

# Small airways ventilation heterogeneity and hyperinflation in COPD: Response to tiotropium bromide

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**Abstract:** In chronic obstructive pulmonary disease (COPD) patients tiotropium bromide has been shown to improve forced expiratory volume in one second ( $FEV_1$ ) and inspiratory capacity (IC). We investigated whether the mechanism leading to these improvements is related to small airways ventilation heterogeneity, assessed by multiple breath washout tests. Forty stable tiotropium-free COPD patients ( $FEV_1$ : 27%–78% predicted) were studied before and 90 min after administration of tiotropium bromide on visit<sub>0</sub>, and following 3 and 6 weeks of tiotropium bromide treatment (visit<sub>3wks</sub>, visit<sub>6wks</sub>). After study completion, COPD patients were classified into two subgroups according to degree of hyperinflation at visit<sub>0</sub> (Hyp–, Hyp+). The Hyp+ group showed significant increases in trough (ie, pre-dose)  $FEV_1$  and IC after 3 and 6 weeks of tiotropium bromide, and the 90 min tiotropium bromide responses of  $FEV_1$  and IC were significant at visit<sub>0</sub> ( $p \leq 0.001$  for both) but not during subsequent visits. The Hyp– group showed significant  $FEV_1$  increases 90 min after tiotropium bromide on all three visits (all  $p < 0.005$ ) but no sustained increase in trough values. In both COPD subgroups, the grossly abnormal ventilation heterogeneity barely showed any significant improvements with tiotropium bromide in the conductive airways (without changes in trough value) and no changes at all in the acinar airways. We conclude that the sustained improvements in trough IC and  $FEV_1$  with tiotropium bromide predominantly observed in COPD patients with considerable hyperinflation, are unrelated to small airways ventilation heterogeneity.

**Keywords:** COPD, hyperinflation, small airways, ventilation heterogeneity

## Introduction

Several clinical trials have investigated the effect of the long-acting anticholinergic bronchodilator, tiotropium bromide (Barnes et al 1995), on lung function and dyspnea at rest (van Noord et al 2000; Vincken et al 2002; Casaburi et al 2002; Celli et al 2003; Brusasco et al 2003; Verkindre et al 2006) and during exercise (O'Donnell et al 2004; Casaburi et al 2005; Maltais et al 2005), and assessed its influence on quality of life (Vincken et al 2002; Casaburi et al 2002; Brusasco et al 2003; Casaburi et al 2005; Verkindre et al 2006) or on the occurrence of exacerbations (Vincken et al 2002; Niewoehner et al 2005; Dusser et al 2006) in chronic obstructive pulmonary disease (COPD). Most of these studies indicated significant and sustained improvements of the primary outcome parameters, and consistently reported improvements in forced expiratory volume in one second ( $FEV_1$ ) and/or inspiratory capacity (IC) after different tiotropium bromide treatment periods up to one year. Given that tiotropium bromide is thought to act through its selective and prolonged binding to  $M_3$  receptors (Gross 2004), which are predominantly located in the large and medium-sized airways (Barnes 2004), it could a priori be expected that the improvement in airway function would be mainly located in these larger conductive airways. While the observed  $FEV_1$  improvements could be brought in agreement with this proposition, the IC improvements have

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prompted several authors to hint at the implication of small airways in this anticholinergic bronchodilation process (van Noord et al 2000; Verkindre et al 2006).

We have recently shown that COPD patients are characterized by a combination of both large and small conductive airways ventilation heterogeneity, as opposed to smokers with similar smoking history but without COPD in whom only small airways are affected (Verbanck et al 2004). This was measured with a model-based analysis of the multiple breath washout test which delivers independent phase III slope-derived indices of ventilation heterogeneity originating in the conductive and acinar lung zone (Scond and Sacin, respectively) (Verbanck et al 1997, 1998). In an earlier study, COPD patients have shown no significant response of Scond or Sacin to 400 µg of salbutamol with a small spacer inhalation aid (Verbanck et al 1999). In the present study, we use Scond and Sacin measurements to test the hypothesis that the small airways situated in the conductive and/or proximal acinar lung zones could participate to some degree in the tiotropium bromide response and thus affect inspiratory capacity, which is linked to dyspnea and exercise limitation in COPD patients (O'Donnell et al 2004). In particular, this mechanistic study aims to investigate whether the reported beneficial effects on pre-dose (trough) and peak values of  $FEV_1$  and IC are paralleled by improvements in small airways heterogeneity. In order to assess whether any potential improvement in small airways ventilation heterogeneity was specifically linked to improvement in hyperinflation, we deliberately included COPD patients with varying degrees of hyperinflation at baseline (which is also representative of a real-life clinical situation).

## Material and methods

### Patients and procedures

The study protocol was approved by the local ethics committee. Forty patients with a clinical diagnosis of COPD for at least 2 years were recruited if they fulfilled the following inclusion criteria: aged over 40 years, smoking history of at least 15 packyears,  $FEV_1$ /forced vital capacity (FVC) < 70% and post bronchodilator  $FEV_1$  < 80%pred. Patients with a history of concomitant asthma or atopy or patients requiring supplemental oxygen therapy were excluded. None of the patients had suffered any exacerbation in the six month period prior to the study, nor any recent upper respiratory tract infection. The COPD patients already on tiotropium bromide treatment were asked to discontinue tiotropium for at least 4 weeks before the screening visit. A written informed consent was obtained from all partici-

pating patients. Patients had been requested not to take any short- or long-acting bronchodilator medication for at least 8 h and 24 h, respectively, before each visit. There was 1 screening visit and 3 visits further referred to as study visits, ie, a baseline visit ( $visit_0$ ) and two visits after respectively 3 and 6 weeks ( $visit_{3wks}$  and  $visit_{6wks}$ ) of tiotropium bromide therapy (18 µg dry powder inhaled via the HandiHaler<sup>®</sup> device once daily in the morning). On  $visit_{3wks}$  and  $visit_{6wks}$ , the last tiotropium bromide intake was on the morning preceding the study day.

On the screening visit, patients underwent lung function and ventilation distribution testing according to standardized procedures. Lung function included spirometry and plethysmographic measurement of specific airway conductance (sGaw) and IC ( $IC_{pl}$ ). Given the potential problems with plethysmographic volume determination in the case of obstructed patients (Rodenstein and Stanescu 1982), we also used the preferred method of IC computation (ie, the difference between open circuit functional residual and total lung capacity volume levels). For the computation and interpretation of ventilation heterogeneity indices Scond and Sacin, we refer to a previous description (Verbanck et al 1997), which is briefly summarized in the Appendix. The screening visit served to characterize patients in terms of lung function and ventilation heterogeneity, and to assess their room for reversibility by the combination of a short-acting anticholinergic and a fast-acting  $\beta_2$ -agonist drug. Sixty minutes after inhalation of 80 µg of ipratropium bromide, an intermediate spirometry was performed, and additional bronchodilation was elicited by inhaling 400 µg of salbutamol. After another 30 min (ie, cumulative post-bronchodilator time: 90 min), lung function and ventilation distribution testing was repeated.

On the baseline study visit ( $visit_0$ ), patients first underwent lung function and ventilation distribution testing, and were then instructed to inhale 18 µg of tiotropium bromide dry powder via the HandiHaler<sup>®</sup> device for the first time. After 60 min, intermediate spirometry was performed, and after 90 min, the study visit was concluded with lung function and ventilation distribution testing. The next two study visits ( $visit_{3wks}$  and  $visit_{6wks}$ ), after respectively 3 and 6 weeks of daily tiotropium bromide treatment, involved exactly the same procedure as that on  $visit_0$ . Given that for these two study visits, the last tiotropium bromide intake had been on the morning preceding the study day, the 90 min effect of tiotropium bromide could be assessed on trough values of lung function and ventilation heterogeneity in the laboratory.

On each visit, patients rated their dyspnea on a visual analogue scale (VAS) and by means of the Medical Research Council (MRC) scale (ranging 1–5). Finally, patients also filled out a symptom score sheet for wheezing, shortness of breath, cough, and chest tightness ranging “not present”, “mild”, “moderate”, “severe” (scored 0–3 and summed to a total symptom score ranging 0–12).

## Statistical analysis

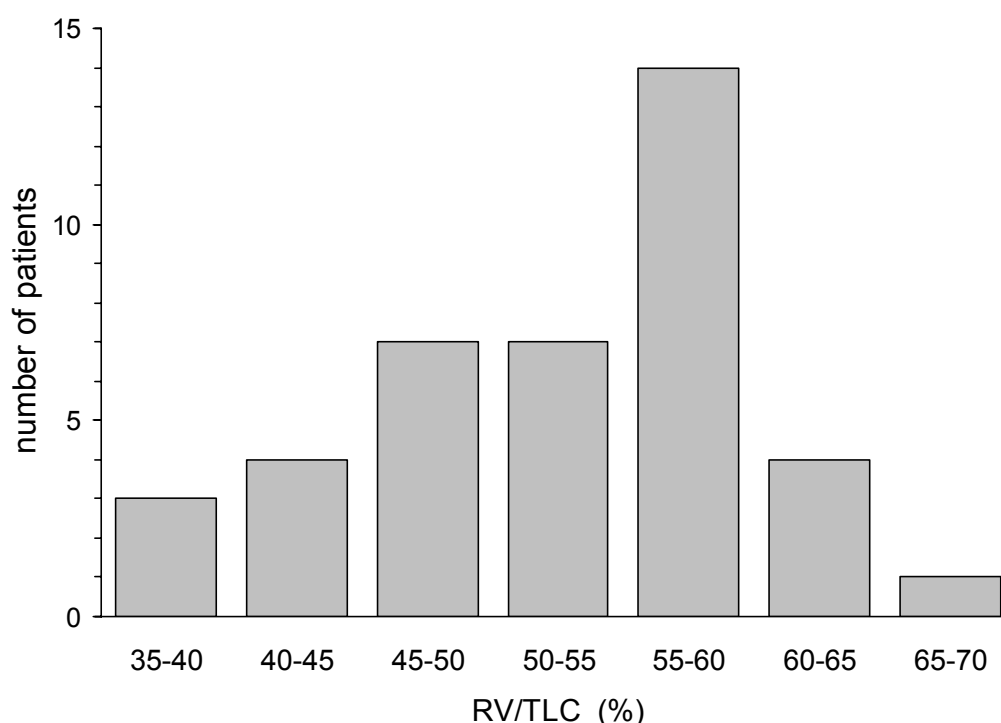
Two-way repeated measures analysis of variance (ANOVA) (Statistica5.5, StatSoft, Tulsa, OK) was used to detect any differences between the 3 study visits prior to and 90 min after tiotropium bromide administration (2 factors: inter-visit and intra-visit tiotropium bromide effect). One-way repeated measures ANOVA was used to detect any inter-visit differences on the symptom scores. Bonferroni adjustment was used to test for post-hoc differences. Spearman rank correlations were performed between changes in symptom or dyspnea scores and changes in lung function indices.

## Results

The 40 patients (36M/4F) participating in this study were stable COPD patients on maintenance treatment mostly

involving inhaled steroids (37/40) in combination with long-acting  $\beta_2$ -agonists (37/40). All patients were also on a combination of short-acting  $\beta_2$ -agonists and ipratropium bromide, but at study entry, ipratropium bromide was discontinued. Eight out of 40 patients were current smokers. Figure 1 shows a histogram of the residual volume over total lung capacity ratio (RV/TLC) measured at the screening visit before bronchodilator administration, in the patient group under study ( $n = 40$ ). The median RV/TLC being 54.8%, the group was split into a subgroup with a high degree of hyperinflation if  $RV/TLC \geq 55\%$  ( $Hyp^+$ ;  $n = 20$ ) and a subgroup with a low degree of hyperinflation if  $RV/TLC < 55\%$  ( $Hyp^-$ ;  $n = 20$ ).

Table 1 shows lung function, ventilation heterogeneity and symptom scores prior to tiotropium bromide administration on the study visits  $visit_0$ ,  $visit_{3wks}$  and  $visit_{6wks}$ . For each parameter in Table 1, change from baseline ( $\Delta$ ) was computed as the difference between the value of  $visit_{6wks}$  and that of baseline  $visit_0$  in order to test for correlations of change. In the  $Hyp^-$  group, none of the correlations between changes in symptom or dyspnea scores and changes in lung function parameters were significant ( $p > 0.1$  for all). In the  $Hyp^+$  group, changes in total symptom or MRC score did not correlate with any lung function or ventilation heterogeneity changes



**Figure 1** Histogram of the distribution of the residual volume (RV) over total lung capacity (TLC) ratio in the COPD group under study ( $n = 40$ ). The 4 left-most bars constitute the  $Hyp^-$  group ( $n = 20$ ) and the 3 right-most bars constitute the  $Hyp^+$  group ( $n = 20$ ).

**Table 1** Characteristics of COPD subgroups with RV/TLC  $\geq 55\%$  (Hyp<sup>+</sup>) OR  $<55\%$  (Hyp<sup>-</sup>) and the effect of tiotropium

	Hyp <sup>+</sup> (n = 20)			Hyp <sup>-</sup> (n = 20)			p-value Hyp <sup>+</sup> vs Hyp <sup>-</sup> at baseline
Age (yrs)	68 $\pm$ 2			65 $\pm$ 2			>0.1
Height (cm)	171 $\pm$ 1			177 $\pm$ 1			0.003
COPD diagnosis (yrs)	7 $\pm$ 1			6 $\pm$ 2			>0.1
Packyears (py)	54 $\pm$ 4			49 $\pm$ 7			>0.1
	Baseline	3 wks tiotropium	6 wks tiotropium	Baseline	3 wks tiotropium	6 wks tiotropium	
FEV <sub>1</sub> (%pred)	50 $\pm$ 3	55 $\pm$ 3†	55 $\pm$ 3†	60 $\pm$ 3	61 $\pm$ 3	62 $\pm$ 3	0.006
FEV <sub>1</sub> (L)	1.38 $\pm$ 0.07	1.52 $\pm$ 0.06†	1.52 $\pm$ 0.07†	1.94 $\pm$ 0.10	1.97 $\pm$ 0.11	1.99 $\pm$ 0.11	<0.001
FEV <sub>1</sub> /FVC (%)	46 $\pm$ 2	47 $\pm$ 2	47 $\pm$ 2	51 $\pm$ 2	50 $\pm$ 2	50 $\pm$ 2	>0.1
FEF <sub>75</sub> (%pred)	13 $\pm$ 1	13 $\pm$ 1	13 $\pm$ 1	18 $\pm$ 1	17 $\pm$ 1	18 $\pm$ 1	0.008
Kco (%pred)	69 $\pm$ 5	69 $\pm$ 5	69 $\pm$ 5	71 $\pm$ 4	69 $\pm$ 4	70 $\pm$ 5	>0.1
sGaw (L/s/cmH <sub>2</sub> O/L)	0.042 $\pm$ 0.005	0.046 $\pm$ 0.003	0.045 $\pm$ 0.004	0.049 $\pm$ 0.004	0.049 $\pm$ 0.003	0.049 $\pm$ 0.003	>0.1
RV <sub>pl</sub> (ml)	3811 $\pm$ 159	3877 $\pm$ 204	3650 $\pm$ 121	3052 $\pm$ 137	3086 $\pm$ 150	2991 $\pm$ 153	0.001
TLC <sub>pl</sub> (ml)	6644 $\pm$ 243	6832 $\pm$ 252	6707 $\pm$ 221	6600 $\pm$ 207	6704 $\pm$ 246	6628 $\pm$ 240	>0.1
IC <sub>pl</sub> (ml)	1889 $\pm$ 95	1956 $\pm$ 84	2026 $\pm$ 119	2392 $\pm$ 148	2367 $\pm$ 141	2428 $\pm$ 140	0.007
IC (ml)	2181 $\pm$ 126	2386 $\pm$ 122*	2434 $\pm$ 130†	2665 $\pm$ 113	2679 $\pm$ 119	2693 $\pm$ 129	0.007
Sacin (L <sup>-1</sup> )	0.425 $\pm$ 0.024	0.399 $\pm$ 0.025	0.411 $\pm$ 0.024	0.339 $\pm$ 0.030	0.365 $\pm$ 0.032	0.340 $\pm$ 0.033	0.03
Scond (L <sup>-1</sup> )	0.086 $\pm$ 0.006	0.083 $\pm$ 0.007	0.090 $\pm$ 0.008	0.086 $\pm$ 0.007	0.081 $\pm$ 0.007	0.095 $\pm$ 0.008	>0.1
FRC <sub>mbw</sub> (ml)	3752 $\pm$ 125	3665 $\pm$ 131	3595 $\pm$ 118	3680 $\pm$ 150	3674 $\pm$ 146	3659 $\pm$ 126	>0.1
Total symptom score (0–12)	3.5 $\pm$ 0.4	2.4 $\pm$ 0.4†	1.5 $\pm$ 0.2†	3.6 $\pm$ 0.4	2.0 $\pm$ 0.3†	1.7 $\pm$ 0.3†	>0.1
MRC dyspnea score (1–5)	2.1 $\pm$ 0.2	2.0 $\pm$ 0.2	1.8 $\pm$ 0.2	1.7 $\pm$ 0.2	1.8 $\pm$ 0.2	1.9 $\pm$ 0.2	>0.1
VAS dyspnea score (0–10)	4.5 $\pm$ 0.5	3.2 $\pm$ 0.4*	2.7 $\pm$ 0.5†	3.0 $\pm$ 0.4	3.0 $\pm$ 0.5	2.7 $\pm$ 0.4	0.03

**Notes:** All averages ( $\pm$  SEM) refer to data prior to tiotropium intake on the study day.

**Abbreviations:** FEV<sub>1</sub>, forced expired volume in one second; FVC, forced expired vital capacity; FEF<sub>75</sub>, forced expiratory flow after expiration of 75% FVC; Kco, carbon monoxide diffusing capacity per lung volume; sGaw, specific airway conductance; RV<sub>pl</sub>, TLC<sub>pl</sub>, IC<sub>pl</sub>, plethysmographic residual volume, total lung capacity, and inspiratory capacity; IC, inspiratory capacity measured in open circuit; Sacin, Scond, index of acinar and conductive ventilation heterogeneity (see text for details); FRC<sub>mbw</sub>, functional residual capacity derived from the multiple breath washout. For symptom and dyspnea scores (see text); Significant changes from baseline (within Hyp<sup>+</sup> and Hyp<sup>-</sup> groups):

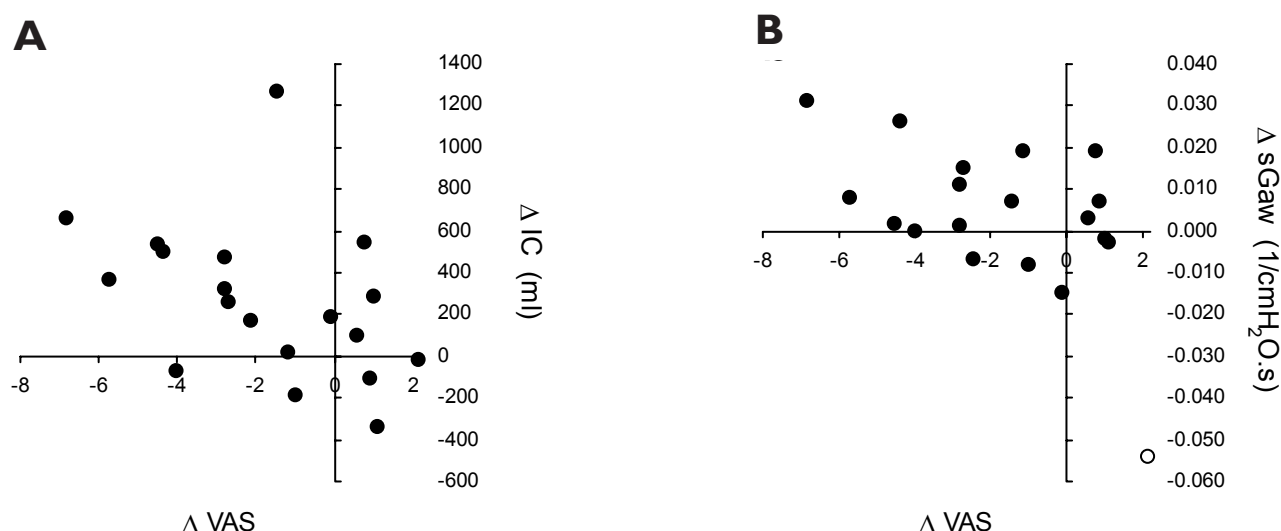
\*p < 0.05; †p < 0.01.

(p > 0.1 for all). However,  $\Delta$ VAS did correlate with  $\Delta$ IC (r = -0.54; p = 0.016) and also with  $\Delta$ sGaw (r = -0.49; p = 0.03) (Figure 2). Figure 2B clearly identifies an outlier at  $\Delta$ sGaw = -0.055 cm<sup>-1</sup>H<sub>2</sub>O • s<sup>-1</sup> (open circle); upon detailed inspection, this was due to the plethysmographic determination of lung volume but there was no objective reason for exclusion.

Panels A in Figures 3–6 illustrate, for the Hyp<sup>+</sup> group, inter- and intra-visit changes in FEV<sub>1</sub>, IC, Scond, or sGaw, ie, those parameters of Table 1 showing any significant inter- or intra-visit change in this subgroup. Panels B in Figures 3–6 display the corresponding data for the Hyp<sup>-</sup> group.

In the Hyp<sup>+</sup> group, FEV<sub>1</sub> (Figure 3A) and IC (Figure 4A) significantly increased 90 min after the first 18  $\mu$ g tiotropium bromide dose on visit<sub>0</sub> (p  $\leq$  0.001 for both), while no such intra-visit increases occurred during visit<sub>3wks</sub> and visit<sub>6wks</sub> (p > 0.1 for both). Intra-visit changes in sGaw (Figure 5A) showed a similarity with FEV<sub>1</sub> and IC

behavior, in that sGaw also increased significantly during the baseline visit<sub>0</sub> (p < 0.001) but not during visit<sub>3wks</sub> or visit<sub>6wks</sub> (p > 0.1). While Figure 5A suggests an increase in trough sGaw value, this was not significant (p > 0.05 for both), yet, when excluding the abovementioned outlier (Figure 2B; open circle), trough sGaw did increase significantly from 0.038 cm<sup>-1</sup>H<sub>2</sub>O • s<sup>-1</sup> (visit<sub>0</sub>) to 0.045 cm<sup>-1</sup>H<sub>2</sub>O • s<sup>-1</sup> (visit<sub>3wks</sub>; p = 0.007) and 0.044 cm<sup>-1</sup>H<sub>2</sub>O • s<sup>-1</sup> (visit<sub>6wks</sub>; p = 0.04). While Figure 6A indicates tendencies for intra-visit Scond decreases (ie, improvements) of the same order as that observed on the screening visit, averaging an 11% decrease over the three study visits, the Scond decrease only reached statistical significance on the last visit (p = 0.02). The complementary indicators of small airway function, end-expiratory flow after 75% of forced expired vital capacity (FEF<sub>75</sub>) and Sacin, did not present any inter- or intra-visit effect in this subgroup (p > 0.1 for all).

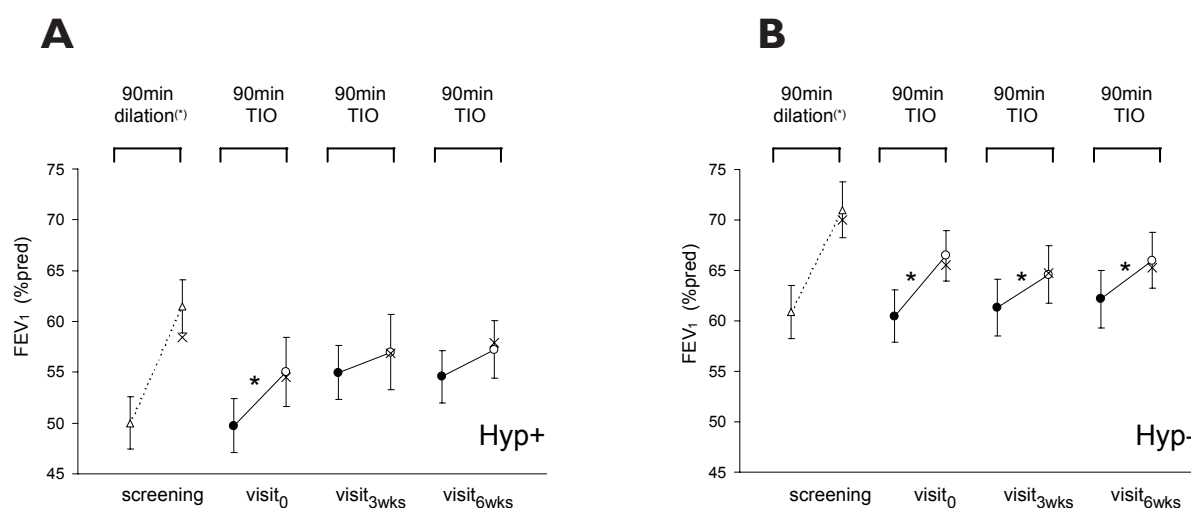


**Figure 2 A:** Scatterplot of the individual change in IC ( $\Delta$ IC) versus the corresponding change in visual analog dyspnea score ( $\Delta$ VAS) in the Hyp+ group where  $\Delta$ IC and  $\Delta$ VAS are computed as the VAS or pre-tiotropium IC value at the 6 weeks tiotropium visit minus the VAS or pre-tiotropium IC value at the baseline study visit. **B:** Scatterplot of the individual change in sGaw ( $\Delta$ sGaw) versus the corresponding change in visual analog score ( $\Delta$ VAS) in the Hyp+ group; same representation as in A.

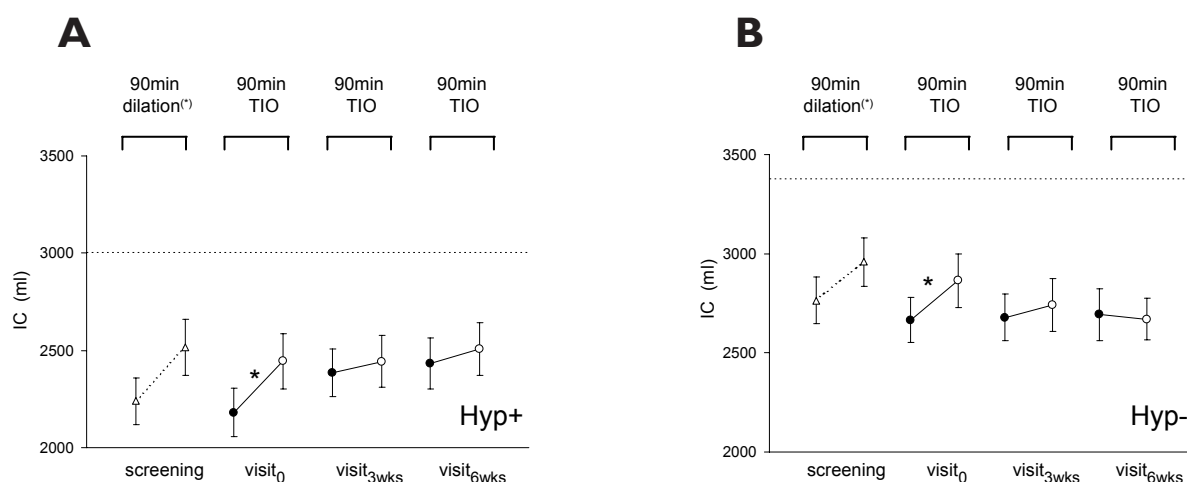
The Hyp<sup>-</sup> group showed no improvements in trough values of any parameter after 3 or 6 weeks of tiotropium bromide treatment. Another striking difference of the Hyp<sup>-</sup> with respect to the Hyp<sup>+</sup> group is that FEV<sub>1</sub> (Figure 3B) and sGaw (Figure 5B) showed highly significant intra-visit increases ( $p < 0.01$  for both) on all study visits. By contrast, IC (Figure 4B) only increased significantly ( $p = 0.03$ ) on the baseline visit ( $p > 0.1$  on visit<sub>3wks</sub> and visit<sub>6wks</sub>). While there were tendencies for intra-visit Scond decreases (ie, improvements) of the same order as that observed at the

screening visit, averaging a 12% decrease over the three study visits, the Scond decrease failed to reach statistical significance (visit<sub>6wks</sub>:  $p = 0.053$ ). As in the Hyp<sup>+</sup> group, FEF<sub>75</sub> and Sacin did not present any inter- or intra-visit effect of tiotropium bromide in the Hyp<sup>-</sup> subgroup ( $p > 0.1$  for all).

Two supplementary analyses were performed to verify whether the observed patterns, that appear to be specifically linked to the presence of hyperinflation, were not a consequence of (a) the expected difference in disease severity (in



**Figure 3 A:** Forced expiratory volume in one second (FEV<sub>1</sub>) as %predicted obtained from the Hyp+ group on the screening visit (dotted line connecting open triangles; pre- and 90 min post-dilation with a combination of salbutamol and ipratropium bromide) and during the three study visits (solid lines connecting open circles (pre-) and closed circles (90 min post-) tiotropium); the crosses refer to intermediate FEV<sub>1</sub> measures after 60 min. On the first study visit, patients were tiotropium-free, while the other two study visits followed 3 and 6 weeks of tiotropium once-daily treatment with the last tiotropium intake on the day prior to the study visit; asterisks indicate any significant change 90 min after tiotropium (2-way ANOVA with Bonferroni;  $p < 0.05$ ). **B:** FEV<sub>1</sub> obtained from the Hyp<sup>-</sup> group.

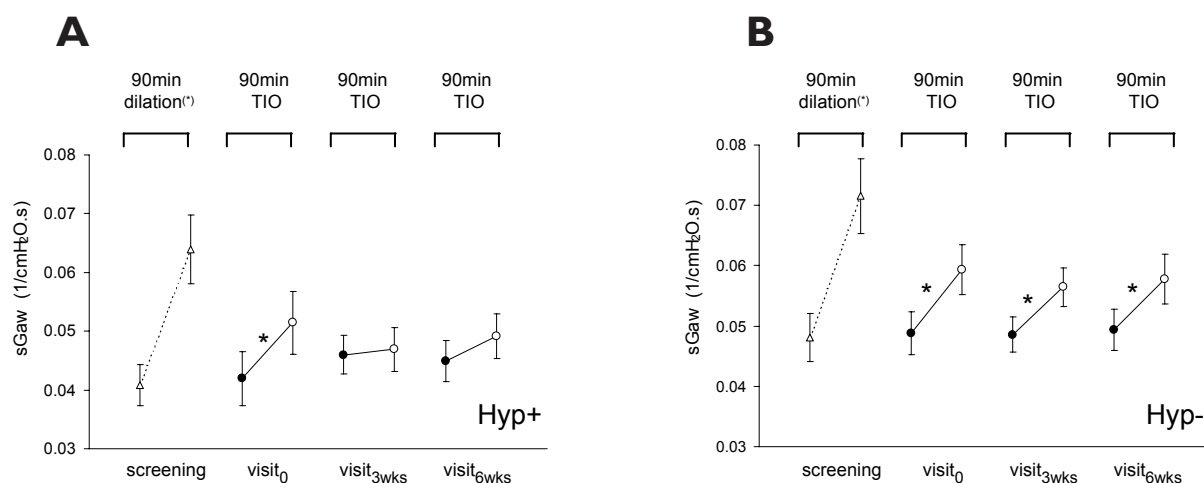


**Figure 4 A:** Inspiratory capacity measured in an open circuit (IC) obtained from the Hyp<sup>+</sup> group on the screening visit (dotted line connecting open triangles; pre- and 90 min post-dilation with a combination of salbutamol and ipratropium bromide) and during the three study visits (solid lines connecting open circles (pre-) and closed circles (90 min post-) tiotropium). On the first study visit, patients were tiotropium-free, while the other two study visits followed 3 and 6 weeks of tiotropium once-daily treatment with the last tiotropium intake on the day prior to the study visit; asterisks indicate any significant change 90 min after tiotropium (2-way ANOVA with Bonferroni;  $p < 0.05$ ); the dotted line represents the predicted IC value for this subgroup. **B:** IC obtained from the Hyp<sup>-</sup> group.

terms of  $FEV_1$  between Hyp<sup>+</sup> and Hyp<sup>-</sup> subgroups, and (b) the arbitrary choice of the median RV/TLC as a cut-off (in casu: RV/TLC = 55%) for the classification into Hyp<sup>+</sup> and Hyp<sup>-</sup> subgroups. Hence we repeated the analysis of Table 1, but by considering  $FEV_1$  matched Hyp<sup>+</sup> and Hyp<sup>-</sup> subgroups ( $n = 15$ ; Table 2), and by considering Hyp<sup>+</sup> and Hyp<sup>-</sup> subgroups ( $n = 10$ ; Table 3) with only those patients corresponding to the 10 lowermost RV/TLC ( $<46\%$ ) and 10 uppermost RV/TLC ( $>59\%$ ) values. Despite some differences, the outcome of both supplementary analyses of Tables 2 and 3 was very similar to that observed for the entire COPD population in Table 1.

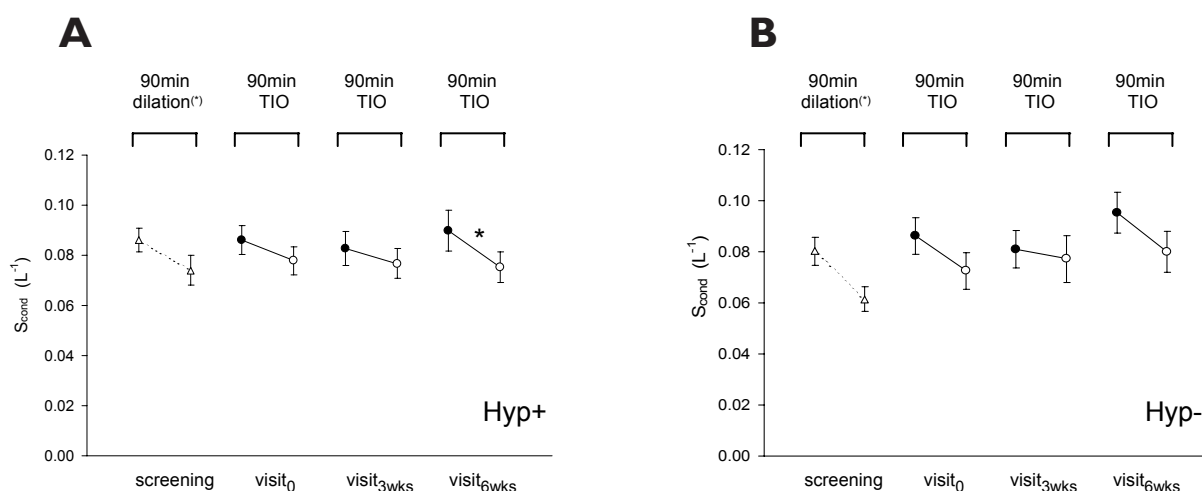
## Discussion

This study shows a very distinct pattern of tiotropium bromide-induced improvement of airflow ( $FEV_1$ ) and IC between COPD patients with a high degree of hyperinflation (Hyp<sup>+</sup>) and COPD patients with low degree of hyperinflation (Hyp<sup>-</sup>), in that only the Hyp<sup>+</sup> group showed significant increases in trough  $FEV_1$  and IC values. No such sustained improvement was observed for ventilation heterogeneity in either group. In fact, reduction in ventilation heterogeneity was confined to the conductive airway compartment, was of limited magnitude, and followed a very similar pattern across both



**Figure 5 A:** Specific conductance (sGaw) obtained from the Hyp<sup>+</sup> group on the screening visit (dotted line connecting open triangles; pre- and 90 min post-dilation with a combination of salbutamol and ipratropium bromide) and during the three study visits (solid lines connecting open circles (pre-) and closed circles (90 min post-) tiotropium). On the first study visit, patients were tiotropium-free, while the other two study visits followed 3 and 6 weeks of tiotropium once-daily treatment with the last tiotropium intake on the day prior to the study visit; asterisks indicate any significant change 90 min after tiotropium (2-way ANOVA with Bonferroni;  $p < 0.05$ ). **B:** sGaw obtained from the Hyp<sup>-</sup> group.





**Figure 6 A:** Conductive ventilation heterogeneity ( $S_{\text{cond}}$ ) obtained from the Hyp<sup>+</sup> group on the screening visit (dotted line connecting open triangles; pre- and 90 min post-dilation with a combination of salbutamol and ipratropium bromide) and during the three study visits (solid lines connecting open circles (pre-) and closed circles (90 min post-) tiotropium). On the first study visit, patients were tiotropium-free, while the other two study visits followed 3 and 6 weeks of tiotropium once-daily treatment with the last tiotropium intake on the day prior to the study visit; asterisks indicate any significant change 90 min after tiotropium (2-way ANOVA with Bonferroni;  $p < 0.05$ ). **B:**  $S_{\text{cond}}$  obtained from the Hyp<sup>-</sup> group.

COPD groups. The discrepancy between the behavior of IC and ventilation heterogeneity in the hyperinflated COPD subgroup, makes it unlikely that small airways are implicated in the sustained improvements of IC observed by us and others. The modest intra-visit  $S_{\text{cond}}$  decreases (reaching  $p = 0.05$  significance on only one occasion) imply that tiotropium bromide action is either affecting heterogeneity of the larger conductive airways (thus not affecting *small airways* heterogeneity) or acting in a very homogeneous way at the level of the small conductive airways (thus not affecting small airways *heterogeneity*). However, the absence of any  $\text{FEF}_{75}$  change with tiotropium bromide makes the latter possibility highly unlikely. Rather, the difference in patterns of sGaw improvements between Hyp<sup>-</sup> and Hyp<sup>+</sup> groups, similar to that observed for IC and  $\text{FEV}_1$ , suggests that larger airways or large lung units subtended by these large airways play a major role in the short- and long-term effect of tiotropium bromide.

The sustained improvement of  $\text{FEV}_1$  and IC after 3 and 6 weeks of tiotropium bromide treatment only occurred in COPD patients with considerable hyperinflation (Hyp<sup>+</sup>). Nevertheless, while the Hyp<sup>-</sup> patients showed no long-term  $\text{FEV}_1$  or IC effects, their short-term  $\text{FEV}_1$  increases (90 min after tiotropium bromide) on each study visit were of the same magnitude as those obtained at baseline visit in Hyp<sup>+</sup> patients. No such similarity between short-term improvements across both COPD subgroups was observed for IC. This indicates that the mechanism and site of

bronchodilation of tiotropium bromide (reflected in  $\text{FEV}_1$ ) are essentially the same in both COPD groups, but that when considerable hyperinflation is present, a continued bronchodilating action has the additional benefit of relieving hyperinflation (reflected in an IC improvement). The similar degree of bronchodilation in both groups suggests that any potential effect of the baseline state of hyperinflation on pulmonary distribution of the tiotropium bromide aerosol was negligible. In addition, the observed large airway effect suggests that either tiotropium bromide deposition was effectively confined to the large conductive airways, or that this was the only effective site of tiotropium bromide action on  $M_3$  receptors.

The trough IC and  $\text{FEV}_1$  values in the Hyp<sup>+</sup> group after 3 and 6 weeks of tiotropium bromide treatment were indistinguishable from the respective peak values seen at the baseline visit 90 min after tiotropium bromide. This contrasts with previous studies (van Noord et al 2000; Vincken et al 2002; Brusasco et al 2003; Celli et al 2003; O'Donnell et al 2004; Maltais et al 2005; Niewoehner et al 2005; Verkindre et al 2006) where trough  $\text{FEV}_1$  and/or IC generally increased to a value intermediate between baseline and peak value, such that study visits after several weeks of tiotropium bromide treatment still showed room for  $\text{FEV}_1$  or IC improvement. Three factors should be considered when comparing our study with previous ones. Firstly, over 90% of the COPD patients under study here were on a combination of a long-acting  $\beta_2$  agonist and inhaled steroid treatment, with tiotropium

**Table 2** Characteristics of FEV<sub>1</sub> matched subgroups with RV/TLC  $\geq$  55% (Hyp<sup>+</sup>) OR < 55% (Hyp<sup>-</sup>) and the effect of tiotropium

	Hyp <sup>+</sup> (n = 20)			Hyp <sup>-</sup> (n = 20)			p-value Hyp <sup>+</sup> vs Hyp <sup>-</sup> at baseline
Age (yrs)	70 $\pm$ 2			65 $\pm$ 2			>0.1
Height (cm)	170 $\pm$ 1			175 $\pm$ 1			0.009
COPD diagnosis (yrs)	7 $\pm$ 2			6 $\pm$ 2			>0.1
Packyears (py)	57 $\pm$ 4			51 $\pm$ 9			>0.1
	Baseline	3 wks tiotropium	6 wks tiotropium	Baseline	3 wks tiotropium	6 wks tiotropium	
FEV <sub>1</sub> (%pred)	55 $\pm$ 2	59 $\pm$ 3 *	59 $\pm$ 2 †	58 $\pm$ 3	58 $\pm$ 3	60 $\pm$ 3	>0.1
FEV <sub>1</sub> (L)	1.46 $\pm$ 0.07	1.56 $\pm$ 0.08 *	1.58 $\pm$ 0.08 †	1.80 $\pm$ 0.11	1.82 $\pm$ 0.11	1.87 $\pm$ 0.12	0.02
FEV <sub>1</sub> /FVC (%)	50 $\pm$ 2	51 $\pm$ 2	50 $\pm$ 2	49 $\pm$ 3	48 $\pm$ 3	49 $\pm$ 3	>0.1
FEF <sub>75</sub> (%pred)	14 $\pm$ 1	14 $\pm$ 2	14 $\pm$ 1	18 $\pm$ 2	16 $\pm$ 2	18 $\pm$ 1	0.09
Kco (%pred)	71 $\pm$ 5	71 $\pm$ 6	71 $\pm$ 6	68 $\pm$ 5	67 $\pm$ 4	67 $\pm$ 5	>0.1
sGaw (L/s/cmH <sub>2</sub> O/L)	0.044 $\pm$ 0.006	0.048 $\pm$ 0.004	0.047 $\pm$ 0.004	0.050 $\pm$ 0.005	0.049 $\pm$ 0.004	0.050 $\pm$ 0.004	>0.1
RV <sub>pl</sub> (ml)	3598 $\pm$ 140	3802 $\pm$ 262	3594 $\pm$ 151	3106 $\pm$ 169	3131 $\pm$ 196	3050 $\pm$ 193	0.03
TLC <sub>pl</sub> (ml)	6272 $\pm$ 219	6573 $\pm$ 302	6433 $\pm$ 243	6535 $\pm$ 260	6603 $\pm$ 319	6518 $\pm$ 303	>0.1
IC <sub>pl</sub> (ml)	1808 $\pm$ 107	1874 $\pm$ 77	1896 $\pm$ 110	2201 $\pm$ 158	2203 $\pm$ 158	2249 $\pm$ 152	0.05
IC (ml)	2088 $\pm$ 103	2232 $\pm$ 96	2315 $\pm$ 122 †	2502 $\pm$ 108	2489 $\pm$ 116	2526 $\pm$ 137	0.01
Sacin (L <sup>-1</sup> )	0.400 $\pm$ 0.027	0.382 $\pm$ 0.028	0.402 $\pm$ 0.029	0.321 $\pm$ 0.029	0.346 $\pm$ 0.031	0.326 $\pm$ 0.037	0.05
Scond (L <sup>-1</sup> )	0.087 $\pm$ 0.006	0.082 $\pm$ 0.008	0.088 $\pm$ 0.010	0.089 $\pm$ 0.008	0.084 $\pm$ 0.008	0.101 $\pm$ 0.009	>0.1
FRC <sub>mbw</sub> (ml)	3685 $\pm$ 151	3595 $\pm$ 161	3547 $\pm$ 154	3721 $\pm$ 170	3737 $\pm$ 171	3703 $\pm$ 136	>0.1
Total symptom score (0–12)	3.2 $\pm$ 0.4	2.1 $\pm$ 0.4 *	1.4 $\pm$ 0.2 †	3.3 $\pm$ 0.3	1.7 $\pm$ 0.2 †	1.3 $\pm$ 0.2 †	>0.1
MRC dyspnea score (1–5)	1.9 $\pm$ 0.2	1.8 $\pm$ 0.2	1.7 $\pm$ 0.2	1.6 $\pm$ 0.2	1.7 $\pm$ 0.2	1.9 $\pm$ 0.3	>0.1
VAS dyspnea score (0–10)	4.6 $\pm$ 0.6	3.0 $\pm$ 0.5 *	2.6 $\pm$ 0.6 †	3.0 $\pm$ 0.6	2.9 $\pm$ 0.6	2.6 $\pm$ 0.5	>0.1

**Notes:** All averages ( $\pm$ SEM) refer to data prior to tiotropium intake on the study day; same abbreviations as in Table 1. Significant changes from baseline (within Hyp<sup>+</sup> and Hyp<sup>-</sup> groups): \*p < 0.05; † p < 0.01.

bromide as an add-on study medication, as opposed to most previous studies where long-acting  $\beta_2$  agonists were not permitted. This may account for some quantitative differences of tiotropium bromide effects with respect to previous studies (here, typical changes in FEV<sub>1</sub> trough value are 10% baseline in Hyp<sup>+</sup> patients). Secondly, in those previous studies where hyperinflation was not part of the inclusion criteria, the COPD groups under study may have been a mixture of patients with a range of hyperinflation. One would then expect a pattern which is intermediate to that seen in our Hyp<sup>+</sup> and Hyp<sup>-</sup> groups, corresponding to a partial increase in trough FEV<sub>1</sub> and IC values between baseline and treatment visits, and an additional intra-visit increase from trough to peak FEV<sub>1</sub> or IC value. Thirdly, the method of IC measurement varied across previous tiotropium bromide studies, using the difference between spirometric volume levels corresponding to FRC and TLC (corresponding to IC here) or that derived from plethysmographic FRC and TLC measurements (corresponding to IC<sub>pl</sub> here). The pitfalls

inherent to plethysmographic volume determination in COPD have been documented before (Rodenstein and Stanescu 1982) and the difference between IC and IC<sub>pl</sub> behavior across Tables 1 to 3 emphasizes the interpretation problems with plethysmographically determined lung volumes.

In conclusion, we have observed a pattern of improved airflow (FEV<sub>1</sub>) and inspiratory lung capacity (IC) with tiotropium bromide in COPD patients, which was linked to their degree of hyperinflation at baseline. In patients with a low degree of hyperinflation, FEV<sub>1</sub> and IC showed consistent increases, but no sustained changes in trough FEV<sub>1</sub> or IC. By contrast, in COPD patients with considerable hyperinflation at baseline, 3 weeks of tiotropium bromide treatment elicited improvements in trough IC and FEV<sub>1</sub>, close to the maximum obtainable value. The concomitant sGaw changes suggest that the main therapeutic effect is elicited in the large conductive airways. Ventilation heterogeneity of the acinar airways was unaffected by tiotropium bromide, and the modest improvements in



**Table 3** Characteristics of COPD subgroups with RV/TLC > 59% (Hyp<sup>+</sup>) or <46% (Hyp<sup>-</sup>) and the effect of tiotropium

	Hyp <sup>+</sup> (n = 20)			Hyp <sup>-</sup> (n = 20)			p-value Hyp <sup>+</sup> vs Hyp <sup>-</sup> at baseline
	Baseline	3 wks tiotropium	6 wks tiotropium	Baseline	3 wks tiotropium	6 wks tiotropium	
Age (yrs)	71 ± 2			63 ± 3			0.04
Height (cm)	171 ± 2			178 ± 2			0.07
COPD diagnosis (yrs)	7 ± 2			5 ± 1			>0.1
Packyears (py)	60 ± 6			50 ± 12			>0.1
FEV <sub>1</sub> (%pred)	49 ± 4	55 ± 4	53 ± 3	65 ± 3	61 ± 4*	64 ± 3	0.002
FEV <sub>1</sub> (L)	1.33 ± 0.07	1.48 ± 0.06	1.44 ± 0.06	2.14 ± 0.15	2.01 ± 0.17*	2.10 ± 0.16	<0.001
FEV <sub>1</sub> /FVC (%)	44 ± 2	45 ± 3	45 ± 3	54 ± 3	51 ± 3	52 ± 3	0.02
FEF <sub>75</sub> (%pred)	13 ± 2	12 ± 2	14 ± 1	20 ± 2	17 ± 3	19 ± 2	0.03
Kco (%pred)	69 ± 8	69 ± 8	68 ± 8	71 ± 6	71 ± 6	71 ± 7	>0.1
sGaw (L/s/cmH <sub>2</sub> O/L)	0.038 ± 0.004	0.044 ± 0.005	0.042 ± 0.005	0.055 ± 0.006	0.050 ± 0.004	0.054 ± 0.005	0.02
RV <sub>pl</sub> (ml)	4028 ± 249	4158 ± 301	3871 ± 164	2577 ± 139	2726 ± 221	2491 ± 142	<0.001
TLC <sub>pl</sub> (ml)	6781 ± 362	7010 ± 294	6879 ± 304	6236 ± 333	6399 ± 434	6236 ± 370	>0.1
IC <sub>pl</sub> (ml)	1817 ± 130	1937 ± 90	2066 ± 188	2572 ± 262	2406 ± 274	2537 ± 265	0.02
IC (ml)	2119 ± 206	2336 ± 171	2395 ± 189 †	2761 ± 215	2655 ± 227	2719 ± 245	0.045
Sacin (L <sup>-1</sup> )	0.432 ± 0.028	0.389 ± 0.025	0.391 ± 0.030	0.283 ± 0.032	0.318 ± 0.043	0.299 ± 0.047	0.003
Scond (L <sup>-1</sup> )	0.090 ± 0.008	0.093 ± 0.011	0.105 ± 0.013	0.086 ± 0.011	0.092 ± 0.013	0.102 ± 0.015	>0.1
FRC <sub>mbw</sub> (ml)	3793 ± 172	3778 ± 207	3636 ± 163	3472 ± 248	3405 ± 208	3498 ± 190	>0.1
Total symptom score (0–12)	3.8 ± 0.6	2.8 ± 0.6*	1.8 ± 0.3†	4.0 ± 0.6	2.3 ± 0.6†	1.9 ± 0.5†	>0.1
MRC dyspnea score (1–5)	2.2 ± 0.2	2.4 ± 0.3	2.0 ± 0.2	2.1 ± 0.2	2.3 ± 0.3	2.2 ± 0.2	>0.1
VAS dyspnea score (0–10)	4.4 ± 0.8	3.6 ± 0.6	3.1 ± 0.9	3.9 ± 0.6	4.0 ± 0.6	3.6 ± 0.6	>0.1

**Notes:** All averages (±SEM) refer to data prior to tiotropium intake on the study day; same abbreviations as in Table 1. Significant changes from baseline (within Hyp<sup>+</sup> and Hyp<sup>-</sup> groups): \*p < 0.05; †p < 0.01.

ventilation heterogeneity of the conductive airways were unrelated to changes in hyperinflation.

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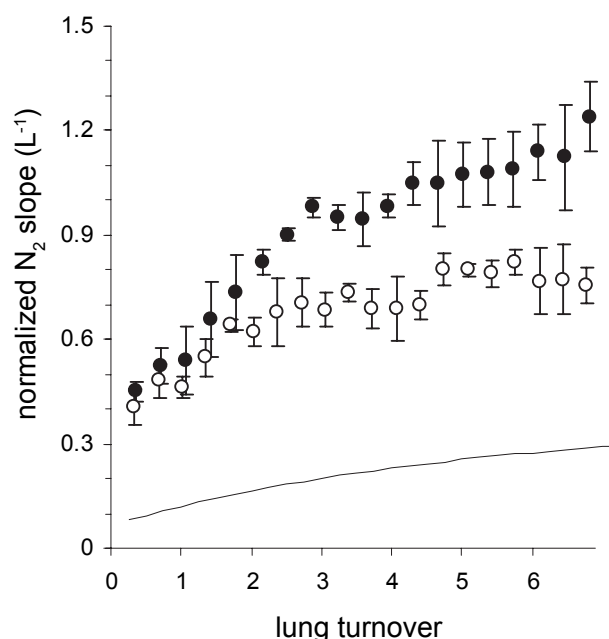
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## Appendix

### Ventilation distribution testing

The ventilation distribution test, ie, the multiple breath washout (MBW), was carried out using a computer-controlled bag-in-box breathing assembly. Tidal volume was targeted at 1 L and after a period of air breathing with stable end-expiratory lung volume at functional residual capacity, inspired air was switched to the test gas mixture. The test gas mixture consisted of pure  $O_2$ , and 1 L tidal breathing continued for 20–25 breaths depending on the subject's lung volume (dilution); exhaled  $N_2$  concentration tracings were analyzed. From the MBW  $N_2$  tracings, indices Scond and Sacin were derived to represent the conductive and acinar components of ventilation heterogeneity, respectively, using an analysis that can be summarized as follows. During each expiration, the  $N_2$  phase III slope is computed and normalized by the mean expired  $N_2$  concentration. This leads to a normalized  $N_2$  slope (Sn) which increases as a function of breath number or lung turnover (Figure A1); lung turnover is determined as the cumulative expired volume divided by ventilated functional residual capacity, which is computed from a mass balance of the MBW test. On theoretical grounds it can be shown that: (a) the rate of rise of the Sn curve is due to the convective flow asynchrony between lung units larger than acini, thus, due to heterogeneity originating in the conductive airways; (b) the offset of the Sn curve is mainly determined by diffusion-convection dependent heterogeneity generated in the acinar airways. Hence, Scond is simply computed as the rate of Sn increase as a function of lung turnover, between 1.5 and 6 lung turnovers. Then, Sacin is computed as the Sn value of the first MBW expiration minus a correction term to discard any conductive lung zone contribution; this correction term equals the lung turnover corresponding to the first breath, multiplied by Scond. Illustrated in Figure A1 are two selected COPD patients who show a similar Sacin ( $0.39\text{ L}^{-1}$  and  $0.41\text{ L}^{-1}$ ) and markedly different Scond (open and closed circles correspond to respectively Scond =  $0.049\text{ L}^{-1}$  and closed circles to Scond =  $0.098\text{ L}^{-1}$ ). A normalized slope curve corresponding to a typical normal subject (dotted line) corresponds to Sacin and Scond values of  $0.072\text{ L}^{-1}$  and  $0.029\text{ L}^{-1}$ , respectively.

The theory of MBW phase III slope analysis leading to indices Scond and Sacin implies that ventilation heterogeneity



**Figure A1** Normalized  $N_2$  phase III slopes (mean  $\pm$  SD) as a function of lung turnover obtained in two patients participating in this study (open and closed circles); also represented is an idealized curve corresponding to normal normalized  $N_2$  phase III slopes (dotted line).

can be attributed to different lung depths, ie, to the conductive and acinar lung zone, respectively, and that Scond and Sacin are intrinsically independent and are not comparable between each other (despite having the same dimension,  $L^{-1}$ ). Finally, since Scond and Sacin are derived from phase III slopes, their value increases when ventilation heterogeneity increases. In particular, Sacin will increase if ventilation heterogeneity is increased in the acinar lung zone, due to an alteration of the intra-acinar asymmetry (eg, unequal narrowing of affected respiratory bronchioles). On the other hand, Scond will increase when heterogeneous narrowing of conductive airways induces an alteration in the specific ventilation and/or flow asynchrony between the lung units subtended by these airways proximal to the terminal bronchioles.

An increase in Scond reflects uneven narrowing of either the large or the small conductive airways, or both. A disproportionate Scond increase with respect to the decrease of large airway parameters such as  $FEV_1$  or specific airway conductance can be used to identify the small airways component of conductive lung zone heterogeneity. An increase in Sacin represents a change in intra-acinar structural asymmetry, due to uneven narrowing of respiratory bronchioles or due to partial destruction of parenchyma, or both. Again, a disproportionate Sacin increase with respect to a decrease of diffusion capacity enables the identification of the nonemphysematous component of acinar lung zone heterogeneity.