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RESEARCH ARTICLE

Acute effects of long-acting bronchodilators on small airways detected in COPD patients by single-breath N_2 test and lung P-V curve

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Pecchiari M, Santus P, Radovanovic D, D'Angelo E. Acute effects of long-acting bronchodilators on small airways detected in COPD patients by single-breath N_2 test and lung P-V curve. *J Appl Physiol* 123: 1266–1275, 2017. First published August 3, 2017; doi:10.1152/japplphysiol.00493.2017.—Small airways represent the key factor of chronic obstructive pulmonary disease (COPD) pathophysiology. The effect of different classes of bronchodilators on small airways is still poorly understood and difficult to assess. Hence the acute effects of tiotropium (18 μg) and indacaterol (150 μg) on closing volume (CV) and ventilation inhomogeneity were investigated and compared in 51 stable patients (aged 70 ± 7 yr, mean ± SD; 82% men) with moderate to very severe COPD. Patients underwent body plethysmography, arterial blood gas analysis, tidal expiratory flow limitation (EFL), dyspnea assessment, and simultaneous recording of single-breath N_2 test and transpulmonary pressure-volume curve (P_L-V), before and 1 h after drug administration. The effects produced by indacaterol on each variable did not differ from those caused by tiotropium, independent of the severity of disease, assessed according to the Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) scale and the presence of EFL. Bronchodilators significantly decreased the slope of phase III and CV (−5 ± 4 and −2.5 ± 2.1%, respectively, both $P < 0.001$), with an increase in both slope and height of phase IV and of the anatomical dead space. Arterial oxygen pressure and saturation significantly improved (3 ± 3 mmHg and 2 ± 2%, respectively, both $P < 0.001$); their changes negatively correlated with those of phase III slope ($r = -0.659$ and $r = -0.454$, respectively, both $P < 0.01$). The vital capacity (VC) increased substantially, but the P_L-V/VC curve above CV was unaffected. In conclusion, bronchodilators reduce the heterogeneity of peripheral airway mechanical properties and the extent of their closure, with minor effects on critical closing pressure. This should lessen the risk of small-airway damage and positively affect gas exchange.

NEW & NOTEWORTHY This is the first study investigating in stable chronic obstructive pulmonary disease patients the acute effects of two long-acting bronchodilators, a β-agonist and a muscarinic antagonist, on peripheral airways using simultaneous lung pressure-volume curve and single-breath N_2 test. By lessening airway mechanical property heterogeneity, both drugs similarly reduced ventilation inhomogeneity and extent of small-airway closure, as indicated by the decrease of phase III slope, increased oxygen saturation, and fall of closing volume, often below expiratory reserve volume.

closing volume; bronchodilators; lung pressure-volume curve; single-breath nitrogen test; chronic obstructive pulmonary disease

THE PHYSIOLOGY OF SMALL AIRWAYS has been the focus of intense research in the sixties and seventies (28). Lately, a growing awareness (4, 27) about the role of small airways in the pathophysiology and progression of chronic obstructive pulmonary disease (COPD) caused a renewed interest in the assessment of small-airway dysfunction (24). Inhaled bronchodilators represent the treatment mainstay in COPD, and their functional target has been always represented by the small airways. However, the evaluation of bronchodilator efficacy has been often limited to the response in terms of dynamic volumes, which are known to poorly reflect small-airway modifications (5, 37). On the other hand, peripheral lung injury and maldistribution of ventilation can be readily assessed by the single-breath nitrogen (SBN) test, reflected in a steeper slope of phase III and an increased closing volume (29).

The effects of bronchodilators on the SBN test have been extensively investigated in normal subjects and asthmatic patients. No effects were observed in normal subjects (6, 10, 11, 22, 23, 33), whereas in asthmatic patients, β-adrenergic agents have been shown not only to lower the slope of phase III (2, 35), reduce the closing volume (12, 26), and increase the vital capacity (2, 26, 36, 39) but also to leave at least one of these variables unchanged (2, 12, 33, 36, 38, 39). No explanation exists for these contrasting results.

Only two studies have evaluated the response to bronchodilators in terms of SBN test in COPD patients. They were conducted on very small samples, using only β-adrenergic drugs, and with contrasting results. No effects were observed by Timmins et al. (40), whereas Olofsson et al. (30) found significant dose-related increases in vital capacity (VC), a decrease in phase III slope, and no change in closing volume (CV). Moreover, the role of bronchodilators has never been investigated in COPD patients who, despite an enhanced small-airway collapse and gas trapping, do not exhibit an appreciable phase IV of the SBN test (16, 17, 19, 20, 32) and in whom the presence of the CV is, however, clearly identifiable from the analysis of the slow deflation transpulmonary pressure-volume (P_L-V) curve (32).

The primary aim of this study was to investigate and compare the effects of indacaterol, a β-adrenergic agonist, and of tiotropium, a long-acting muscarinic antagonist, on the SBN test in a large group of COPD patients. The simultaneous assessment of the CV from the P_L-V curve allowed us to extend the examination to patients in whom the SBN test lacks phase IV.

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METHODS

Subjects. Patients with an established diagnosis of COPD were consecutively enrolled while referring to an academic outpatient clinic. Inclusion criteria were as follows: >60 yr of age; forced expired volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 and FEV_1 <80% of predicted value; current or former smokers (>20 pack·yr); stable clinical conditions. Exclusion criteria were as follows: known unstable or moderate-severe heart disease; history of asthma or active pulmonary disease other than COPD; neuromuscular or disabling cognitive problems; body mass index (BMI) ≥30 kg/m^2 and restrictive-obstructive pattern; drug abuse. Staging of the disease was according to Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD) 1–4 (21). Using the ABCD assessment (21), 12 patients (24%) belonged to *group A*, 10 (20%) to *group B*, 10 (20%) to *group C*, and 18 (36%) to *group D*. The study was approved by the local ethics committee (Fondazione Salvatore Maugeri 629 CEC). All subjects gave written informed consent. The trial has been registered at https://clinicaltrials.gov/ (NCT01437748).

Measurements. Lung volumes and total specific airway resistance (sRAW) were assessed by means of a constant-volume body plethysmograph (Master Screen Body; Erich Jaeger, Würzburg, Germany). Intrathoracic gas volume (ITGV) was measured close to the end-expiratory lung volume during quiet breathing. Sampling the radial artery allowed the measurement of carbon dioxide (Pa_{CO_2}) and oxygen partial pressure (Pa_{O_2}), oxygen saturation (So_2), and pH (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA). Chronic dyspnea was rated after the modified Medical Research Council (mMRC) scale (18), and dyspnea sensation was rated using the visual analog scale (VAS) according to American Thoracic Society recommendations (1).

N_2 washout and P_L-V curves were simultaneously recorded during slow expiratory vital capacities. The SBN test was performed with the VMAX Encore (Viasys Health Care, Yorba Linda, CA). A detailed description of the maneuver can be found elsewhere (32). A balloon-tipped catheter (inner diameter 1 mm, length 70 cm) and pressure transducer system (Celesco LCVR-0100; Raytech Instruments, Vancouver, BC, Canada) allowed esophageal pressure (P_{es}) measurements. The balloon (Microtek Medical, Zutphen, The Netherlands; circumference 2.1 cm; length 10 cm; filled with 0.8–1 ml of air) was advanced through the nose and placed in the lower third of the esophagus. Correct positioning was checked as suggested by Baydur et al. (3). Pressure at the mouth (P_{ao}) was measured with a Celesco LCVR-0100 transducer, P_L being computed as $P_{ao} - P_{es}$.

SBN test and P_L-V curve were separately analyzed using a custom-made program (LabView Software; National Instruments, Austin, TX). Tests were repeated three times before and after bronchodilator administration, respectively, and data were collected in a blind fashion on two occasions several weeks apart. For each condition, the analysis of the P_L-V curve was performed on the proof with the highest end-inspiratory P_L and an expired volume within 50 ml of the largest VC measured in that subject. For any given variable and condition, the difference between the two readings never exceeded 2.5% of their mean, which was therefore used to quantify the variable. The validation of the procedures used to analyze the P_L-V curve and a graphic description of how the program works are given elsewhere (32).

Assessment of tidal expiratory flow limitation was done in 33 patients using the negative expiratory pressure method (18). Pressure and flow signals were processed and analyzed as previously described (31).

Experimental protocol. After 48 h of pharmacological washout, during which only salbutamol metered dose inhaler was allowed, though discouraged, patients were randomized according to a computer-generated list to take either tiotropium inhalation powder 18 μg via HandiHaler (Boehringer Ingelheim, Ingelheim am Rhein, Germany) or indacaterol inhalation powder 150 μg via Breezhaler (Novartis International, Basel, Switzerland). The investigators were blind to the type of drug administered, and patients were instructed and given the experimental drug by an uninformed nurse, who also checked the inhalation procedure. During the pharmacological washout, the 17 patients that had inhaled corticosteroids as part of their therapy were left with the equivalent daily dose of fluticasone. Patients were instructed to discontinue salbutamol for at least 12 h before the day of testing.

Routine lung function tests and assessment of dyspnea sensation were performed first, followed by SBN tests and P_L-V curves. The same sequence of tests was repeated 1 h after inhalation of either tiotropium or indacaterol.

Statistics. Data, presented as means ± SD, were analyzed using SPSS 19.0 (SPSS, Chicago, IL). Normal distribution of data was assessed by means of the Kolmogorov-Smirnov test. Predicted normal values of spirographic, plethysmographic, and P_L indexes are from Quanjer (34), those for SBN tests are from Buist and Ross (7, 8), and those for Pa_{O_2} are from Cerveri et al. (9). Comparisons were performed using either the Student's *t*-test or a two-way mixed between-within-groups ANOVA; when significant differences were found, values of each variable were compared using paired or unpaired Student's *t*-test with Bonferroni correction. Relationships between variables were assessed by means of linear regression analysis. Statistical significance was taken at $P \leq 0.05$.

RESULTS

Indacaterol vs. tiotropium. Table 1 shows the anthropometric data and variables of patients receiving either indacaterol or tiotropium. At baseline, no variable differed between the two groups. Spirographic and plethysmographic variables improved after the administration of bronchodilators except for total lung capacity (TLC), expiratory reserve volume (ERV)/VC, and FEV_1/FVC, which did not change. These effects were paralleled by a significant improvement in dyspnea sensation. Standard parameters of the SBN test were also significantly affected by bronchodilators. Indacaterol and tiotropium produced similar effects, except for inspiratory capacity (IC) and VC changes, which were larger with indacaterol. Data from the two groups were therefore pooled.

Bronchodilators did not affect the breathing pattern; tidal volume, inspiratory duration, and expiratory duration were 0.69 ± 0.21 liters, 1.18 ± 0.25 s, and 1.94 ± 0.37 s at baseline and 0.69 ± 0.21 liters, 1.17 ± 0.21 s, and 1.92 ± 0.42 s after bronchodilator administration, respectively.

According to the GOLD categories based on clinical observations (21), 12 patients (24%) belonged to *group A*, 10 (20%) to *group B*, 10 (20%) to *group C*, and 18 (36%) to *group D*. No significant difference occurred in the categorical distribution between patients receiving indacaterol or tiotropium.

SBN test. Variables of the SBN test are summarized in Table 2. At baseline, a clearly discernable phase IV, i.e., CV_{SBN}, was present in 22 patients (43%). The slope of phase III and the closing capacity-to-TLC ratio (CC_{SBN}/TLC) were markedly greater than predicted, the latter reflecting the substantial increase in the residual volume (RV)-to-TLC ratio. In contrast, the anatomical dead space (aDS) and the CV_{SBN}-to-VC ratio were within normal limits. Values of VC were similar to those assessed by spirometry (Table 1). Pa_{O_2} and So_2 were significantly lower than predicted (Table 2).

Bronchodilators caused a significant decrease of phase III slope and a significant increase of both slope and height of phase IV and aDS. Elevation of phase III and N_2 concentration at RV were unchanged. The within-subject SD of phase III

Table 1. *Anthropometric and lung function data and variables of the SBN test*

	Indacaterol			Tiotropium			
	Pre	Δ	*P*	Pre	Δ	*P*	*P**
M/F	22/3			19/6			
Age, yr	69 ± 7			71 ± 8			
Height, m	1.70 ± 0.10			1.69 ± 0.10			
BMI, kg/h^2	25 ± 4			25 ± 4			
mMRC	1.8 ± 1.1			1.8 ± 1.0			
VAS, %	16 ± 13	−11 ± 9	<0.001	14 ± 13	−8 ± 8	<0.001	0.274
IC, liters	2.23 ± 0.81	0.26 ± 0.32	<0.001	2.18 ± 0.60	0.07 ± 0.17	0.005	0.011
IC, %predicted	83 ± 23			85 ± 15			
ERV, liters	0.81 ± 0.32	0.10 ± 0.23	0.036	0.58 ± 0.36	0.10 ± 0.18	0.013	0.918
ERV, %predicted	63 ± 20			55 ± 23			
VC, liters	3.02 ± 1.02	0.34 ± 0.29	<0.001	2.79 ± 0.75	0.19 ± 0.18	0.067	0.024
VC, %predicted	76 ± 19			77 ± 10			
ERV/VC, %	26 ± 10	−0 ± 8	0.900	21 ± 11	2 ± 6	0.155	0.363
ERV/VC, %predicted	83 ± 23			73 ± 29			
FEV_1, liters	1.12 ± 0.56	0.09 ± 0.11	<0.001	1.13 ± 0.47	0.06 ± 0.11	0.011	0.296
FEV_1, %predicted	41 ± 18			42 ± 15			
FVC, liters	2.47 ± 0.85	0.13 ± 0.31	0.046	2.36 ± 0.69	0.17 ± 0.25	0.002	0.589
FVC, %predicted	70 ± 18			68 ± 13			
FEV_1/FVC, %	45 ± 13	2 ± 5	0.053	48 ± 15	−1 ± 8	0.495	0.100
FEV_1/FVC, %predicted	51 ± 15			55 ± 17			
TLC, liters	6.81 ± 1.14	0.14 ± 0.51	0.162	6.43 ± 1.24	−0.04 ± 0.58	0.145	0.253
TLC, %predicted	106 ± 15			106 ± 18			
ITGV, liters	4.75 ± 1.07	−0.23 ± 0.46	0.019	4.40 ± 0.96	−0.13 ± 0.44	0.016	0.438
ITGV, %predicted	138 ± 32			132 ± 29			
RV, liters	3.92 ± 1.07	−0.33 ± 0.42	0.001	3.72 ± 0.85	−0.27 ± 0.52	<0.001	0.641
RV, %predicted	179 ± 52			163 ± 44			
sRAW, kPa·s	4.32 ± 2.56	−1.15 ± 1.16	<0.001	4.48 ± 2.48	−1.04 ± 1.29	0.042	0.745
sRAW, %predicted	373 ± 222			413 ± 253			
ΔIII, N_2%/l	9.8 ± 4.9	−2.1 ± 2.0	<0.001	12.1 ± 5.1	−2.9 ± 4.2	<0.001	0.215
ΔIII, %predicted	686 ± 321			796 ± 309			
CV, liters†	0.85 ± 0.33	−0.05 ± 0.18	0.361	0.88 ± 0.30	−0.14 ± 0.14	0.023	0.224
VC, liters	3.08 ± 0.79	0.31 ± 0.26	<0.001	2.74 ± 0.83	0.18 ± 0.16	<0.001	0.030
CV, %VC†	24 ± 6	−3 ± 5	0.042	29 ± 9	−5 ± 4	0.011	0.253
CV, %predicted	105 ± 26			118 ± 48			
aDS, liters	0.18 ± 0.05	0.02 ± 0.04	0.004	0.18 ± 0.05	0.04 ± 0.04	<0.001	0.210

Values are means ± SD. SBN test, single-breath nitrogen test; Δ, change; M, male; F, female; BMI, body mass index; mMRC, modified Medical Research Council dyspnea scale; VAS, visual analog scale; IC, inspiratory capacity; ERV, expiratory reserve volume; VC, slow expiratory vital capacity; FEV_1, forced expiratory vital capacity in 1 s; FVC, forced vital capacity; TLC, total lung capacity; ITGV, intrathoracic gas volume; RV, residual volume; sRAW, total specific airway resistance; ΔIII, slope of phase III; CV, closing volume; aDS, anatomic dead space. *Between-group changes. †Values refer to 22 patients in whom phase IV was present both before and after bronchodilator administration.

slope was 0.36 N_2%/l, the confidence limits amounting to ±0.71 N_2%/l. In 80% of patients, the fall of phase III slope was >0.71 N_2%/l, averaging −3.17 ± 1.86 N_2%/l. CV_{SBN} was also significantly reduced. Owing to the concomitant increase of VC, fall of RV, and constancy of TLC, there was a highly significant decrease of both CV_{SBN}/VC and CC_{SBN}/TLC. Among the 29 patients with no phase IV at baseline, this phase became clearly discernable after bronchodilator administration in 9 patients.

Both Pa_{O_2} and So_2 improved significantly with bronchodilator administration, whereas pH and Pa_{CO_2} were unaffected. Changes in Pa_{O_2} and So_2 were significantly correlated with those of the slope of phase III (Fig. 1). No correlation was found between changes in CV_{SBN} and Pa_{O_2} ($r = 0.029$; $P = 0.917$) or So_2 ($r = 0.021$; $P = 0.940$).

P_L-V curve. Variables of the slow deflationary P_L-V curve are reported in Table 3. P_L at TLC ($P_{L,TLC}$) was within normal limits. However, $P_{L,TLC}$ and *K*, an index of overall specific lung compliance, were significantly lower (2.09 ± 0.48 vs. 2.62 ± 0.57 kPa; $P = 0.0002$) and larger (2.95 ± 1.08 vs. 1.72 ± 0.43 kPa^{-1}; $P < 0.0001$), respectively, than the corresponding values of healthy subjects of similar age (32), indicating loss of lung recoil in COPD patients. P_L at RV ($P_{L,RV}$) was markedly negative, implying substantial lung compression; it was significantly more negative than that of healthy elderly subjects (−2.87 ± 1.31 vs. −1.96 ± 1.0 kPa; $P = 0.007$) (32), indicating increased air trapping in these patients, consistent with the augmentation of RV.

An inflection was invariably and clearly present in the slow deflation P_L-V curve both before and after bronchodilator administration (Fig. 2) allowing for the assessment of CV (CV_{exp}) in all patients (Table 3). P_L at which CV took place ($P_{L,CV}$) did not differ from that observed in a large group of young and elderly healthy subjects and COPD patients (32). CV_{exp} and CV_{SBN} values were well correlated, this being the case also for the nine patients in whom phase IV was present only after bronchodilator administration (Fig. 3). CV_{SBN} and CV_{exp} at baseline and their changes with bronchodilator administration were essentially coincident (Tables 2 and 3).

Dependency on airway resistance, GOLD stage, and tidal expiratory flow limitation. At baseline, the slope of phase III was significantly correlated both with FEV_1 and, more closely,

Table 2. *Variables of the SBN test and arterial blood gases and pH*

	Pre	Post	Δ	*P*
Phase III				
Slope, N_2%/l	10.9 ± 5.1	8.4 ± 4.3	−2.5 ± 2.1	<0.001
Slope, %predicted	741 ± 317			
Elevation, N_2%	19 ± 6	19 ± 6	0 ± 3	0.924
Phase IV*				
Slope, N_2%/l	12.1 ± 4.8	14.4 ± 5.4	2.3 ± 1.5	<0.001
Height, N_2%	7.8 ± 2.8	9.3 ± 3.7	1.5 ± 1.8	0.001
CV_{SBN}, liters	0.86 ± 0.31	0.78 ± 0.24	−0.08 ± 0.17	0.038
CV_{SBN}/VC, %	26 ± 8	23 ± 6	−4 ± 5	0.001
CV_{SBN}/VC, %predicted	107 ± 36			
CC_{SBN}/TLC, %	64 ± 6	60 ± 7	−4 ± 7	0.022
CC_{SBN}/TLC, %predicted	131 ± 14			
VC, liters	2.91 ± 0.82	3.16 ± 0.86	0.25 ± 0.22	<0.001
$[N_2]_{RV}$, N_2%	48 ± 7	48 ± 7	−1 ± 5	0.466
aDS, liters	0.18 ± 0.05	0.21 ± 0.05	0.03 ± 0.04	<0.001
$\dot{V}$, l/s	0.27 ± 0.13	0.27 ± 0.14	0.00 ± 0.15	0.951
Arterial blood				
Pa_{CO_2}, mmHg	43 ± 7	42 ± 6	−0.4 ± 2.1	0.160
Pa_{O_2}, mmHg	70 ± 7	73 ± 7	3 ± 3	<0.001
Pa_{O_2}, %predicted	87 ± 9			
So_2, %	93 ± 2	95 ± 2	2 ± 2	<0.001
pH	7.42 ± 0.03	7.42 ± 0.03	0.00 ± 0.02	0.945

Values are means ± SD. CV_{SBN}, closing volume assessed from the onset of phase IV; VC, slow expiratory vital capacity; CC_{SBN}, closing capacity assessed from the onset of phase IV; TLC, total lung capacity; $[N_2]_{RV}$, expired nitrogen concentration at residual volume; aDS, anatomic dead space; $\dot{V}$, mean expiratory flow; Pa_{CO_2} and Pa_{O_2}, carbon dioxide and oxygen partial pressure, respectively; So_2, oxygen saturation. *Values refer to 22 patients in whom phase IV was present both before and after bronchodilator administration.

with sRAW (Fig. 4). However, its changes with bronchodilator administration were not related to those of FEV_1, whereas the correlation with sRAW changes was very close to significance. No correlation occurred between CV or aDS and FEV_1 or sRAW at baseline, nor among their changes with bronchodilator administration.

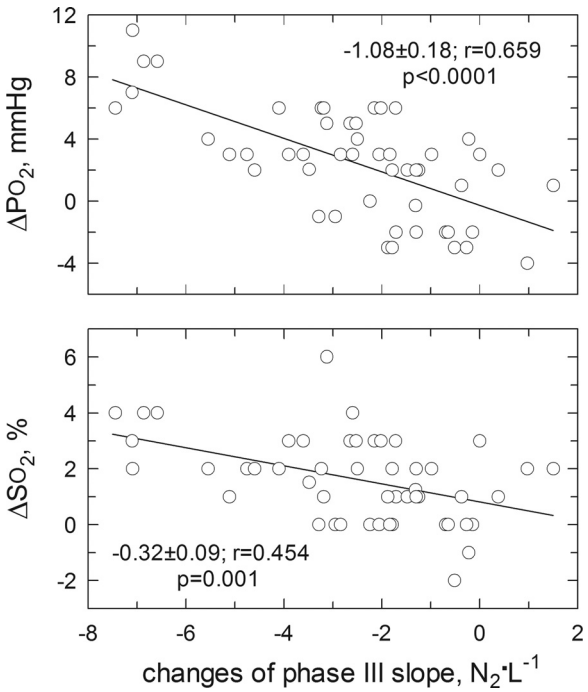

Fig. 1. Relationships between changes of phase III slope and those of arterial oxygen partial pressure (ΔPo_2) or saturation (ΔSo_2) with bronchodilator administration. Numbers are slopes ± SE.

Except for elevation of phase III slope, CV_{exp}/VC, and TLC, all lung function parameters were impaired as the severity of disease identified by GOLD stages progressed, but only FEV_1, RV %predicted, and expired nitrogen concentration at residual volume ($[N_2]_{RV}$) differed significantly between groups (Table 4). With bronchodilator administration, the slope of phase III, CV_{exp}, and CV_{exp}/VC decreased, while aDS and VC increased in all groups by nearly the same amount. A significant improvement in RV and sRAW was present in all groups and was paralleled by a reduction in dyspnea sensation, whereas FEV_1 increased significantly in GOLD 3 and GOLD 4 patients only.

At baseline, the slope of phase III and $[N_2]_{RV}$ were significantly larger in tidal expiratory flow limitation (EFL) patients,

Table 3. *Variables of the slow deflationary P_L-V curve*

	Pre	Post	Δ	*P*
$P_{L,TLC}$, kPa	2.09 ± 0.48	2.13 ± 0.48	0.04 ± 0.18	0.114
$P_{L,TLC}$, %predicted	95 ± 24			
$P_{L,RV}$, kPa	−2.87 ± 1.31	−2.78 ± 1.36	0.09 ± 0.51	0.224
	Exponential fit			
P_o, kPa	0.02 ± 0.09	0.02 ± 0.10	0.01 ± 0.05	0.065
K, kPa^{-1}	2.95 ± 1.08	3.02 ± 1.17	0.07 ± 0.25	0.082
CV_{exp}, liters	0.78 ± 0.31	0.69 ± 0.27	−0.10 ± 0.16	<0.001
CV_{exp}/VC, %	27 ± 7	22 ± 7	−5 ± 4	<0.001
CV_{exp}/VC, %predicted	107 ± 31			
CC_{exp}/TLC, %	68 ± 7	63 ± 8	−5 ± 4	<0.001
CC_{exp}/TLC, %predicted	137 ± 17			
$P_{L,CV}$, kPa	0.15 ± 0.10	0.14 ± 0.10	−0.01 ± 0.05	0.063

Values are means ± SD. $P_{L,TLC}$ and $P_{L,RV}$, transpulmonary pressure at total lung capacity and at residual volume, respectively; P_o and *K*, coefficients of the exponential function $V/VC = 1 - e^{-K\cdot(P_L - P_o)}$ used to fit the transpulmonary pressure-volume (P_L-V) curve; CV_{exp}, closing volume estimated from the inflection point or departure from the exponential fit; VC, slow expiratory vital capacity; CC_{exp}, closing capacity, assessed using CV_{exp}; $P_{L,CV}$, transpulmonary pressure at the inflection point.

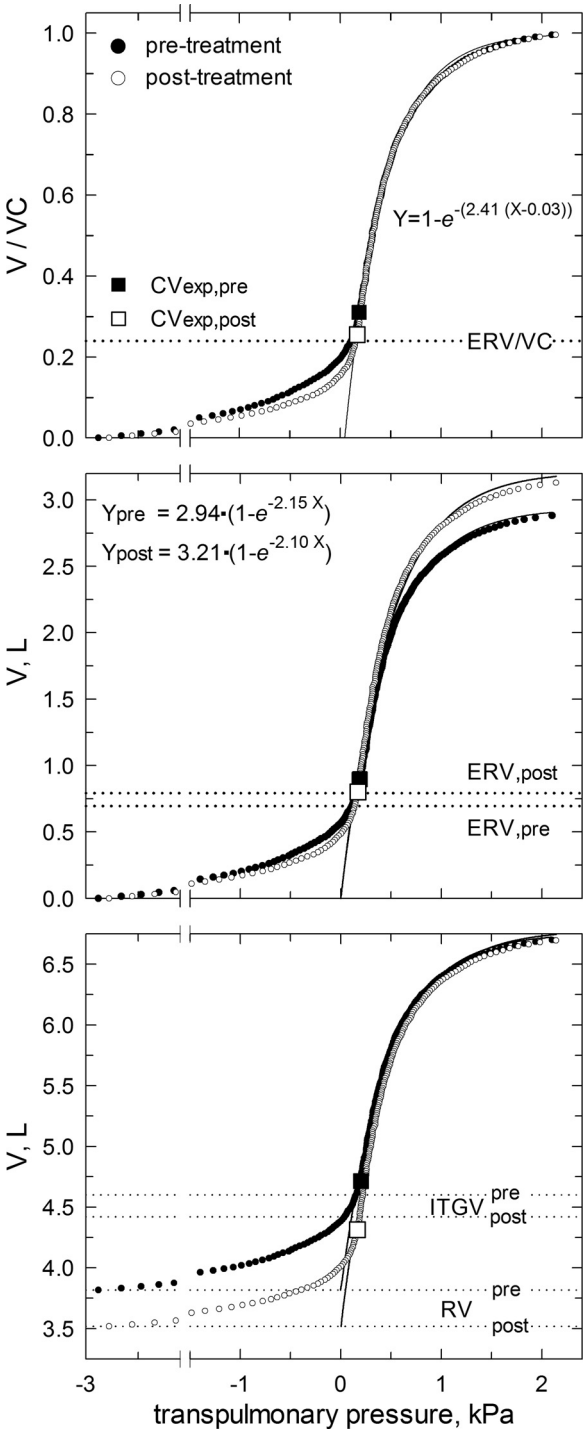

Fig. 2. Average slow deflation P_L-V curve before (pre) and after bronchodilator administration (post) presented in relative and absolute terms with the position of the inflection point. The equations are best exponential fit (solid curves) of the curve above the inflection point. The same equation fitted the pretreatment and posttreatment P_L-V curve when volume was expressed as percent vital capacity. The dotted lines indicate the specified pulmonary volumes before and after bronchodilator administration.

whereas elevation of phase III, CV_{exp}/VC, VC, and aDS were similar in both groups. TLC, ITGV, RV, and sRAW %predicted were significantly larger whereas FEV_1 was significantly lower in EFL than in non-EFL patients. Bronchodilator administration decreased the slope of phase III, CV_{exp}, CV_{exp}/VC, RV, and sRAW and increased VC, aDS, and FEV_1; these changes were significant and similar in both groups. In contrast, the elevation of phase III, $[N_2]_{RV}$, TLC, and ITGV were unaffected. Although baseline dyspnea was greater in EFL patients, it improved similarly in both groups.

Closing volume vs. end-expiratory volume. In the 22 patients with phase IV, the difference between ERV and CV_{SBN} before and after bronchodilator administration averaged 0.06 ± 0.42 and 0.23 ± 0.39 liters ($P = 0.013$), respectively, whereas the difference between ERV and CV_{exp} was −0.10 ± 0.36 and 0.09 ± 0.39 liters, respectively ($P = 0.059$ and $P = 0.147$). Nevertheless, bronchodilator administration increased the number of patients who were breathing above their CV. The within-subject SD of CV_{SBN} was 0.046 liters, the confidence limits amounting to ±0.091 liters. Taking this value as the criterion for the relevance of CV changes, the percentage of patients with positive ERV-CV_{exp} increased from 24 to 40% with bronchodilators, whereas that of patients with a negative ERV-CV_{exp} decreased from 46 to 36%.

The proportion of patients with positive or negative ERV-CV depended on the severity of the disease (Fig. 5). At baseline, the percentage of negative ERV-CV_{exp} values was 21, 54, and 58% in GOLD 2, GOLD 3, and GOLD 4 patients, respectively, whereas the corresponding percentages after bronchodilator administration decreased to 14, 46, and 42%. Conversely, the corresponding percentages of positive ERV-CV_{exp} values, which were 29, 29, and 8% at baseline, became 57, 33, and 33% after bronchodilator administration.

DISCUSSION

This is the first study that has assessed and compared the acute effects of a long-acting β-agonist and a muscarinic antagonist in terms of lung volume heterogeneity, gas exchange, and closing volume, using both the SBN test and the lung P-V curve in stable COPD patients. It has shown that bronchodilators significantly affect all parameters of the SBN test, causing *1*) a decrease of the slope of phase III paralleled by an increase of arterial oxygen partial pressure and satura-

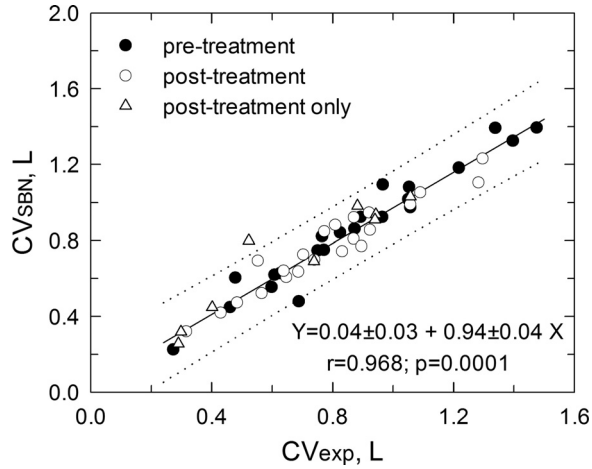

Fig. 3. Relationship between closing volumes obtained from the P_L-V curve according to the exponential approach (CV_{exp}) and the corresponding values assessed from the onset of phase IV (CV_{SBN}). The dotted lines indicate 99% confidence intervals. For elevation and slope, ±SE is reported.

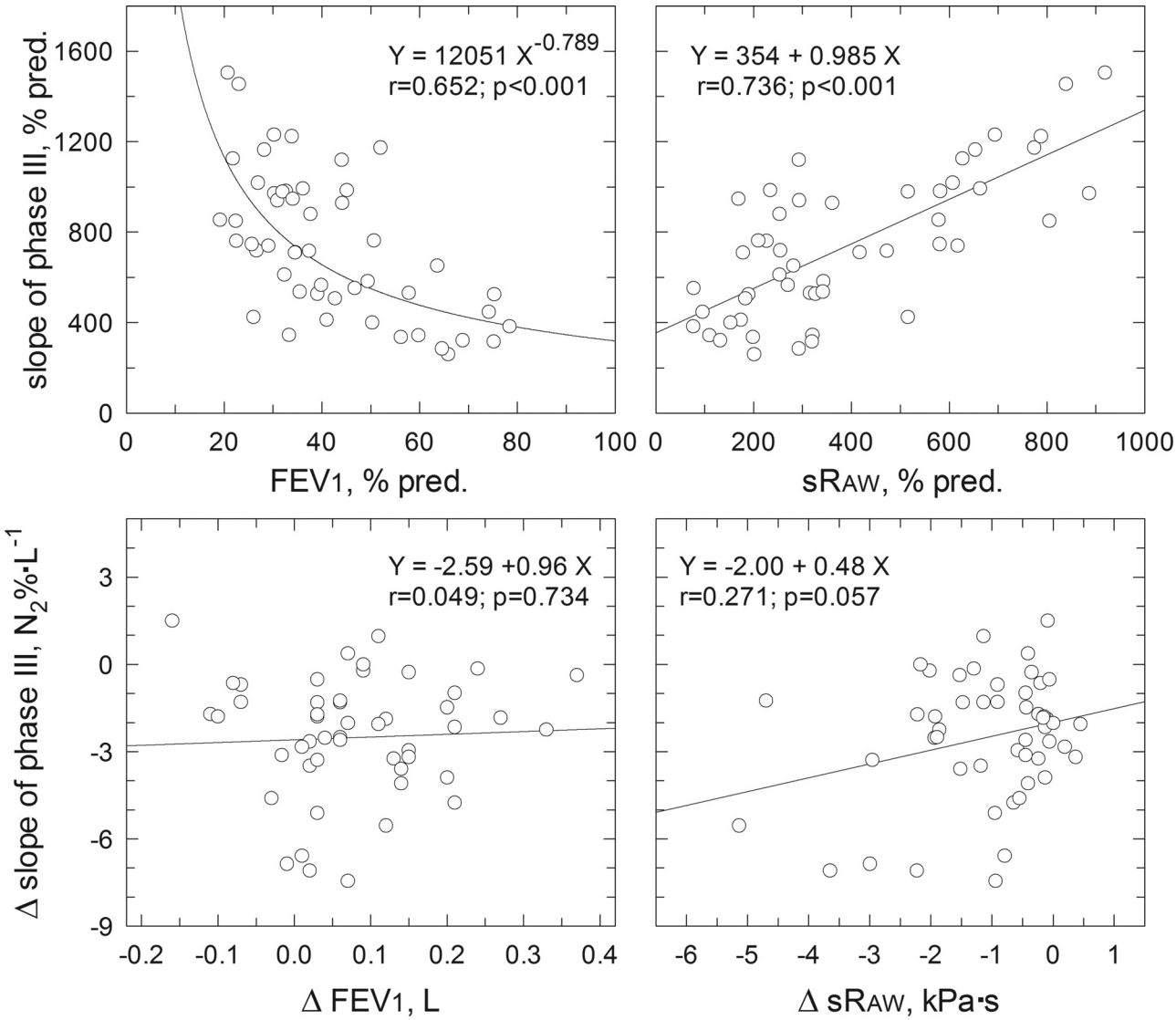

Fig. 4. *Top*: relationships between forced expired volume in 1 s (FEV_1) or specific total airway resistance (sRAW) and corresponding values of phase III slope obtained before bronchodilator administration; pred., predicted. *Bottom*: relationships between the changes of FEV_1 or sRAW and those of phase III slope with bronchodilator administration.

tion; *2*) a decrease of the closing volume, whether assessed from the SBN test or the P_L-V curve; *3*) an increase of vital capacity and expiratory reserve volume with no change in the P_L-V/VC curve; and *4*) an increase of the anatomical dead space. Furthermore, the effects of tiotropium and indacaterol were essentially similar, independent of the severity of the disease and the presence of tidal EFL. The small increase of aDS, likely due to the dilation of central airways, was similar to that occurring in normal subjects (22), whereas the decrease of phase III slope and the increase of VC are in line with Olofsson et al.'s (30) observations.

The slope of phase III depends on the presence of a nonuniform distribution of regional RV/TLC, and the progressively increasing contribution to expiration of regions with higher regional RV/TLC when exhaling to RV after a full inspiration. In normal subjects with grossly uniform lung mechanical properties, this is mainly ensured by the vertical gradient of P_L. In COPD patients, the slope of phase III becomes increasingly dependent on the enhanced heterogeneity of lung elastic and resistive properties, leading to a wider range of regional RV/TLC and peripheral airway resistance. Indeed, the difference between elevation of phase III and $[N_2]_{RV}$ (Table 2), an index of the amplitude of the RV/TLC range, was significantly larger than that observed in elderly healthy subjects (32). Moreover, this difference increased progressively from GOLD 2 to GOLD 4, as well as with tidal EFL, paralleling the behavior of phase III slope (Tables 4 and 5). This and the satisfactory relation with the degree of obstruction (Fig. 4) support phase III slope as an index of disease severity.

Bronchodilator administration decreased the slope of phase III, independent of the severity of the disease and the presence of tidal EFL; its fall could be rated as effective in 80% of the patients. The slope of phase III is currently taken as an overall index of ventilation heterogeneity: consistently, its fall was paralleled by a significant, though small, increase of both Pa_{O_2}

Table 4. *Effects of bronchodilator administration on SBN test, P_L-V curve, and lung function in patients stratified according to GOLD scale*

	GOLD 2		GOLD 3		GOLD 4		
	Pre	Δ	Pre	Δ	Pre	Δ	*P**
M/F	10/4		21/3		10/2		
VAS	7 ± 10	−5 ± 6	18 ± 12	−12 ± 9	20 ± 13	−9 ± 7	0.056
			Phase III				
Slope, N_2%/l	8.0 ± 4.9	−2.3 ± 1.8	11.3 ± 4.6	−2.6 ± 2.3	13.7 ± 4.7	−2.6 ± 2.3	0.912
Slope, %predicted	530 ± 271		761 ± 273		947 ± 318		
Elevation, N_2%	21 ± 5	1 ± 2	17 ± 6	−0 ± 3	20 ± 7	0 ± 5	0.443
$[N_2]_{RV}$, N_2%	43 ± 4	1 ± 3	49 ± 6	−1 ± 4	53 ± 6	0 ± 6	0.599
aDS	0.19 ± 0.04	0.03 ± 0.03	0.18 ± 0.05	0.03 ± 0.04	0.17 ± 0.04	0.02 ± 0.03	0.525
VC, liters	3.11 ± 0.91	0.26 ± 0.25	3.01 ± 0.84	0.22 ± 0.23	2.47 ± 0.63	0.28 ± 0.20	0.680
VC, %predicted	82 ± 8		79 ± 13		66 ± 12		
CV_{exp}, liters	0.79 ± 0.33	−0.15 ± 0.12	0.79 ± 0.31	−0.08 ± 0.19	0.74 ± 0.30	−0.05 ± 0.12	0.197
CV_{exp}/VC, %	26 ± 7	−6 ± 3	26 ± 7	−4 ± 5	27 ± 9	−4 ± 3	0.912
CV_{exp}/VC, %predicted	106 ± 32		102 ± 29		111 ± 37		
TLC, liters	6.24 ± 1.27	−0.09 ± 0.40	6.78 ± 1.20	0.12 ± 0.51	7.02 ± 1.07	−0.09 ± 0.75	0.513
TLC, %predicted	102 ± 14		105 ± 16		113 ± 20		
ITGV, liters	3.81 ± 0.81	−0.27 ± 0.52	4.60 ± 0.81	−0.15 ± 0.45	5.41 ± 1.00	−0.15 ± 0.39	0.700
ITGV, %predicted	115 ± 21		134 ± 23		161 ± 33		
RV, liters	3.03 ± 0.56	−0.23 ± 0.45	3.82 ± 0.72	−0.28 ± 0.44	4.73 ± 1.18	−0.43 ± 0.57	0.543
RV, %predicted	135 ± 27		170 ± 34		216 ± 63		
FEV_1, liters	1.72 ± 0.48	0.04 ± 0.13	1.01 ± 0.27	0.10 ± 0.10	0.66 ± 0.17	0.08 ± 0.09	0.257
FEV_1, %predicted	64 ± 10		37 ± 6		24 ± 3		
sRAW, kPa·s	3.00 ± 2.30	−0.76 ± 1.25	4.01 ± 1.88	−0.91 ± 1.02	6.81 ± 2.22	−1.86 ± 1.30	0.039
sRAW, %predicted	281 ± 246		354 ± 181		602 ± 207		

Values are means ± SD. GOLD, Global Initiative for Chronic Obstructive Pulmonary Disease; M, male; F, female; VAS, visual analog scale; $[N_2]_{RV}$, nitrogen concentration at residual volume; aDS, anatomic dead space; VC, slow expiratory vital capacity; CV_{exp}, closing volume assessed from the P_L-V curve; TLC, total lung capacity; ITGV, intrathoracic gas volume; RV, residual volume; FEV_1, forced expiratory vital capacity in 1 s; sRAW, total specific airway resistance. *Among-group changes.

and S_{O2} (Table 2 and Fig. 1), likely attributable to a better distribution of regional ventilation-to-perfusion ratios. Bronchodilators did not change the lung elastic properties and the range of regional RV/TLC, because both the P_L-V/VC curve above CV and the difference between $[N_2]_{RV}$ and the elevation of phase III were unaffected. This suggests that the main cause of the fall of the phase III slope should be represented by a reduced heterogeneity of regional airway resistances. A connection between the changes of phase III slope and distribution of peripheral airway resistances can be supported by the quasi-significant relationship between the changes of phase III slope and those of sRAW produced by bronchodilators, based on the assumption that larger reductions of regional resistance heterogeneity are more likely to occur in patients who exhibit greater falls of airway resistance. No such relation occurred between the changes of phase III slope and those of FEV_1; this should be not surprising because FEV_1 changes are poor evaluators of those of small-airway resistance (5, 37).

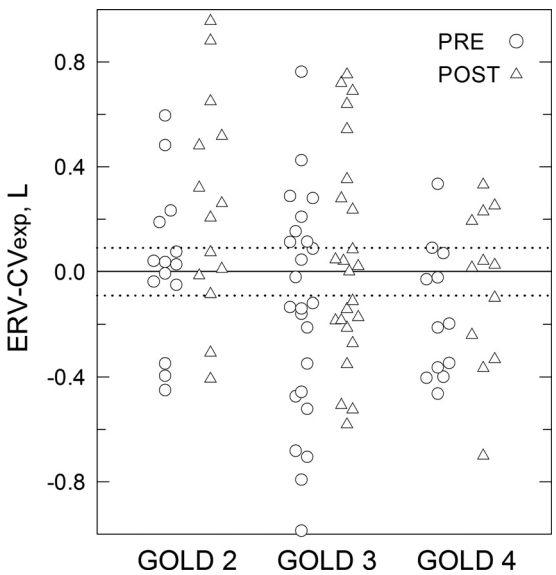

Fig. 5. Difference between expiratory reserve (ERV) and closing volume assessed from the deflation P_L-V curve according to the exponential approach (CV_{exp}) in COPD patients stratified according to GOLD stage. The dotted lines represent the suggested limit for the significance of these differences.

Phase III to IV transition is currently thought to indicate the volume at which an appreciable amount of peripheral airways closes. In our sample, a clearly discernable phase IV was present both before and after bronchodilator administration in only 43% of patients, this percentage being greater in GOLD 2 than in GOLD 4 stage (57 vs. 17%) and in non-EFL than in EFL patients (63 vs. 24%). The possibility of assessing the closing volume using the SBN test is therefore limited, in line with previous conclusions (20, 32). In contrast, an inflection was invariably and clearly present in the slow deflation P_L-V curve (Fig. 2), whereby the closing volume could be assessed in all patients from this curve using the monoexponential criterion (32). The validity of this approach is supported by the good coincidence between CV_{exp} and CV_{SBN} (Fig. 3).

Bronchodilator administration decreased the closing volume by a modest amount; in fact, in only 40% of patients this reduction was larger than the confidence limits of baseline CV_{SBN} values. It is therefore not surprising that previous studies performed on small numbers of COPD patients could not demonstrate appreciable changes of the closing volume

Table 5. *Effects of bronchodilator administration on SBN test, P_L-V curve, and lung function in patients stratified according to the presence of tidal expiratory flow limitation*

	EFL			No EFL				
	Pre	Δ	*P*	Pre	Δ	*P*	*P**	*P*†
M/F	−13/4			−14/2				
VAS	19 ± 13	−12 ± 9	<0.001	10 ± 12	−5 ± 5	0.002	0.033	0.012
				Phase III				
Slope, N_2%/l	13.3 ± 5.6	−2.1 ± 2.7	0.005	8.5 ± 4.1	−2.6 ± 1.8	<0.001		0.580
Slope, %predicted	901 ± 350			582 ± 249			0.005	
Elevation, N_2%	20 ± 7	−1 ± 5	0.454	18 ± 6	1 ± 2	0.116	0.381	0.164
$[N_2]_{RV}$, %	52 ± 7	−1 ± 6	0.591	44 ± 4	0 ± 4	0.839	0.006	0.777
aDS, liters	0.17 ± 0.04	0.02 ± 0.04	0.034	0.19 ± 0.05	0.02 ± 0.03	0.005	0.839	0.699
VC, liters	2.64 ± 0.98	0.44 ± 0.30	<0.001	3.27 ± 1.01	0.25 ± 0.25	<0.001		0.870
VC, %predicted	71 ± 18			81 ± 14			0.073	
CV_{exp}, liters	0.68 ± 0.24	−0.08 ± 0.15	0.032	0.90 ± 0.33	−0.12 ± 0.19	0.028		0.599
CV_{exp}/VC, %	26 ± 8	−5 ± 5	<0.001	27 ± 7	−5 ± 5	0.002		0.895
CV_{exp}/VC, %predicted	104 ± 31			110 ± 33			0.605	
TLC, liters	6.93 ± 1.29	0.08 ± 0.77	0.661	6.57 ± 1.04	0.05 ± 0.41	0.635		0.877
TLC, %predicted	114 ± 17			81 ± 14			0.015	
ITGV, liters	5.17 ± 1.09	−0.21 ± 0.45	0.073	4.29 ± 0.66	−0.16 ± 0.47	0.183		0.781
ITGV, %predicted	156 ± 32			123 ± 19			0.002	
RV, liters	4.36 ± 0.98	−0.47 ± 0.51	0.002	3.33 ± 0.58	−0.19 ± 0.36	0.058		0.081
RV, %predicted	202 ± 53			146 ± 29			0.001	
FEV_1, liters	0.85 ± 0.37	0.09 ± 0.08	0.001	1.41 ± 0.62	0.09 ± 0.13	0.019		0.932
FEV_1, %predicted	32 ± 11			50 ± 18			0.003	
sRAW, kPa·s	6.40 ± 2.08	−1.67 ± 1.29	<0.001	3.21 ± 2.16	−0.84 ± 1.21	0.014		0.065
sRAW, %predicted	576 ± 203			285 ± 212			<0.001	

Values are means ± SD. M, male; F, female; VAS, visual analog scale; $[N_2]_{RV}$, expired nitrogen concentration at residual volume; aDS, anatomic dead space; VC, slow expiratory vital capacity; CV_{exp}, closing volume assessed from the P_L-V curve; TLC, total lung capacity; ITGV, intrathoracic gas volume; RV, residual volume; FEV_1, forced expiratory vital capacity in 1 s; sRAW, total specific airway resistance. *Between baseline (pre) values. †Between-group changes.

(30, 40) and that in asthmatic patients a significant fall was found only occasionally (12, 26). Moreover, bronchodilators did not appreciably affect $P_{L,CV}$, i.e., the pressure at which substantial airway closure starts to take place (Table 3), which was similar to that of normal subjects (32). Nevertheless, bronchodilators did reduce the extent of airway closure at any $P_L < P_{L,CV}$, thus allowing for greater lung emptying and VC augmentation (Tables 2 and 3 and Fig. 2), independent of the severity of the disease or the presence of EFL (Tables 4 and 5). These effects are attributable to the reduced muscle tone and increased airway compliance, which improve peripheral airway stability ensured by the tethering action of the surrounding parenchyma. Hence the measure of the closing volume alone does not allow a correct appreciation of bronchodilator action on airway closure, which is instead reflected by the decrease of CV-to-VC and CC-to-TLC ratios.

Unexpectedly, a clearly discernable phase IV appeared in nine patients after bronchodilator administration, independent of any of the measured parameters, severity of the disease, and EFL. CV_{exp} and CV_{SBN} after bronchodilators were similar, indicating that tiotropium and indacaterol did not interfere with the method used for the assessment of the closing volume. The reason why phase IV is not observable in patients in whom airway closure certainly occurs well above RV is currently unknown (32). A model analysis has suggested that absence of phase IV might be related to an enlarged range of closing pressures (25). It could be hypothesized that bronchodilators cause the reappearance of phase IV by decreasing the mechanical heterogeneity of peripheral airways, thus reducing the range of closing pressures.

Peripheral lung injury leading to maldistribution of ventilation and impaired gas exchange can result from an abnormally elevated closing volume (29). In animals, mechanical ventilation at low lung volume induces peripheral airway injury with increased airway resistance (13, 15). Further damage and substantial inflammatory reaction occur when small-airway collapse is enhanced by higher surface tension (14), thus supporting the suggestion that also in humans, opening and closing of small airways causes lung injury (29). Cyclic airway opening and closing during tidal breathing should occur whenever the closing volume exceeds ERV. At baseline, a significantly negative ERV-CV_{exp} occurred in 46% of patients. It appears, therefore, that many COPD patients are potentially exposed to risk of peripheral airway damage, and this seems to be enhanced by disease severity, as demonstrated by the increased proportion of patients with negative ERV-CV_{exp} from GOLD 2 to GOLD 4 stages. Bronchodilator administration reduced the percentage of patients with negative ERV-CV_{exp} values by ~20%. This resulted from the modest fall of the closing volume and the substantial increase of ERV (Fig. 2), due to the larger reduction of RV than of ITGV. The reduced airway closure and dependent air trapping can explain the fall of RV, whereas that of ITGV was likely of dynamic origin. In fact, the breathing pattern was not modified by bronchodilator administration, and a decrease of the end-expiratory lung volume might be therefore expected as a consequence of the fall of airway resistance. At any rate, by keeping the closing volume below ERV, bronchodilators should prove effective in reducing the risk of peripheral airway damage during tidal breathing.

The main limitation of the present study is related to the acute evaluation; long-term studies are therefore required to confirm that the observed effects are maintained over time.

Furthermore, an investigation involving a greater number of moderate and very severe patients might be warranted.

In conclusion, for the first time it was demonstrated that both muscarinic antagonists and β-adrenergic agonists are similarly able to express their bronchodilator effect by improving the heterogeneity of the mechanical properties of peripheral airways, positively affecting gas exchange and the extent of small-airway closure. This should represent a key factor to reduce the risk of small-airway damage in subjects with COPD.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.D'A. conceived and designed research; M.P., P.S., and D.R. performed experiments; M.P., D.R., and E.D'A. analyzed data; M.P., D.R., and E.D'A. interpreted results of experiments; E.D'A. prepared figures; E.D'A. drafted manuscript; M.P., P.S., D.R., and E.D'A. edited and revised manuscript; M.P., P.S., D.R., and E.D'A. approved final version of manuscript.

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