-3EQ}$), measured from four equal and sequential aliquots of the total alveolar gas sample, was also affected by

manoeuvres that altered the distribution of ventilation in the lung periphery in normal nonsmoking subjects (16). Compared with the predicted carbon monoxide washout for a uniform lung model, the carbon monoxide concentration was higher than expected early in exhalation and lower than expected later in exhalation in normal subjects for SBW_{SM} manoeuvre without breath holding. This resulted in a reduced DLCO^{SB}-3EQ when calculated from the first alveolar sample early in exhalation but an increased DLCO^{SB}-3EQ when measured from the fourth sample at near residual volume (RV) in normal subjects. These differences in DLCO^{SB}-3EQ among small alveolar gas samples were attributed to time-dependent carbon monoxide concentration gradients in the lung periphery because the effect largely disappeared with short periods of breath holding (16).

We wished to determine whether the changes in DLCO^{SB}-3EQ among small alveolar gas samples were abnormal in smokers with otherwise relatively normal lung function. We hypothesized that smoking would magnify the degree of nonuniformity (D_I) of DLCO^{SB}-3EQ measured from small alveolar gas samples at 0 s of breath holding in smokers, particularly for SBW_{SM} manoeuvres. As well we thought that D_I would correlate with increases in peripheral ventilation inhomogeneity in smokers using the normalized phase III helium slope (11), but would disappear with short periods of breath holding (16).

PATIENTS AND METHODS

Study group: Twenty-one smokers and 21 nonsmokers were recruited from the community and hospital personnel. In both groups as previously reported (11), subjects denied recent respiratory symptoms. Spirometric testing revealed that the forced vital capacity (FVC), the forced expired volume in 1 s (FEV₁) and the maximal mid-expiratory flows (FEF₂₅₋₇₅) were within the 95% confidence limits of normal using previously reported regressions (17). Smokers had a current cigarette consumption of at least 10 cigarettes per day and a cumulative exposure of at least four pack-years. Nonsmokers had no current exposure and had smoked less than 100 cigarettes in their lifetime.

Apparatus and equipment: Using equipment previously described (11,18), seated subjects, at rest, breathed test gas containing 0.3% carbon monoxide, 10% helium, 21% oxygen and the balance nitrogen through a low dead space two-way valve. Flow and volume were measured continuously with a pneumotach mounted in the wall of a bag-in-box system, helium concentration with a mass spectrometer and carbon monoxide concentration with a rapidly responding infrared analyzer. Throughout each SBW, including two to three preceding tidal breaths and a standardized deep breath, flow, volume, carbon monoxide and helium signals were stored digitally (50 Hz) for later computer analysis.

Protocol: The protocol used in this study has been previously reported (11). All SBWs were preceded by a standardized deep breath of room air (19) and were performed singly in random order on one study day. An individualized volume versus time 'template' of the prescribed manoeuvre (18) was

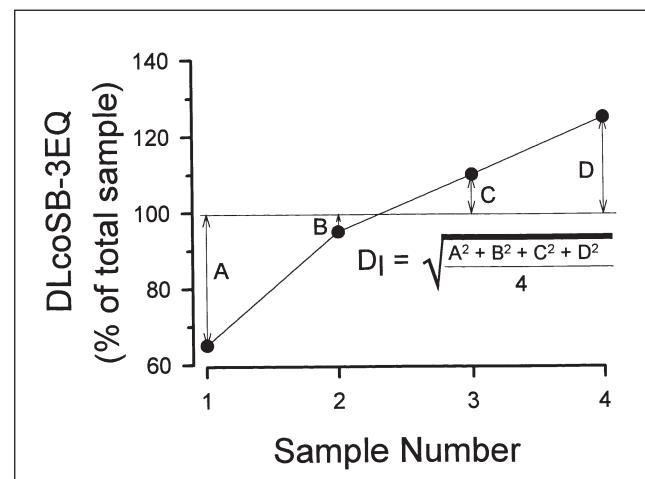


Figure 1) Three-equation diffusing capacity for carbon monoxide ($DLco^{SB}$ -3EQ) values measured from the four equal alveolar gas samples are normalized by expressing them as a percentage of $DLco^{SB}$ -3EQ measured from the total alveolar gas sample (y axis). Values (sample 1 to 4) are shown for a representative submaximal single breath washout manoeuvre at 0 s of breath holding. $DLco^{SB}$ -3EQ value for sample 1, measured at high expired lung volumes, is below 100% whereas subsequent $DLco^{SB}$ -3EQ values rise progressively above 100%. For each of the four normalized $DLco^{SB}$ -3EQ values measured from the four equal alveolar gas samples, the difference between each of the small samples and the normalized $DLco^{SB}$ -3EQ from the total alveolar gas sample was calculated (A, B, C, D). The root mean square difference of the four samples, the degree of diffusion nonuniformity (D_I) is calculated using A, B, C and D as shown in the insert. D_I is 27% in this example.

displayed on a monitor to assist subjects in performing the following SBWs: SBW_{SM} manoeuvres consisting of slow inhalation from FRC to one-half IC, with either 0 s or 10 s of breath holding and slow exhalation to RV; and conventional SBW_{VC} manoeuvres consisting of slow inhalation from RV to total lung capacity, and without breath holding, slow exhalation to RV. All inspired and expired flows for the preceding deep breath and for all SBW manoeuvres were maintained at 0.5 L/s.

Analysis: For each SBW, the inspired and expired volumes, inspiratory and expiratory times, and the breath hold time were measured (11). RV was calculated by measuring the mass of helium inhaled and exhaled to determine the mass of helium remaining in the lung, as previously described (18,20,21). It was assumed that the mean helium concentration in the lung at RV was equal to the measured helium concentration at end expiration.

Because SBW manoeuvres were analyzed after storage of the flow, carbon monoxide and helium signals in digital form, $DLco^{SB}$ -3EQ from any one or a number of simulated alveolar samples from the same manoeuvre could be analyzed (18). In this analysis the three-equation diffusing capacity using the simulated entire alveolar gas sample was computed (13,18,20). $DLco^{SB}$ -3EQ from the mean carbon monoxide concentration in each of four simulated sequential and equal alveolar gas samples (16) was calculated, and each $DLco^{SB}$ -3EQ value was normalized by expressing it as a percentage of $DLco^{SB}$ -3EQ calculated from the entire alveolar

TABLE 1
Group mean values (\pm SD) of single breath washout manoeuvres for nonsmokers and smokers during conventional vital capacity manoeuvres without breath holding (SBW_{VC}) and submaximal (SBW_{SM}) manoeuvres at 0 and 10 s of breath holding

Breath hold time	SBW _{VC}		SBW _{SM}			
	Nonsmoker	Smoker	0 s		10 s	
			Non-smoker	Smoker	Non-smoker	Smoker
Expired volume (L)	4.46 \pm 0.95	4.34 \pm 1.1	2.99 \pm 0.71	3.00 \pm 0.98	2.96 \pm 0.77	2.97 \pm 0.98
Absolute maximum end-inspired volume (L)	6.13 \pm 1.27	6.18 \pm 1.60	4.74 \pm 1.09	4.92 \pm 1.42	4.79 \pm 1.15	4.99 \pm 1.40
Absolute end-inspired volume (L)	1.67 \pm 0.42	1.83 \pm 0.50	3.14 \pm 0.93	3.38 \pm 1.17	3.14 \pm 0.97	3.41 \pm 1.15
Breath hold time (s)	1.4 \pm 0.5	1.3 \pm 0.5	0.8 \pm 0.2	0.8 \pm 0.2	9.8 \pm 0.8	9.8 \pm 0.35
DLco ^{SB} -3EQ	33.5 \pm 7.6	29.0 \pm 6.4*	34.3 \pm 8.2	28.9 \pm 7.8*	33.5 \pm 8.0	29.9 \pm 6.8
D _I (%)	5.1 \pm 2.7	7.6 \pm 4.3*	17.8 \pm 6.7	42.4 \pm 29.9***	3.4 \pm 1.1	5.0 \pm 2.8*
ΔD_I (%)			14 \pm 6	37 \pm 28***		
S _n (mL ⁻¹)	-14.4 \pm 7.9	-16.1 \pm 8.3	-64.9 \pm 29.5	-134.5 \pm 5.3***	-27.7 \pm 15.4	-59.2 \pm 43.5**
Mixing efficiency (%)	96.8 \pm 1.3	96.0 \pm 1.6	89.9 \pm 2.6	87.9 \pm 4.2	93.1 \pm 1.7	91.6 \pm 3.2

Significance of comparisons between smokers and nonsmokers: *P<0.05, **P<0.01, ***P<0.001. DLCO^{SB}-3EQ Three equation diffusing capacity for carbon monoxide; D_I Degree of uniformity, root mean square difference of DLCO^{SB}-3EQ calculated from the four equal alveolar gas samples that are normalized by expressing them as a percentage of DLCO^{SB}-3EQ measured from the total alveolar gas sample; S_n Normalized phase III slope for the tracer gas helium

gas sample (18). For all DLCO^{SB}-3EQ calculations, the mean carbon monoxide concentration in the exhaled alveolar gas of the deep breath of room air immediately preceding the single breath manoeuvre was used as the estimate of the carbon monoxide back pressure for the subsequent SBW manoeuvre (22).

D_I described the degree to which the four DLCO^{SB}-3EQ values measured from the four alveolar gas samples deviated from the single DLCO^{SB}-3EQ value measured from the entire alveolar gas sample by calculating the root-mean-square difference (18) of the normalized DLCO^{SB}-3EQ values (Figure 1). D_I quantified the degree of nonuniformity of DLCO^{SB}-3EQ measured from the four small samples, each normalized by DLCO^{SB}-3EQ measured from the total sample.

As previously reported (11) the phase III helium slope ($\Delta He/L$) between 33% and 67% of the exhaled volume was measured, and the phase III slope (S_n) was normalized by dividing $\Delta He/L$ by the predicted ideal, mean end-inspired alveolar helium concentration ([He]_{A PRED}) (13,18). The mixing efficiency (E_{mix}) was calculated using a previously described computer algorithm (18).

Statistics: Group mean data for smokers and nonsmokers were compared using unpaired Student's *t* tests. Differences due to the SBW manoeuvre and to breath hold time were assessed using paired Student's *t* tests in both smokers and nonsmokers. Correlations were performed using linear regression analysis.

RESULTS

The characteristics of the smoking and nonsmoking groups, which have been previously reported (11), were similar in terms of sex distribution (smokers: 12 women and nine men; nonsmokers: 10 women and 11 men), age (smokers 39 \pm 8 years; nonsmokers 38 \pm 7 years, mean \pm SD) and height (smokers 172 \pm 10 cm; nonsmokers 174 \pm 10 cm). There

were no significant differences in FVC (smokers 105 \pm 10% predicted; nonsmokers 101 \pm 8% predicted) or FEV₁ (smokers 100 \pm 9% predicted; nonsmokers 102 \pm 8% predicted). The FEF₂₅₋₇₅ was smaller (P<0.05) in smokers (90 \pm 24% predicted) than in nonsmokers (106 \pm 21% predicted). The smokers had a mean of 21 \pm 12 pack-years of cigarette smoking (range of four to 55 pack-years). There were no differences in the expired volume, maximum end-inspired volume, pre-inspiratory lung volume or breath hold time for comparable manoeuvres between smokers and nonsmokers (Table 1).

SBW_{SM} at 0 s of breath holding: DLCO^{SB}-3EQ from the total alveolar sample was slightly reduced in smokers (Table 1; P<0.05). DLCO^{SB}-3EQ measured from small alveolar gas samples was reduced in the first alveolar sample, measured at high lung volumes just after alveolar dead space clearance, but increased progressively in subsequent samples (Figure 2, left graph). D_I was therefore greater in smokers (42 \pm 29%) than in nonsmokers (18 \pm 7%) (Table 1; P<0.001). D_I correlated with age in smokers (Figure 3; r²=0.23; P=0.026) and pack-years of smoking (Figure 3; r²=0.36; P=0.004). S_n, but not E_{mix}, was also markedly increased in smokers (Table 1; P<0.001). D_I at 0 s of breath holding correlated significantly with S_n at 0 s of breath holding in both smokers (r²=0.63; P<0.001) and nonsmokers (r²=0.38; P<0.01) (Figure 4). The day-to-day coefficient of variation of D_I for this manoeuvre was 8.0 \pm 4.6% in 10 normal subjects whose value were repeated on five successive days (22).

Effect of 10 s of breath holding on SBW_{SM}: DLCO^{SB}-3EQ from the total alveolar gas sample was similar between smokers and nonsmokers (Table 1). For DLCO^{SB}-3EQ measured from small alveolar gas samples there was markedly less deviation of all four samples from DLCO^{SB}-3EQ measured from the total sample (Figure 2). However, the pattern of change remained similar to that observed at 0 s of breath

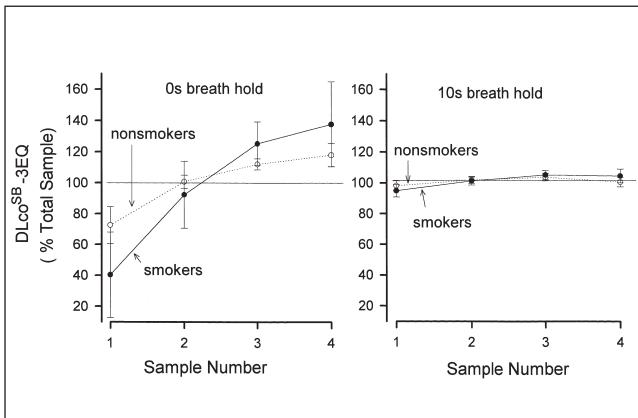


Figure 2) Group mean values for the four normalized three-equation diffusing capacity for carbon monoxide ($\text{DLCO}^{\text{SB}}\text{-3EQ}$) values (error bars, $\pm \text{SD}$) for nonsmokers (\circ) and smokers (\bullet) at 0 s of breath holding (left diagram) and after 10 s of breath holding (right diagram). Because the $\text{DLCO}^{\text{SB}}\text{-3EQ}$ values deviate more from $\text{DLCO}^{\text{SB}}\text{-3EQ}$ measured from the whole sample in smokers, degree of nonuniformity (D_I) is larger in this group ($42 \pm 29\%$, mean $\pm \text{SD}$) than in nonsmokers ($18 \pm 6\%$) at 0 s of breath holding. At 10 s of breath holding the four samples are almost identical to $\text{DLCO}^{\text{SB}}\text{-3EQ}$ from the total alveolar gas sample. The degree of uniformity dramatically decreases with breath holding in both groups, but remains significantly higher in smokers

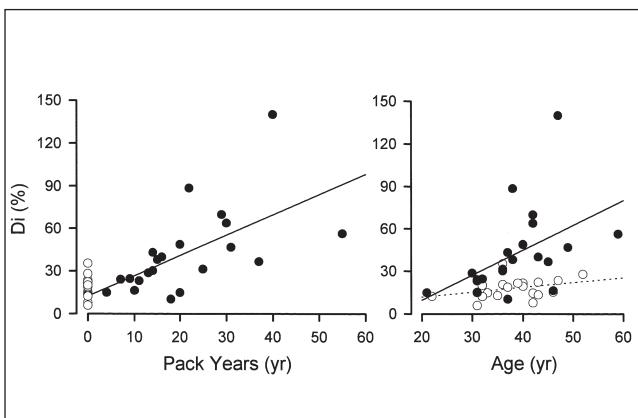


Figure 3) For submaximal single breath washout manoeuvres degree of diffusion nonuniformity (D_I) correlates with pack-years of smoking (left diagram) in smokers ($r^2=0.36$; $P=0.004$) and with age in smokers ($r^2=0.23$; $P=0.026$), but not in nonsmokers (right diagram, $r^2=0.12$). \circ with dashed line Nonsmokers; \bullet with solid line Smokers

holding with $\text{DLCO}^{\text{SB}}\text{-3EQ}$ being lower for sample 1 but higher for sample 4. Whereas D_I was markedly reduced, compared with those measured at 0 s of breath holding, it remained slightly, but significantly higher in smokers ($5 \pm 3\%$) than nonsmokers ($3 \pm 1\%$) (Table 1) and correlated with pack-years of smoking ($r^2=0.51$; $P<0.001$) and with S_n ($r^2=0.22$; $P<0.05$).

Change in D_I with breath holding for SBW_{SM}: The decrease in D_I from 0 s to 10 s of breath holding correlated with S_n measured at 0 s of breath holding in both nonsmokers ($r^2=0.37$; $P<0.01$) and smokers ($r^2=0.61$; $P<0.01$), but did not correlate with S_n at 10 s of breath holding in either group. In smokers the decrease in D_I with breath holding time (ΔD_I)

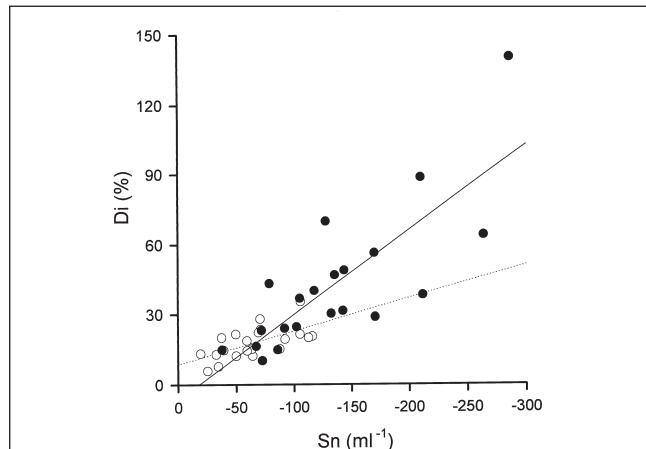


Figure 4) Degree of diffusion nonuniformity (D_I) (%) correlates with the normalized phase III helium slope (S_n) for submaximal single breath washout (SBW_{SM}) manoeuvres at 0 s of breath holding in both smokers ($r^2=0.63$, $P<0.001$, solid line) and in nonsmokers ($r=0.38$, $P<0.01$, dashed line). For the nonsmokers and smokers combined, ($r^2=0.69$, $P<0.001$). \circ Nonsmokers; \bullet Smokers

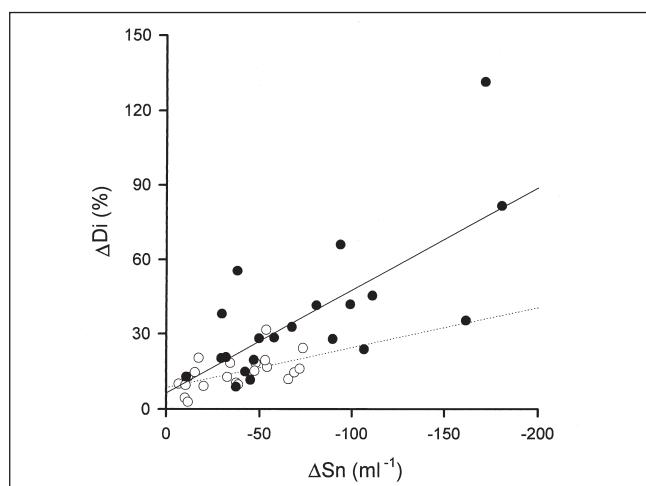


Figure 5) The reduction in degree of diffusion nonuniformity (D_I) from 0 s to 10 s of breath holding (ΔD_I) for submaximal single breath washout (SBW_{SM}) (y axis) correlates with the change in normalized phase III helium slope (S_n) from 0 to 10 s of breath holding (ΔS_n) for SBW_{SM} manoeuvres in both smokers ($r^2=0.51$, $P<0.001$, solid line) and nonsmokers ($r^2=0.31$, $P<0.01$, dashed line). \circ Non-smokers; \bullet Smokers

was significantly greater in smokers than nonsmokers (Table 1) and correlated with the corresponding change in S_n (ΔS_n) (Figure 5, $r^2=0.51$; $P<0.001$). In nonsmokers ΔD_I and ΔS_n were both less than in smokers, but the correlation between them was also significant ($r^2=0.31$; $P<0.01$).

SBW_{VC}: $\text{DLCO}^{\text{SB}}\text{-3EQ}$ measured from the whole sample was slightly lower in smokers (Table 1). D_I was significantly increased in smokers (Table 1; $P<0.05$) and correlated with age in smokers ($r^2=0.48$; $P<0.001$) but not in nonsmokers, and with pack-years of smoking ($r^2=0.55$; $P<.001$) (Figure 6). However, there were no differences between smokers and nonsmokers for either S_n or E_{mix} (Table 1) as previously reported (11). Furthermore, D_I did not correlate with S_n in either

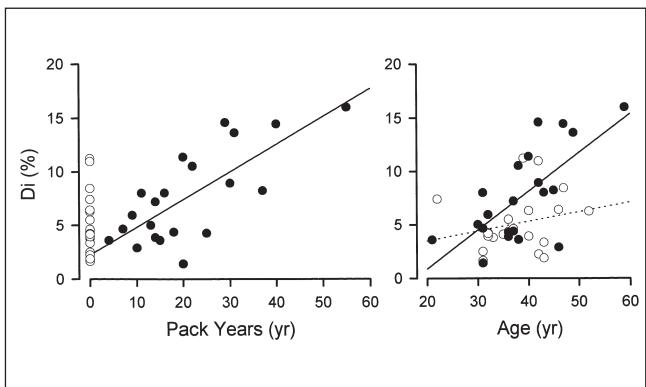


Figure 6 For vital capacity single breath washout manoeuvres degree of diffusion nonuniformity (D_l) correlates with pack-years of smoking (left diagram) in smokers ($r^2=0.55, P<0.001$) and with age in smokers ($r^2=0.48, P<0.01$) but not in nonsmokers (right diagram). ○ Nonsmokers; ● Smokers; yr Years

smokers or nonsmokers. The day-to-day coefficient of variation of D_l for this manoeuvre was $1.6\pm 5.9\%$ in 10 normal subjects who repeated the manoeuvre on five successive days (22).

DISCUSSION

The most remarkable finding in this study was that D_l was significantly higher in smokers for SBW_{SM} at 0 s of breath holding and correlated with cumulative pack-years of smoking. The fact that, for these manoeuvres, D_l correlated with both age and pack-years of smoking in smokers, but was not affected by age in nonsmokers (Figure 3), implied a specific effect of smoking on D_l that could not be explained by ageing. Although the precise pathological changes responsible for the increase in D_l in smokers for SBW_{SM} manoeuvres at 0 s of breath holding must await future structure-function correlations, the effects of breath holding on both D_l and simultaneous measurements of ventilation inhomogeneity allow us to draw some inferences about the possible mechanisms for the effects of smoking on D_l .

Smoking produces a number of pathological lesions in the lung (1-3,23,24). Recent observations using computed tomography (CT) revealed surprisingly common abnormalities in smokers with relatively normal lung function. The abnormalities consisted of ground glass attenuation, micronodules and diffuse emphysematous changes in smokers, but these changes were not found in nonsmokers (24). Pathologically, ground glass attenuation correlated with alveolitis and interstitial inflammation; parenchymal micronodules correlated with bronchiolectasis and peribronchial fibrosis, similar to those previously found in smokers (3); and emphysema exceeded the extent observed from CT analysis (1).

For SBW_{SM} at 0 s of breath holding the increase in D_l with smoking pack-years could be related to any one or a combination of these abnormalities. Macroscopic emphysema was unlikely to have caused the increase in D_l in this group of smokers. Previous postmortem studies in smokers found no relationship between either emphysema grade, or the destructive index, and the single breath phase III slope (25) in

the present study. Therefore, inflammation and fibrosis in small airways most likely accounted for the increase in D_l in these healthy smokers in the present study. This was supported by the correlation of D_l with S_n in smokers (Figure 4) for SBW_{SM} manoeuvres at 0 s of breath holding. Moreover, breath holding dramatically decreased D_l for SBW_{SM} manoeuvres (Figure 2), and the decrease in D_l with breath holding correlated with the change in S_n with breath holding (Figure 4). This observation further supported the concept that the effects of smoking on D_l occurred primarily in the pulmonary parenchyma distal to the membranous bronchioles (26).

Two possible mechanisms could explain this observation. First, the decrease in D_l and the shallower S_n with breath holding for SBW_{SM} may both be explained by the common effect of small airway inflammation and/or regional loss of elastic recoil on intraregional convective dependent inhomogeneity, the interaction of convection and diffusion at peripheral branch points, or both phenomena (12). Second, the breath holding effects on D_l and S_n may not necessarily have been causally linked to alterations in ventilation inhomogeneity as previously suggested to explain the effects of breath holding on D_l in normal subjects (16). Rather, both may have been altered by time, but for different reasons. S_n may have been steeper in smokers because of increased peripheral ventilation inhomogeneity. D_l may have been increased, at least in part, by an additional effect of an exaggerated serial gradient in alveolar-capillary diffusion in smokers, such that diffusion was preferentially reduced in the proximal versus distal region of the terminal respiratory unit. Without breath holding, such a gradient in diffusion within the acinus would have created a higher carbon monoxide concentration in proximal regions that emptied early in exhalation, accounting for the higher than expected carbon monoxide concentration early in exhalation (sample 1) and the lower than expected carbon monoxide concentration later in exhalation (16). However, with breath holding this serial gradient in carbon monoxide concentration within the acinus would have rapidly decreased over time because of cardiogenic and diffusive gas mixing within the air phase of the terminal respiratory unit. Early centrilobular emphysema (1,24) could have caused such a preferential decrease in diffusion in the proximal (alveolar ducts), compared with the distal (alveole), regions of the terminal respiratory units, thus exaggerating the effects of breath holding on D_l seen in normal subjects (16).

Abnormalities in the phase III nitrogen slope in a group of healthy smokers with otherwise normal lung function have been previously reported for SBW_{VC}, but only when the smokers forcibly exhaled to RV immediately before performing the SBW_{VC} manoeuvre (27). The proposed mechanism for this effect of smoking was that the rapid forced exhalation just preceding the SBW_{VC} manoeuvre delayed regional emptying in peripheral regions distal to small airway inflammation and narrowing in the lungs of smokers. This reveals the presence of this disease process by transiently altering the spatial distribution ventilation by increasing specific

ventilation in regions distal to the airway narrowing. In the present study, SBW_{VC} manoeuvres were preceded by a deep breath, consisting of slow exhalation to RV, so that we would not have expected changes in ventilation inhomogeneity in otherwise healthy smokers due to the former mechanism (27); indeed, S_n and E_{mix} for SBW_{VC} manoeuvres in smokers were normal. However, D_I was unexpectedly increased and did not correlate with changes in S_n in smokers suggesting that an additional factor, other than alterations in ventilation inhomogeneity, was involved. The increase in D_I due to smoking for these SBW_{VC} manoeuvres may have been due to reductions in gas diffusion in macroscopic regions of emphysema that remained ventilated, but that emptied preferentially early in expiration. This appeared unlikely because macroscopic emphysema was thought to be ventilated poorly and largely by collateral channels (28). Alternatively, and more likely, the effect of smoking on D_I for these SBW_{VC} manoeuvres may have been due to the same mechanism proposed for SBW_{SM} manoeuvres as described above. That is, centrilobular emphysema could have caused proximal destruction of terminal respiratory units, resulting in an exaggeration of the serial gradients in diffusion from the proximal (reduced diffusion) to the distal (increased diffusion) segments of terminal respiratory units.

Previous reports found that the conventional single breath diffusing capacity for carbon monoxide (DLCO^{SB}) (29) was reduced in smokers (5,30) and correlated with macroscopic disease, as detected by CT scan techniques (31). However, in seated patients at rest DLCO^{SB} was insensitive to the inter-regional nonuniformity in the distribution of emphysema that often preferentially affects the apex of the lung in smokers (5). Conventional DLCO^{SB} testing, as distinct from the DLCO^{SB}-3EQ (20), was also less useful as a screening tool because the method was spuriously affected by the variations in the way the single breath test was performed (32). Interpretation of DLCO^{SB} in smokers has also been potentially confounded by uncertainties about precise corrections of DLCO^{SB} for carbon monoxide back pressure, carboxy-hemoglobin and the binding affinity of hemoglobin (29). In the present study we corrected all measurements of DLCO^{SB}-3EQ for the carbon monoxide back pressure by measuring the carbon monoxide in the exhaled gas during the exhalation phase of the deep breath of room air preceding each DLCO^{SB}-3EQ manoeuvre (18). The infrared carbon monoxide analyzer was sufficiently sensitive to allow measurement of background carbon monoxide in nonsmokers at levels of 0 to 70 ppm (18). The carbon monoxide analyzer was linear in this range and was checked daily (18).

Although we did not measure carboxy-hemoglobin (29) in the smokers in this study, most of whom reported smoking in the 2 h before testing, it would likely have been elevated, accounting for most of the apparent reduction in DLCO^{SB}-3EQ measured from the total sample in smokers compared with nonsmokers (22). DLCO^{SB}-3EQ measured from the total sample in smokers would have been affected by both carbon monoxide back pressure and carboxy-hemoglobin. However, we found that D_I required no corrections because it was not

affected by increases in carboxy-hemoglobin in normal subjects (22).

The present method of measuring DLCO^{SB}-3EQ from four sequential alveolar gas samples has a number of potential limitations. An analyzer with appropriate instrumentation and software is not currently commercially available. We employed a carbon monoxide analyzer that was modified to decrease its response time to 110 ms (18). The use of mass spectrometry for analysis of helium, while providing excellent signal characteristics, is too expensive for routine implementation. The manoeuvres also required instantaneous biofeedback to ensure reproducibility. This is feasible using available computer technology, and the software has been developed. In our experience, naive subjects are able to complete these manoeuvres with minimal prior coaching, but the reproducibility is less than in trained subjects (33). Finally, although this report demonstrates clear changes in smokers, the effect of smoking on D_I is not necessarily specific. Other pathological processes that lead to small airway obstruction (34) such as bronchiolitis obliterans following lung transplant, could have similar effects.

CONCLUSIONS

We have shown that D_I was increased in smokers in whom FEV₁ was normal. The effect, which was much greater for SBW_{SM} manoeuvres, largely disappeared with breath holding and correlated with a greater change in S_n with breath holding, indicating that the increase in D_I was caused by events occurring in the lung periphery. However, the precise structural correlates require definition. Because D_I was also increased in smokers for SBW_{VC} manoeuvres, in the absence of smoking-induced alterations in ventilation inhomogeneity, the effects of smoking on D_I may have been due to intra-regional changes in diffusion across the air-blood barrier. D_I may be a more sensitive indicator of changes in the lung periphery due to smoking.

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