TIDAL EXPIRATORY FLOW-LIMITATION INDUCES EXPIRATORY LOOPING OF THE ALVEOLAR PRESSURE-FLOW RELATION IN COPD PATIENTS

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Running head: Plethysmographic loops and tidal expiratory flow-limitation

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CONTRIBUTIONS

DR, MP and PS conceived the study; DR and MP made the experiments; CZ, DR, ED, GS LS and MP analyzed the data; CZ, DR, and MP drafted the manuscript. CZ, DR, ED, GS, LS, MP, and PS critically revised the manuscript and gave final approval.

Abstract

During spontaneous breathing at rest the alveolar pressure (P_{alv}) - flow ($\dot{V}$) relation exhibits a prominent expiratory loop in many COPD patients. Among the possible determinants of the loop, tidal expiratory flow-limitation (tEFL) may be the main responsible. To compare the characteristics of the expiratory loop in COPD patients with flow-limitation (FL) and without flow-limitation (NFL), tEFL was assessed with the negative expiratory pressure technique in stable, mild to very severe COPD patients, undergoing body-plethysmography before and after bronchodilation (BD), an intervention which is able to reduce mechanical heterogeneity, recruitment/derecruitment and gas trapping, but rarely abolishes tEFL. The magnitude of the expiratory loop was indexed by the integral of P_{alv} on $\dot{V}$ during expiration (A_{exp}). Before BD, A_{exp} was 360% greater in FL (n=35) than in NFL (n=25) patients (P<0.001). After BD, A_{exp} was unchanged in NFL patients (ΔA_{exp} 0%, P=0.882) and slightly decreased in FL patients who remained FL (n=32, ΔA_{exp} -17%, P=0.064). Three FL patients became NFL after BD, and their A_{exp} decreased markedly (ΔA_{exp} -61%), reaching values similar to those observed in NFL patients at baseline. In conclusion, the greater A_{exp} measured in FL relative to NFL COPD patients, its relative invariance after BD when flow-limitation persists, and its fall when flow-limitation is abolished indicate that tEFL is a major determinant of the magnitude of the expiratory loop. Furthermore, A_{exp} can be used as a predictor of the presence of tEFL.

New & Noteworthy

In stable COPD patients spontaneously breathing at rest, tidal expiratory flow-limitation is the major determinant of the occurrence of expiratory looping in the plethysmographic flow-alveolar pressure diagram. In these patients the magnitude and the characteristics of the loop can be used as predictors of the presence of tidal expiratory flow-limitation.

KEYWORDS: plethysmographic loops, expiratory flow-limitation, chronic obstructive pulmonary disease, respiratory function tests.

Introduction

Recently, the shape of the relation recorded at rest during spontaneous breathing between flow ($\dot{V}$) and shift volume (ΔV_S) recorded by a plethysmograph has attracted considerable attention (30, 36). As ΔV_S is the change in lung volume due only to compression or decompression of gas inside of the lung, independently of mass flow (8), the ΔV_S-$\dot{V}$ relation mirrors that between flow and alveolar pressure (P_{alv}), which is abnormal in respiratory diseases such as chronic obstructive pulmonary disease (COPD) and may reflect pathophysiological processes taking place in the lungs (30).

One of the most striking features of the P_{alv}-$\dot{V}$ relation present in some COPD patients during spontaneous breathing at rest is a prominent expiratory loop running counterclockwise (Fig. 1) (30). Looping of the P_{alv}-$\dot{V}$ relation can be induced by several mechanisms. In expiration, mechanical heterogeneity, air trapping, recruitment/derecruitment of lung units, and tidal expiratory flow-limitation (tEFL) all induce a counterclockwise rotating loop. In inspiration, mechanical heterogeneity and air trapping cause a counterclockwise rotating loop, while that produced by recruitment/derecruitment rotates clockwise (17, 21).

Among these mechanisms, tEFL, a condition in which the iso-volume expiratory flow becomes independent of the pressure difference between the mouth and the alveoli during tidal breathing (16, 28, 40), is of paramount importance in the development of the symptoms and signs which characterize COPD. Indeed, tEFL induces dynamic hyperinflation (DH) with a concomitant marked increase of inspiratory muscles work, and adverse effects on hemodynamics (34). All these factors may contribute to the increase of dyspnea sensation, limiting the exercise capacity of patients with COPD (9, 31). Moreover, the presence of tEFL may worsen the prognosis, being associated with the progression of the disease (1).

Some lines of evidence suggest that tEFL is the main factor responsible for the disproportionate increase of the expiratory loops in COPD patients (30). In healthy young subjects mechanical heterogeneity is minimal and air trapping, recruitment-derecruitment, and tEFL are

absent (30). Aging and COPD cause mechanical heterogeneity to increase, as reflected by an increase of the slope of the phase III of the single breath nitrogen test (7, 26), besides recruitment-derecruitment and gas trapping (26). However, the increase of the area of the expiratory loop (A_{exp}) is much greater (+198%) in COPD patients relative to healthy elderly in whom tEFL is absent, and only modestly greater (38%) in elderly than in young subjects, consistent with the idea that the prominent expiratory loop seen in COPD patient is caused mainly by tEFL (30).

The aim of the present research is to verify the hypothesis that tEFL is the major factor responsible for the disproportionate increase of the expiratory loop seen in many COPD patients. This objective will be pursued in three ways.

First, if tEFL has a prominent role in causing expiratory looping in COPD patients during spontaneous breathing at rest, than COPD patients with tEFL should exhibit greater expiratory looping than those without tEFL.

Second, it is known that bronchodilators are able to affect several mechanisms potentially responsible of the expiratory loop in COPD patients, reducing the heterogeneity of peripheral airway mechanical properties, the extent of their closure and gas trapping, independently of the presence of tEFL (27). However, these drugs do not abolish tEFL in the majority of COPD patients (5, 25, 35). Therefore, bronchodilator administration should not reduce substantially expiratory looping in COPD patients, as long as tEFL remains unchanged.

Third, a prominent role of tEFL in the genesis of the expiratory loop in COPD patients suggests that loop-derived parameters can be useful predictors of tEFL at rest in these patients. This will be tested using the receiver operating characteristic curve (ROC curves).

Methods

The inclusion criteria of this prospective observational study were: (a) a confirmed diagnosis of COPD, (b) stable clinical conditions, and (c) the ability to perform pulmonary function tests adequately. Patients were excluded if they had (a) impaired cognitive function (Mini-Mental State

Examination score <26), (b) previous lobectomy or a current diagnosis of neoplastic or musculoskeletal diseases, (c) a mixed obstructive-restrictive ventilatory pattern, (d) history of asthma, (e) a confirmed diagnosis of obstructive sleep apnea, (f) a BMI >34 Kg m^{-2}, and (g) recent cardiothoracic surgery or NYHA III or IV functional class heart failure.

At the time of evaluation, none of the patients was treated with oral β_2-agonists, theophylline or systemic corticosteroids; short and long acting bronchodilators were withdrawn respectively 8 and 24 hours before the study. COPD patients were attending a bronchodilation test as part of their clinical evaluation at the Pulmonary Rehabilitation Unit of Fondazione Salvatore Maugeri, University of Milan (Italy), and were enrolled when the experimenters (DR and MP) were present in the Pulmonary Rehabilitation Unit. The study was conducted in accordance with the amended Declaration of Helsinki and was approved by the local ethics committee (Fondazione Salvatore Maugeri, Comitato Etico Centrale, -629 CEC, Italy). Written informed consent was obtained from all patients. Information regarding the flow of patients through the study are available in the Supplement.

Experimental sequence

Before and after four inhalations of salbutamol (100 μg each) through a metered-dose inhaler and a spacer, lung function tests and assessment of tEFL were performed in random order. Dyspnea was assessed at rest before and after the administration of salbutamol by means of the modified Borg scale (3).

Static and dynamic lung volumes, together with specific airway resistance (sR_{aw}) were measured with a constant-volume plethysmograph (MasterScreen Body Plethysmograph, Erich Jaeger GmbH, Würzburg, Germany), following the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (39). Additional information is available in the Supplement.

tEFL was assessed by means of the NEP method (20). Subjects, wearing a nose clip, breathed quietly through a flanged mouthpiece, a heated pneumotachometer (3700; Hans Rudolph, Kansas City, MO, USA), connected to a differential pressure transducer (Celesco LCVR-0005; Raytech Instruments, Vancouver, BC, Canada), in series with a Venturi device. The Venturi device was connected via a solenoid valve to a high-pressure source, and a regulator allowed for a pre-set pressure (-5 cmH_2O) at the airway opening that was measured with a pressure transducer (Celesco LCVR-0100; Raytech Instruments). The pneumotachograph, calibrated with a 3-L syringe, was linear over the experimental flow range. Pressure and flow signals were amplified, low-pass filtered at 50 Hz and digitized at 100 Hz by a 16-bit analogue-to-digital converter (Direc Physiologic Recording System; Raytech Instruments). The volume was obtained by numerical integration of the flow signal. The digitized data were stored on a computer.

Data analysis

A detailed description of the measurement of plethysmographic loop-derived parameters is given elsewhere (30).

Briefly, after retrieving the ASCII files recorded during the measurement of airway resistance containing plethysmographic ΔV_S and $\dot{V}$, sampled at 50 Hz for ten consecutive breaths, a custom-built LabView program (National Instruments, Austin, TX),

a) converted ΔV_S into P_{alv} for all breaths, according to the following equation: $P_{alv,t} = -\frac{P_B \Delta V_{S,t}}{V_{rs,t} + \Delta V_{S,t}}$ where P_{alv} is alveolar pressure minus P_B, P_B barometric minus vapor pressure, and V_{rs} the volume of the respiratory system, calculated as the sum of the intrathoracic gas volume and the time integral of the flow;

b) allowed the elimination of abnormal breaths (cough or sigh) by an operator blind to the NEP results;

c) averaged the acquired inspirations and expirations after normalization with respect to their duration.

Subsequently, on the subject's representative breath, the following parameters were assessed:

a) tidal volume (V_T), duration of inspiration (T_I) and of expiration (T_E),

b) area of the inspiratory (A_{ins}) and expiratory (A_{exp}) loop by numerical integration of P_{alv} on $\dot{V}$ for the inspiration and expiration respectively; the beginning and the end of the inspiration were defined in terms of zero $\dot{V}$.

d) expiratory (R_{exp}) airway resistance (37).

Moreover, A_{exp} was divided by peak expiratory flow, yielding a rough index of the mean pressure during expiration (ΔP^{mean}), and the width of the expiratory loop measured at the flow corresponding to the maximal alveolar pressure (ΔP^{atPmax}) (Fig. 1). R_{exp}, A_{exp}, ΔP^{mean} and ΔP^{atPmax} are collectively referred to in the text as loop-derived parameters.

The presence of tEFL according to the NEP technique was assessed offline by an operator blind to the identity of the subjects as previously described (20).

Statistics

Qualitative and quantitative variables were summarized with absolute and relative (percentage) frequencies and means (standard deviations, SD) or medians (interquartile ranges, IQR), depending on their parametric and non-parametric distribution. Qualitative variables were compared with chi-squared or Fisher exact test, when appropriate, whereas quantitative variables were compared with Student t or Mann-Whitney test for normal or non-normal distribution, respectively. The comparison for more than two groups was performed with Kruskal-Wallis test for non-normally distributed quantitative variables. Comparison of paired quantitative data was carried out with Wilcoxon signed-rank test. A Spearman's correlation was adopted to quantitatively assess the relationship between variables. Furthermore, the relationship between quantitative dependent

variables and covariates was evaluated with linear regression models. The diagnostic accuracy was measure with ROC curve analysis. A two-tailed p-value less than 0.05 was considered statistically significant. Cut-off values were calculated using Youden's J statistic.

The assumptions behind the computation of the sample size included data previously collected on 20 elderly healthy subjects and 130 COPD patients (30). Based on an estimated parametric distribution of A_{exp} between COPD patients without and with tEFL and on a statistical power of 0.95 and an alpha error of 0.05, a sample of at least 24 individuals per single group (COPD with or without tEFL) was planned.

All statistical analyses were performed with the statistical softwares STATA version 15 (StataCorp, Texas, US), MedCalc (MedCalc Software, Ostend, Belgium) and G*Power (13).

Results

Anthropometric characteristics, spirometric and plethysmographic parameters, and dyspnea sensation at rest of 60 COPD patients stratified according to tEFL absence or presence before salbutamol administration are shown in Table 1.

All non flow-limited patients at baseline remained non flow-limited after salbutamol administration (NFL_{pre}-NFL_{post}). Of the flow-limited patients, 32 remained flow-limited after bronchodilation (FL_{pre}-FL_{post}), and 3 became non flow-limited (FL_{pre}-NFL_{post}). For sake of simplicity, FL_{pre}-NFL_{post} were pooled with FL_{pre}-FL_{post} for the purpose of the analysis of the effects of salbutamol on dyspnea and spirometric and plethysmographic volumes, while they were considered separately to track the relation between the presence of tEFL and the characteristics of the loop.

Static and dynamic lung volumes, specific airway resistance and dyspnea sensation

Relative to non flow-limited patients, flow-limited patients had more severe obstruction as assessed by FEV_1, FVC, and sR_{aw} (-28% pred; -20 %pred; +228 %pred; $P<0.001$ for all), more

prominent hyperinflation (ΔITGV=+39 %pred; ΔIC=-26 %pred; P<0.001 for both), and more gas trapping (ΔRV=+52 %pred, P<0.001). Breathlessness at rest was more severe in patients with than without tEFL (Table 1).

Salbutamol inhalation increased FEV_1, and decreased RV, and ITGV similarly in patients with and without tEFL (Table 2). IC did not change in non flow-limited patients (P=0.196), but increased significantly in flow-limited ones by 0.2 L (P<0.001). sR_{aw} decreased both in patients without and with tEFL, but more in the latter than in the former (-1.6 versus -9.7 cmH_2O s, P<0.001). Similarly, dyspnea sensation decreased more in flow-limited that in non flow-limited patients (P<0.001).

Breathing pattern

No systematic differences were detected between the breathing pattern assessed during NEP application and plethysmographic measurements, independent of bronchodilator administration and presence of tEFL.

Before salbutamol V_T, T_E, respiratory rate (RR) and ventilation ($\dot{V}_E$) were similar in patients with or without tEFL, whereas flow-limited patients exhibited a shorter T_I (Table 3) and a smaller mean expiratory flow (0.33 (0.31; 0.45) versus 0.44 (0.35; 0.52) L s^{-1}, P=0.031).

Salbutamol inhalation did not change the breathing pattern (Table 3).

Loop-derived parameters

Before salbutamol A_{ins} was greater in flow-limited than in non-flow limited patients (0.45 (0.37; 0.77) versus 0.22 (0.14; 0.30) cmH_2O L^{-1}, P<0.001) and in both groups substantially smaller than the corresponding A_{exp} (P<0.001). A positive correlation was present between A_{ins} and A_{exp} in both flow-limited and non flow-limited patients (P=0.002 and <0.001, respectively), but the amount of the variance of A_{exp} explained by the variance of A_{ins} was greater in non flow-limited than flow-

239 limited patients, as indicated by the significant difference in the corresponding R^2 (0.80 and 0.25,
240 respectively, P<0.001).

241 At baseline, all loop-derived parameters were markedly greater in FL_{pre}-NFL_{post}, and FL_{pre}-
242 FL_{post}, than in NFL_{pre}-NFL_{post} patients (Fig. 2 and 3). Relative to NFL_{pre}-NFL_{post} patients R_{exp}, A_{exp},
243 ΔP^{mean} and ΔP^{atPmax} increased in FL_{pre}-NFL_{post} patients by 298 (P=0.043), 360 (P=0.043), 444
244 (P=0.024), and 600% (P=0.004), respectively, and in FL_{pre}-FL_{post} patients by 372, 360, 389, and
245 533%, respectively (P<0.001).

246 The effects of salbutamol on plethysmographic P_{alv}-$\dot{V}$ relations and loop-derived parameters
247 are shown in Fig.2 and 3, respectively.

248 In NFL_{pre}-NFL_{post} loop-derived parameters were unaffected by salbutamol. In FL_{pre}-FL_{post}
249 salbutamol caused a modest decrease of R_{exp} (-12%, P=0.001), A_{exp} (-17%, P=0.064), ΔP^{mean} (-19%,
250 P=0.013) and ΔP^{atPmax} (-19%, P=0.001). Greater changes were elicited by salbutamol in FL_{pre}-
251 NFL_{post}: R_{exp} declined by 34%, A_{exp} by 61%, ΔP^{mean} by 40%, and $\Delta P^{atP\ max}$ by 30% (P=0.109 in all
252 cases).

253

254 *Predicting the presence of tEFL*

255 The ability of dyspnea sensation, static and dynamic lung volumes, and loop-derived
256 parameters to predict the presence of tEFL in stable COPD patients at rest before and after
257 salbutamol administration is shown in Table 4. Before salbutamol inhalation, dyspnea sensation and
258 static and dynamic lung volumes were good predictors of tEFL (33), while loop-derived parameters
259 (R_{exp}, A_{exp}, ΔP^{mean} and ΔP^{atPmax}) were even better. After salbutamol inhalation the predicting ability
260 of all parameters decreased somewhat. The predicting ability of R_{exp}, ΔP^{mean} and ΔP^{atPmax} was better
261 than that of FEV_1 %pred before salbutamol administration (ΔAUC (95% CI); 0.12 (0.03; 0.20),
262 P=0.001; 0.14 (0.05; 0.24), P=0.003; 0.16 (0.06; 0.25), P=0.002; respectively), but these differences
263 decreased after salbutamol (ΔAUC 0.09 (-0.00; 0.19), P=0.059; 0.09 (-0.03; 0.21), P=0.129; 0.09 (-
264 0.03; 0.21), P=0.132; respectively). Table 4 also reports sensibility and specificity measured at a

threshold corresponding to the maximal vertical distance of the ROC curve from the diagonal for FEV_1 and loop-derived parameters. The same information is represented graphically for FEV_1, R_{exp}, ΔP^{mean}, and ΔP^{atPmax} in Fig. S2 of the Supplement. The change in threshold after the bronchodilator expressed in percentage of the pre value was trivial for R_{exp} and ΔP^{mean} (-3 and 1%, respectively), somewhat higher for FEV_1 and A_{exp} (13 and -11%, respectively) and large for ΔP^{atPmax} (50%).

Discussion

The novel finding of this study is that in stable COPD patients with tEFL, assessed with the NEP technique, looping of the expiratory portion of the P_{alv}-$\dot{V}$ relation is greater than in patients without tEFL, indicating that, among the possible determinants of expiratory looping, tEFL plays a paramount role. Indeed, in patients in whom salbutamol does not abolish tEFL, expiratory looping decreases modestly after salbutamol, while in patients who become non flow-limited after salbutamol there is a marked reduction of expiratory looping. Furthermore, it is shown that loop-derived parameters are excellent predictors of the presence of tEFL at rest according to the classification of Swets (33), especially before bronchodilator administration.

Earlier studies attributed the appearance of expiratory looping to abnormal compression and collapse of the airways during expiration (2) or to compression of non-ventilated airspaces (15). On the other hand, airway compression does not necessarily lead to airway collapse. Moreover, an inspiratory loop should also be generated when non-ventilated or poorly ventilated lung units are compressed in expiration and decompressed during inspiration (30). The key role of tEFL in the genesis of a prevalently expiratory looping was recognized by Van de Woestijne and coll. (38), although no experimental proof has been ever given. In line with this concept we observed that in our patients with tEFL loop-derived parameters (R_{exp}, A_{exp}, ΔP_{mean}, and ΔP^{atPmax}) were significantly greater compared with patients without tEFL (Fig. 2 and 3).

The presence of regional tEFL (29) should increase the magnitude of the expiratory loop by enhancing the mechanical heterogeneity, and cause a greater increase of A_{exp} than A_{ins}. This could

be one of the reasons why A_{exp} was greater than A_{ins} also in subjects in whom no tEFL was detected by the NEP method. Alternatively, dynamic compression not resulting in flow-limitation together with abnormal volume-dependence of airway resistance may explain this observation. However, the effectiveness of these two mechanisms in producing expiratory looping should be modest, also in view of the ability of A_{exp}, like the other loop-derived parameters, to predict the presence of tEFL (Table 4).

In contrast with tEFL, heterogeneity of time constants, air trapping and recruitment/derecruitment of lung units should produce both inspiratory and expiratory looping (30). Indeed, A_{ins} and A_{exp} were correlated both in flow-limited and non flow-limited patients. However, the amount of variance of A_{exp} explained by A_{ins} was significantly larger for non flow-limited (80%) than flow-limited subjects (25%), coherently with the idea that the major contributor to A_{exp} is tEFL.

After salbutamol administration, three patients with pre-bronchodilator tEFL became non flow-limited (Fig. 2). Using the NEP technique, abolition of tEFL by bronchodilators occurs in minority of COPD patients, ~10% when tEFL is assessed by NEP (5, 10, 25, 27, 35), and ~35% when assessed with FOT (10, 11). Due to the limited number of subjects investigated, it is possible that the different incidence of tEFL abolition by bronchodilators obtained by two techniques is simply related to differences in the composition of the experimental groups.

Salbutamol had limited effects on A_{exp} in NFL_{pre}-NFL_{post} and FL_{pre}-FL_{post} patients (ΔA_{exp}=0% and -17%, P=0.882 and 0.064, respectively) (Fig. 2 and 3). To the contrary A_{exp} declined substantially in FL_{pre}-NFL_{post} (ΔA_{exp}=-61%) (Fig. 2 and 3), even if statistical significance was not reached due to the small number of subjects, and A_{exp} after salbutamol became of the same order of magnitude as that of NFL_{pre}-NFL_{post} patients. It is tempting to speculate that in these patients the expiratory looping which remains after salbutamol corresponds to the effect of heterogeneity, recruitment-derecruitment and gas trapping.

In this study, dyspnea sensation, static and dynamic lung volumes were good predictors of the presence of tEFL in stable COPD patients at rest (Table 4). Before salbutamol administration R_{exp}, ΔP^{mean} and ΔP^{atPmax} were significantly better predictors of the presence of tEFL than FEV_1 %pred, the best predictor of all the standard pulmonary function test parameters in our study sample. After salbutamol, the predictive power of all the parameters deteriorated somewhat. The AUC of loop-derived parameters remained higher than those of static or dynamic volumes but statistical significance was lost. This is most likely due to the fact that salbutamol-induced changes of loop-derived and spirometric parameters were greater or tended to be greater in flow-limited than in non flow-limited patients, decreasing the difference between the two groups.

Even if the analysis of loop-derived parameters appear a promising tool for tEFL detection, it is worth pointing out that its validation was made on the basis of the NEP test. The choice of this test as the reference standard was primarily determined by the fact that this technique constitutively detects tEFL (19), because in a subject with normal upper airway elastance, a lack of increase of the expiratory flow at iso-volume in front of an artificial increase of the driving pressure necessarily indicates the presence of tEFL (16, 28, 40). The conceptual simplicity of this technique is not shared by the subtraction method (22) or the forced oscillation technique (12), which exploit secondary effects of tEFL. An additional advantage of using NEP relative to the subtraction method in the present research is that tEFL detection by NEP is based on a principle completely different from the principle on which the method we were going to evaluate is based, in other words the NEP and the presently proposed plethysmographic techniques are truly independent. Indeed, the NEP method detects the presence of tEFL as a lack of increase of the expiratory flow when the driving pressure is artificially increased by the application at the mouth of a small negative pressure (20), while our plethysmographic method detects tEFL when loop-derived parameters exceed certain thresholds. In contrast, the subtraction method (12, 22) detects the presence of tEFL when the expiratory flow decreases with increasing dynamic pressure. This dynamic pressure is a surrogate of alveolar pressure, the same variable which is estimated by the plethysmograph (30). Moreover the

342 decrease of flow with increasing dynamic pressure on which the Mead-Whittenberger method is
343 based very often coexists with the presence of an expiratory loop, the feature of the P_{alv}-$\dot{V}$ relation
344 used by the present method to detect the presence of tEFL. We recognize that despite its conceptual
345 simplicity a number of practical issues can complicate the use of the NEP technique (10), possibly
346 impeding the assessment of tEFL in single breaths; however, these issues prevent the classification
347 of a subject as flow-limited or not only rarely, as shown by the impressive number of studies in
348 which this technique has been applied in healthy subjects and patients with various diseases (4, 6, 9,
349 14, 18, 23).

350 The subtraction method, also called Mead-Whittenberger method, detects the presence tEFL
351 when during part of the expiration flow decreases in spite of an increase of dynamic pressure. This
352 might not take place at iso-volume, and therefore its presence, though highly suggestive of tEFL, is
353 not probative (28). Moreover, dynamic pressure reflects alveolar pressure only if a number of
354 conditions are met, namely that pendelluft is absent when the flow at the mouth is zero, the
355 measured value of esophageal pressure reflects the overall pleural pressure acting on the lungs (24),
356 elastance is constant in the tidal volume range, and viscoelasticity contributes trivially to
357 transpulmonary pressure. Violation of these assumptions leads to distortion of the expiratory
358 dynamic pressure-flow trajectory, complicating or even preventing the interpretation of the test
359 (12). Furthermore, the specificity of the subtraction method is limited by the fact that whatever
360 mechanism causing the resistance to increase in the course of expiration is potentially able to elicit a
361 decrease of flow in spite of an increase of the driving pressure.

362 FOT identifies tEFL as a within-breath difference between inspiration and expiration in
363 terms of reactance (ΔX_{rs}). The particular ΔX_{rs} threshold (2.8 cmH_2O s L^{-1}) used to detect the
364 presence of tEFL has been empirically obtained comparing FOT results with those of the
365 subtraction method (12), a technique which cannot be considered a reference standard for tEFL
366 detection, as discussed above. However, direct comparison between FOT and NEP method resulted
367 in an acceptable degree of agreement (10). As for the Mead Whittenberger method and the present

loop-derived parameters, FOT is not specific for tEFL recognition, and cyclic opening and closing of small airways can, in line of principle, contribute to the swings of X_{rs} during inspiration and expiration, even if the time course of X_{rs} during the respiratory cycle suggests that this phenomenon is not a preponderant determinant of the changes of X_{rs} in the majority of COPD patients (12). Anyway, the lack of specificity should not prevent the use of these indexes for the detection of tEFL in COPD patients, as they are found able to recognize the presence of tEFL in an very high number of cases.

Limitations

This is a monocentric observational study, and the placebo effects on plethysmographic loops were not studied. However it is unlikely that placebo has a major effect on plethysmographic loop, as sR_{aw}, which is calculated on the loop, does not change after saline inhalation (32).

A major limitation of this study is that it is technically impossible to apply the NEP technique inside of the plethysmograph, so that the presence of tEFL was separately assessed with the NEP technique after a few minutes from the plethysmographic test. Even if efforts were made to maintain similar conditions inside and outside the plethysmograph, and no systematic differences in the breathing pattern were found between the two situations, some variability was introduced, as indicated by the coefficients of determination of V_T, T_I and T_E (Table 3). Anyway, differences in the presence of tEFL between the measurements inside and outside the plethysmograph might have affected the strength of the relation between loop-derived parameters and tEFL.

A detailed list of the limitations of this plethysmographic technique has been previously published (30).

Conclusions

This study has shown that a primary determinant of expiratory looping of the plethysmographic P_{alv}- $\dot{V}$ relation is the presence of tEFL, as indicated by the greater magnitude of

the expiratory loop in flow-limited than in non flow-limited patients, by the relative invariance of the loop in COPD patients in whom flow-limitation is unaffected by salbutamol and by the fall of the area of the loop in those few patients in whom tEFL is abolished by the bronchodilator. It was therefore possible to show that there are values of expiratory loop-derived parameters beyond which the probability of the presence of tEFL becomes very high. These values obviously apply to the present population and type of plethysmograph; further studies are therefore needed to establish the general validity of this observation. Given the widespread diffusion of the plethysmograph, and the fact that the measurement of loop-derived parameters does not require additional maneuvers apart from those performed to measure airway resistance, the present plethysmographic approach for the prediction of the presence of tEFL may represent a convenient tool for a more complete functional characterization of COPD patients, especially in clinical studies involving a large number of participants.

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TABLE 1 Anthropometric characteristics, spirometric and plethysmographic parameters of 60 COPD patients non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before salbutamol admistration

	NFL_{pre} **(n= 25)**	**FL_{pre}** **(n= 35)**	**P**
Males, n (%)	15 (60)	22 (63)	*0.822*
age, years	71±6	73±7	*0.434*
height, cm	164±9	164±10	*0.811*
weight, kg	72±13	67±16	*0.151*
BMI, kg/m^2	26.7±3.3	24.7±4.2	*0.057*
BORG pre	0 (0-2)	3 (1; 4)	*<0.001*
FEV_1, % pred	62 (48; 76)	34 (24; 55)	*<0.001*
FVC, % pred	86 (71; 96)	66 (50; 79)	*<0.001*
FEV_1/FVC, %	57 (52; 70)	41 (37; 54)	*<0.001*
IC, % pred	92 (74; 106)	66 (60; 81)	*<0.001*
ERV, % pred	89 (73; 150)	75 (55; 104)	*0.154*
VC, % pred	96 (84; 114)	75 (67; 93)	*<0.001*
TLC, % pred	110 (98; 126)	123 (109; 140)	*0.039*
RV, % pred	132 (114; 156)	184 (150; 226)	*<0.001*
ITGV, % pred	126 (118; 145)	165 (134; 185)	*<0.001*
sR_{aw}, % pred	183 (126; 208)	471 (320; 625)	*<0.001*

Values are mean±SD or median (IQR), unless otherwise specified. FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; IC: inspiratory capacity; ERV: expiratory reserve volume; VC: slow vital capacity; TLC: total lung capacity; RV: residual volume; ITGV: intrathoracic gas volume; sR_{aw}: specific airway resistance. P: probability of a difference between COPD patients who were non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before salbutamol admistration.

TABLE 2 Effects of salbutamol administration on dyspnea sensation at rest, spirometric and plethysmographic parameters in COPD patients who were non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before salbutamol admistration.

	NFL_{pre} (n= 25)			**FL_{pre} (n= 35)**			
	Pre	**Δ**	**P(Δ)**	**Pre**	**Δ**	**P(Δ)**	**p***
BORG	0 (0;2)	0 (-0.5; 0.0)	*0.005*	3 (1.0; 4.0)	-1 (-2.0; -0.5)	*<0.001*	*<0.001*
FEV_1, L	1.25 (1.07;1.76)	0.06 (-0.01; 0.14)	*0.026*	0.74 (0.58; 0.98)	0.05 (0.02; 0.11)	*<0.001*	*0.898*
FVC, L	2.4 (1.9; 3.1)	0.0 (-0.1; 0.2)	*0.306*	1.7 (1.4; 2.2)	0.1 (0.0; 0.3)	*<0.001*	*0.033*
FEV_1/FVC, %	57 (52; 70)	1.3 (-0.6; 3.5)	*0.125*	41 (37; 54)	0 (-2.0; 1.7)	*0.408*	*0.102*
IC, L	2.0 (1.8; 2.5)	0.0 (-0.1; 0.1)	*0.196*	1.6 (1.3; 2.3)	0.2 (0.0; 0.3)	*<0.001*	*0.043*
ERV, L	0.8 (0.5; 1.0)	0.0 (-0.1; 0.1)	*0.798*	0.6 (0.4; 0.8)	0.0 (-0.2; 0.2)	*0.844*	*0.922*
VC, L	3.0 (2.4; 3.7)	0.0 (-0.2; 0.2)	*0.979*	2.3 (1.9; 3.1)	0.1 (-0.1; 0.3)	*0.015*	*0.115*
TLC, L	5.9 (5.4; 7.4)	-0.3 (-0.4; 0.0)	*0.053*	7.3 (5.8; 7.9)	-0.2 (-0.5; 0.1)	*0.003*	*0.919*
RV, L	3.1 (2.7; 3.5)	-0.2 (-0.5; 0.0)	*0.013*	4.3 (3.7; 5.2)	-0.3 (-0.7; -0.0)	*<0.001*	*0.467*
ITGV, L	4.0 (3.3: 4.5)	-0.2 (-0.6; 0.0)	*0.026*	5.0 (4.2; 5.9)	-0.3 (-0.6; -0.1)	*<0.001*	*0.343*
sR_{aw}	18.8 (14.1;22.9)	-1.6 (-3.6; -0.6)	*0.028*	50.2 (34.8; 69.5)	-9.7 (-19.5; -4.3)	*<0.001*	*<0.001*

Values are median (IQ). FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; IC: inspiratory capacity; ERV: expiratory reserve volume; VC: slow vital capacity; TLC: total lung capacity; RV: residual volume; ITGV: intrathoracic gas volume; sR_{aw}: specific airway resistance. Δ: change of a parameter upon salbutamol administration. P(Δ): probability of the change of a parameter before and after salbutamol inhalation; P(Δ):

probability of a difference between pre and post salbutamol. P*: probability of a difference of the effect of salbutamol between COPD patients who were non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before the administration of salbutamol.

TABLE 3 Effects of salbutamol administration on the breathing pattern measured by the plethysmograph in COPD patients who were non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before salbutamol admistration.

	NFL_{pre} (n= 25)					FL_{pre} (n= 35)					
	Pre	**Δ**	**P(Δ)**	**R^2 pre**	**R^2 post**	**Pre**	**Δ**	**P(Δ)**	**R^2 pre**	**R^2 post**	**p***
V_T, L	0.8 (0.7; 1.0)	0.0 (0.0; 0.1)	*0.737*	0.534	0.518	0.7 (0.6; 0.9)	0.0 (0.0; 0.1)	*0.381*	0.723	0.752	*0.444*
T_I, s	1.4 (1.2; 1.6)	0.1 (-0.2; 0.2)	*0.706*	0.359	0.466	1.1 (0.9; 1.4)	0.0 (-0.1; 0.1)	*0.713*	0.716	0.757	*0.840*
T_E, s	1.9 (1.7; 2.4)	0.0 (-0.1; 0.3)	*0.443*	0.360	0.399	1.9 (1.6; 2.6)	0.0 (-0.1; 0.2)	*0.635*	0.709	0.783	*0.621*
RR, breaths/min	18 (14; 20)	0 (-3; 1)	*0.353*	0.407	0.457	20 (16; 23)	0 (-2; 1)	*0.456*	0.681	0.785	*0.621*
$\dot{V}_E$, L/min	14.5 (12.1; 17.5)	-0.5 (-2.1; 0.3)	*0.226*	0.598	0.479	13.4 (12.0; 15.8)	-0.1 (-.9; 1.1)	*0.928*	0.599	0.531	*0.210*

Values are median (IQ). R^2pre and R^2post: coefficients of determination of the correlations between plethysmographic and NEP measurement of the same parameter; V_T: tidal volume; T_I: inspiratory duration; T_E: expiratory duration; RR: respiratory rate; $\dot{V}_E$: pulmonary ventilation. Δ: change of a parameter upon salbutamol administration. P(Δ): probability of the change of a parameter before and after salbutamol inhalation; P*: probability of a difference of the effect of salbutamol between COPD patients who were non flow-limited (NFL_{pre}) or flow-limited (FL_{pre}) before the administration of salbutamol.

TABLE 4. Ability of loop-derived parameters, dyspnea sensation and static and dynamic lung volumes to predict the presence of tidal expiratory flow-limitation at rest in COPD patients before (pre) and after (post) salbutamol administration.

	AUC (95% CI) Pre	P	Threshold	Sensitivity	Specificity
R_{exp}, cmH_2O s/L	0.95 (0.86-0.99)	<0.001	9.2	64.3	88.0
A_{exp}, cmH_2O L/s	0.92 (0.82-0.97)	<0.001	1.09	82.9	92.0
ΔP^{mean}, cmH_2O	0.97 (0.89-1.00)	<0.001	1.75	94.3	92.0
ΔP^{atPmax}, cmH_2O	0.99 (0.92-1.00)	<0.001	1.67	94.3	96.0
Borg	0.79 (0.66-0.88)	<0.001	-	-	-
FEV_1, %pred	0.83 (0.71-0.92)	<0.001	36.8	57.1	96.0
FVC, %pred	0.76 (0.63-0.86)	<0.001	-	-	-
FEV_1/FVC	0.79 (0.67-0.89)	<0.001	-	-	-
IC, %pred	0.78 (0.65-0.87)	<0.001	-	-	-
VC, %pred	0.78 (0.65-0.87)	<0.001	-	-	-
RV, %pred	0.79 (0.66-0.88)	<0.001	-	-	-
ITGV, %pred	0.77 (0.64-0.86)	<0.001	-	-	-

	AUC (95% CI) Post	P	Threshold	Sensitivity	Specificity
R_{exp}, cmH_2O s/L	0.90 (0.79-0.96)	<0.001	8.9	81.2	81.1
A_{exp}, cmH_2O L/s	0.83 (0.71-0.92)	<0.001	0.97	75.0	85.7
ΔP^{mean}, cmH_2O	0.90 (0.79-0.96)	<0.001	1.77	75.0	96.4
ΔP^{atPmax}, cmH_2O	0.89 (0.79-0.96)	<0.001	2.50	68.7	96.4
Borg	0.73 (0.60-0.84)	0.002	-	-	-
FEV_1, %pred	0.81 (0.68-0.90)	<0.001	41.4	62.5	82.1
FVC, %pred	0.71 (0.58-0.82)	0.002	-	-	-
FEV_1/FVC	0.76 (0.63-0.86)	<0.001	-	-	-
IC, %pred	0.72 (0.59-0.83)	0.001	-	-	-
VC, %pred	0.73 (0.60-0.84)	<0.001	-	-	-
RV, %pred	0.75 (0.62-0.85)	<0.001	-	-	-
ITGV, %pred	0.71 (0.58-0.82)	0.002	-	-	-

R_{exp}: expiratory airway resistance; A_{exp}: area of the expiratory part of the P_{alv}-$\dot{V}$ relation; mean; ΔP^{mean}: mean width of the expiratory part of the P_{alv}-$\dot{V}$ relation; ΔP^{atPmax}: maximal width of the expiratory part of the P_{alv}-$\dot{V}$ relation; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; IC: inspiratory capacity; VC: slow vital capacity; RV: residual volume; ITGV:

intrathoracic gas volume;. P: probability that AUC is significantly different from 0.5. Threshold, sensitivity and specificity have been calculated only for loop-derived parameters and FEV_1.

Legends

Figure 1: Time course of flow ($\dot{V}$) (Panel A) and alveolar pressure (P_{alv}) (Panel B) in a patient with severe COPD spontaneously breathing at rest. The P_{alv}-$\dot{V}$ diagram (Panel C) shows a prominent expiratory loop running counterclockwise. The integral of P_{alv} on $\dot{V}$ is A_{exp} (shaded area). ΔP^{mean} is A_{exp} divided by peak expiratory flow. The black bar indicates ΔP^{atPmax}, that is the width of the expiratory loop at the flow corresponding to the maximal expiratory P_{alv}.

Figure 2: Average P_{alv}-$\dot{V}$ diagrams measured during spontaneous breathing at rest in non flow-limited COPD patients who remained non flow-limited after salbutamol administration (NFL_{pre}-NFL_{post}) (Panel A), flow-limited patients who became non-flow limited after salbutamol administration (FL_{pre}-NFL_{post}) (Panel B), and flow-limited patients who remained flow-limited after salbutamol administration (FL_{pre}-FL_{post}) (Panel C).

Figure 3: Effects of salbutamol administration on some loop-derived parameters in COPD patients who were flow-limited before and after (FL_{pre}-FL_{post}), flow-limited before and not flow-limited after (FL_{pre}-NFL_{post}) and not flow-limited before and after (NFL_{pre}-NFL_{post}) salbutamol admistration. R_{exp}: expiratory resistance, A_{exp}: area of the expiratory part of the P_{alv}-$\dot{V}$ relation; ΔP^{mean}: mean width of the expiratory part of the P_{alv}-$\dot{V}$ relation; ΔP^{atPmax}: maximal width of the expiratory part of the P_{alv}-$\dot{V}$ relation.

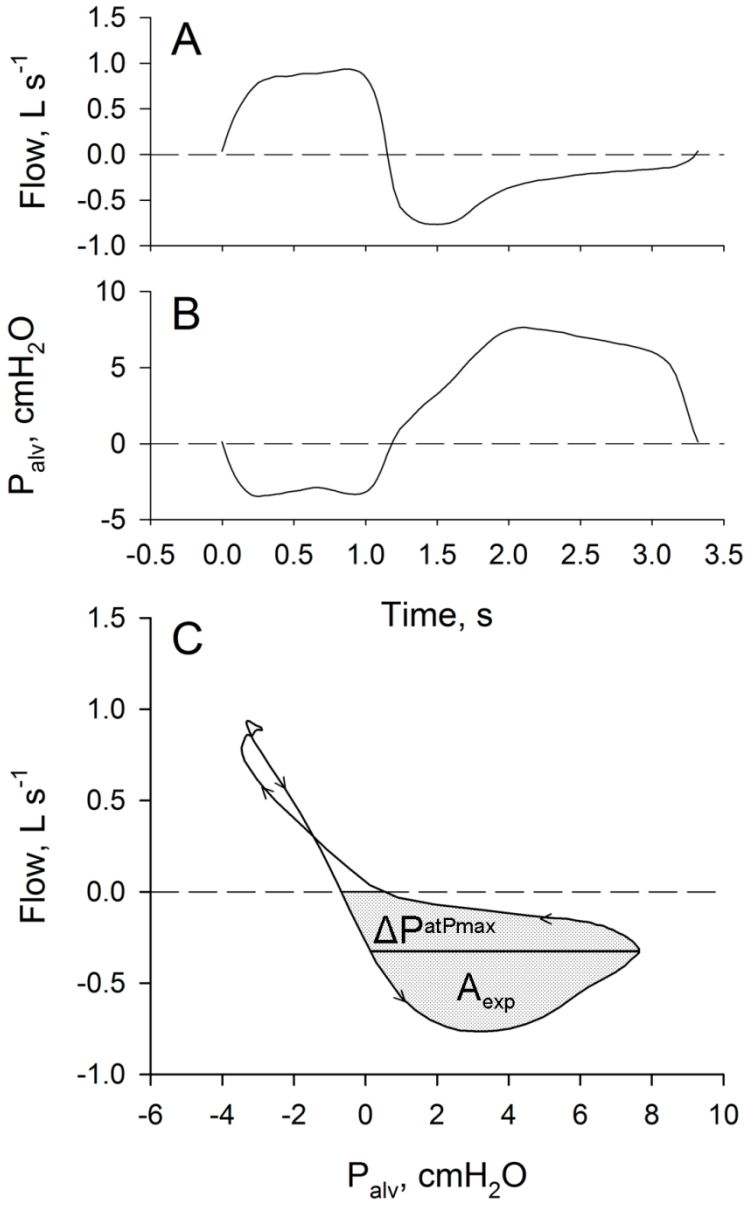

Figure 1

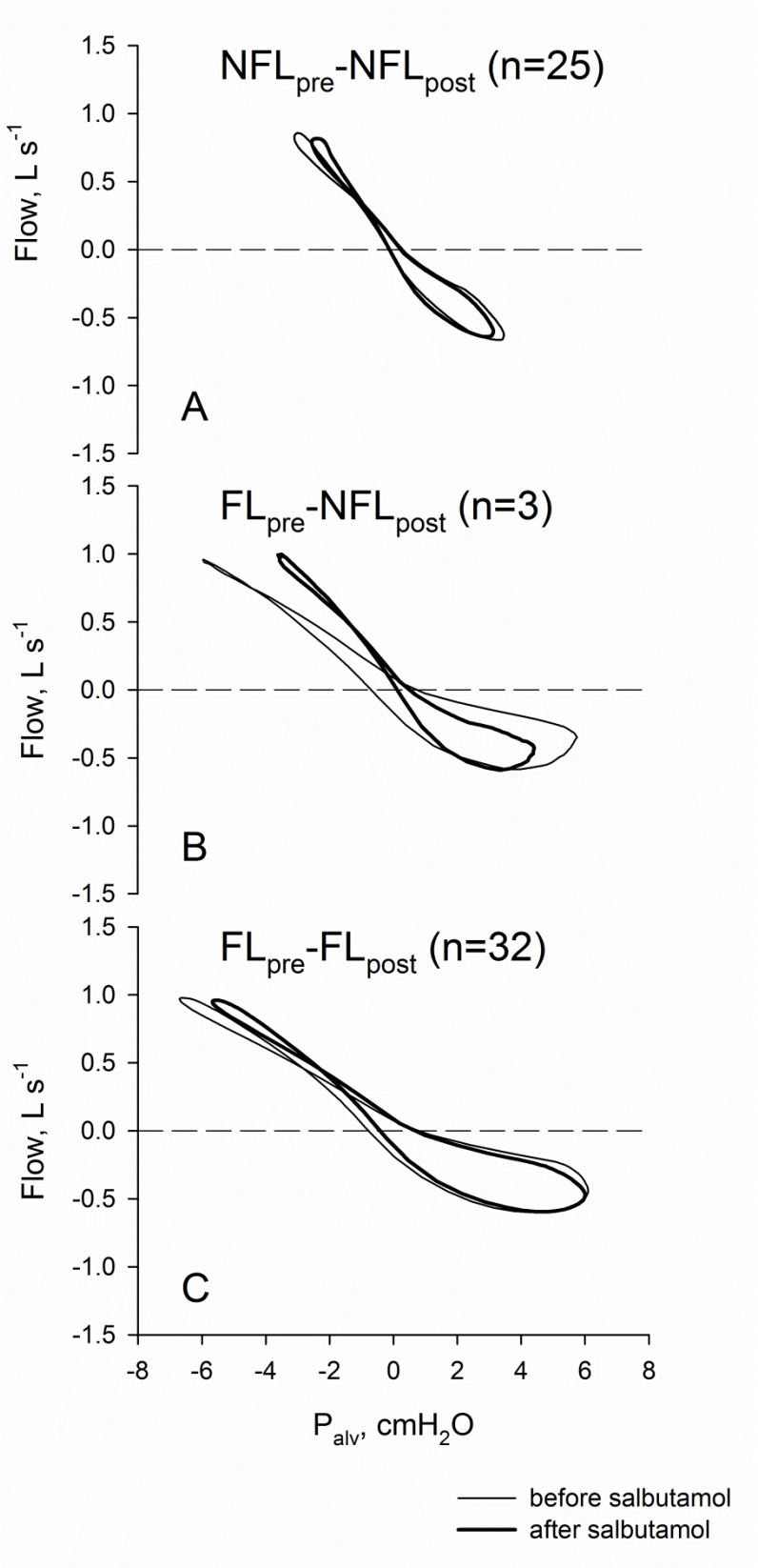
NFL$_{pre}$-NFL$_{post}$ (n=25)
FL$_{pre}$-NFL$_{post}$ (n=3)
FL$_{pre}$-FL$_{post}$ (n=32)
Flow, L s^{-1}
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
A
B
C
-8
-6
-4
-2
0
2
4
6
8
P$_{alv}$, cmH$_2$O
before salbutamol
after salbutamol

Figure 2

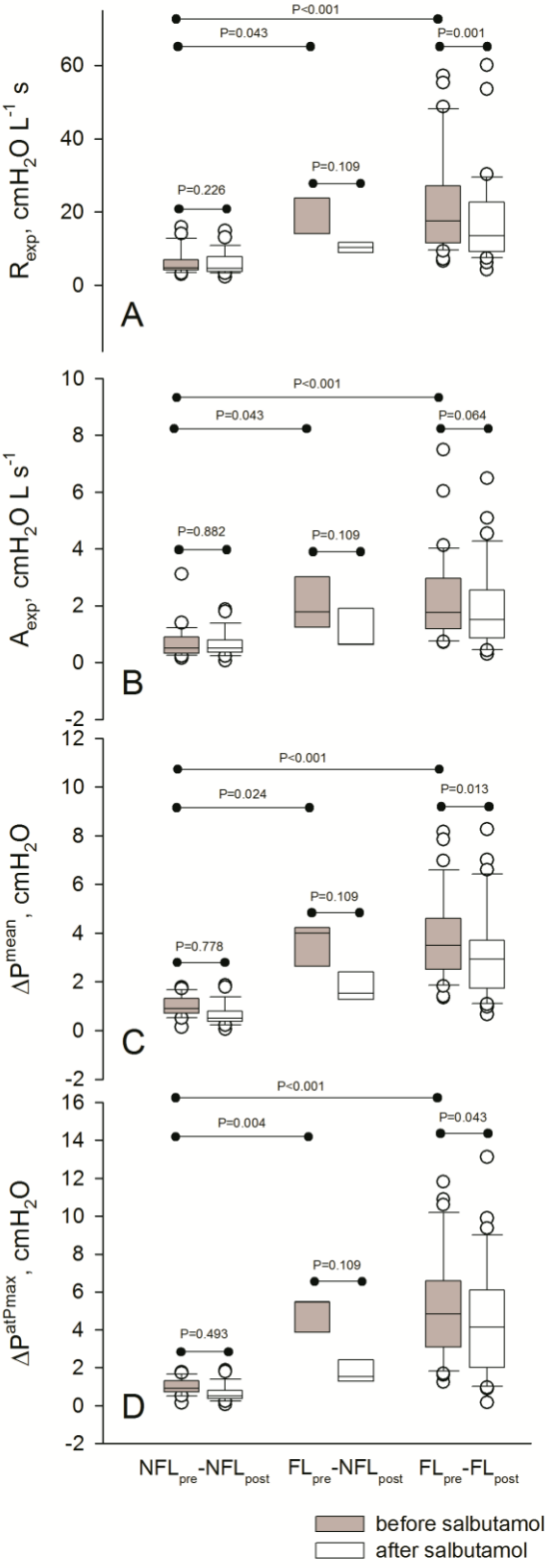
P<0.001
P=0.043
P=0.001
P=0.109
P=0.226
R_{exp}, $cmH_2O\ L^{-1}\ s$
A
P<0.001
P=0.043
P=0.064
P=0.109
P=0.882
A_{exp}, $cmH_2O\ L\ s^{-1}$
B
P<0.001
P=0.024
P=0.013
P=0.109
P=0.778
ΔP^{mean}, cmH_2O
C
P<0.001
P=0.004
P=0.043
P=0.109
P=0.493
ΔP^{atPmax}, cmH_2O
D
NFL_{pre}-NFL_{post}
FL_{pre}-NFL_{post}
FL_{pre}-FL_{post}
before salbutamol
after salbutamol

Figure 3

SUPPLEMENT

TIDAL EXPIRATORY FLOW-LIMITATION INDUCES EXPIRATORY LOOPING OF THE ALVEOLAR PRESSURE-FLOW RELATION IN COPD PATIENTS

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Supplemental methods

Experimental sequence: recording of flow and shift volume during spontaneous breathing

Static and dynamic lung volumes, together with specific airway resistance (sR_{aw}) were measured with a constant-volume plethysmograph (MasterScreen Body Plethysmograph, Erich Jaeger GmbH, Würzburg, Germany), following the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (1). In particular, the time-course of flow and shift volume were recorded for each of ten consecutive breaths during the plethysmographic measurement of sRaw. The sequence of the maneuvers required for this measurement is similar to that required for the plethysmographic assessment of intrathoracic gas volume (ITGV) and is described in details in (1). Briefly, after an adequate warm-up period and calibration of the plethysmograph, the levels of the mouthpiece and of the seat were adjusted so that the patient was able to seat comfortably without the need to flex or extend the neck. Thereafter, a detailed explanation of the procedure was given, the door of the plethysmograph was closed, and time allowed for temperature to stabilize. The patient, wearing a noseclip, was then instructed to attach to the mouthpiece and to breath quietly. At this point the plethysmograph started displaying automatically the time-course of volume and the shift-volume plots. When a stable breathing pattern was reached, the operator activated the shutter at end-expiration for ITGV measurement. Immediately the system automatically stores the time-course of flow, volume and shift-volume of preceding ten breaths. Upon reopening of the shutter, the patient was invited to perform a a slow exhalation to residual volume followed by a slow inflation to total lung capacity.

The tracings corresponding to the last ten breaths before the occlusion were exported as ASCII, together with the measured value of ITGV and of environmental parameters (ambient pressure, temperature and water vapor saturation).

Supplemental results

Fig. S1 shows the flow of patients through the study.

76 patients were considered for eligibility. Of these 9 were excluded because they met the exclusion criteria. Of the 67 remaining patients, 5 did not give the informed consent, in three cases because of claustrophobia. In two cases, NEP malfunctioning prevented the execution of the test. The two subjects were therefore excluded from the analysis.

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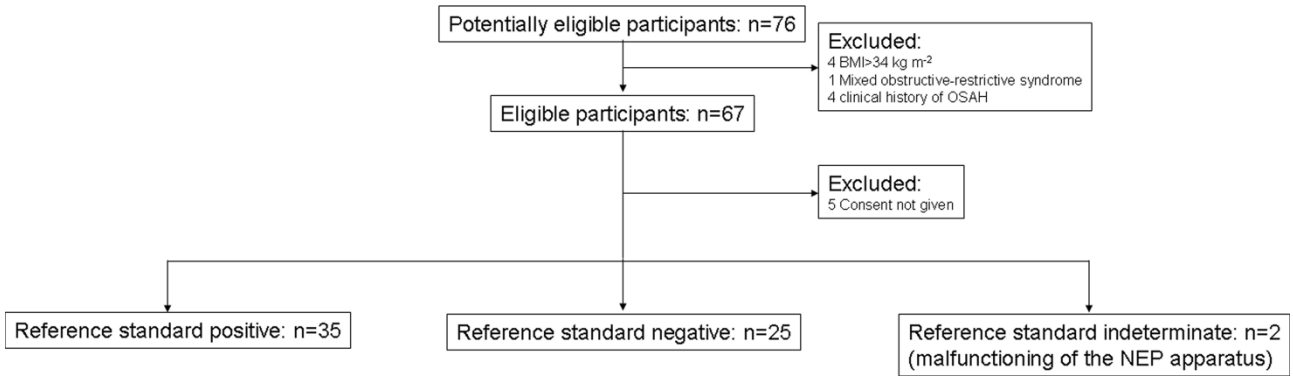

Figure S1: Flow of patients through the study.

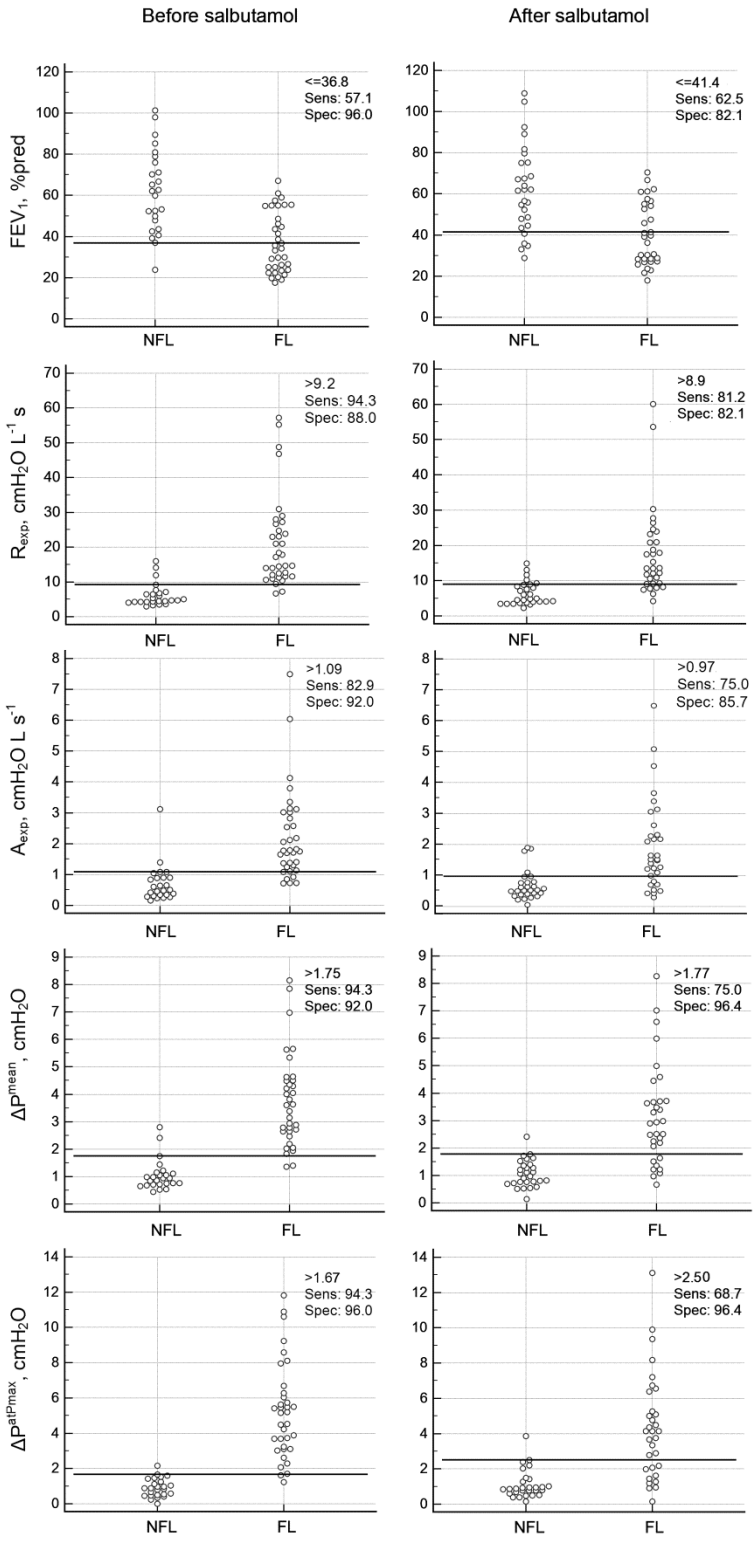

Figure S2: FEV_1, R_{exp}, A_{exp}, ΔP^{mean} and ΔP^{atPmax} in stable COPD patients who were non flow-limited (NFL) or flow-limited (FL) before and after salbutamol inhalation. The threshold which best discriminates between NFL and FL patients has been calculated according to maximum Youden index. The corresponding sensitivity (sens) and specificity (spec) are indicated.