

## How to interpret reduced FEV<sub>1</sub>/VC ratio with normal FEV<sub>1</sub>

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### SHORT TITLE

Reduced FEV<sub>1</sub>/VC ratio with normal FEV<sub>1</sub>

## **ABSTRACT**

Whether the combination of low FEV<sub>1</sub>/VC ratio with normal FEV<sub>1</sub> represents a physiological variant or a sign of early airflow obstruction is unknown.

We studied 40 subjects presenting with low FEV<sub>1</sub>/VC but FEV<sub>1</sub> within the range of normality predicted by ERS reference equations and 10 healthy controls. All subjects completed two questionnaires and underwent comprehensive pulmonary function testing , which included methacholine challenge and single-breath nitrogen wash-out.

According to the questionnaires, the subjects were assigned to three groups, *i.e.* rhinitis (n=8), bronchial asthma (n=13) and COPD (n=12). Subjects with negative responses to questionnaires were assigned to an asymptomatic group (n=7). Airway hyperresponsiveness was found in 4 subjects of the rhinitis group, all of the asthma group, 10 of the COPD group and was associated in the last two groups with signs of increased airway closure and gas trapping. Bronchodilator response to salbutamol was positive in only few individuals across groups. In the subjects of the asymptomatic group, no significant functional changes were observed, possibly suggesting dysanaptic lung growth.

In subjects with low FEV<sub>1</sub>/VC and normal FEV<sub>1</sub>, questionnaires on respiratory symptoms together with additional pulmonary function tests may help to clarify the nature of this pattern of lung function.

**KEYWORDS:** Atopic rhinitis, bronchial asthma, chronic obstructive pulmonary disease, dysanaptic lung growth, methacholine challenge, single-breath nitrogen wash-out

## INTRODUCTION

The assumption that a decrease in forced expiratory volume in one second ( $FEV_1$ ) and its ratio to vital capacity (VC) below the 5<sup>th</sup> percentile of predicted normal value indicates an obstructive pulmonary abnormality is a useful simple approach [1]. However, in some individuals with VC normal or higher than normal, the  $FEV_1/VC$  ratio may lie below the normal range while the  $FEV_1$  is still above the lower limit of normality [2-5]. Whether this spirometric pattern represents a physiological variant, possibly due to dysanaptic lung growth [6-8], or an early sign of airflow obstruction, possibly due to increased airway resistance [9] or loss of elastic recoil [10], is unknown. As the treatment of obstructive pulmonary diseases is based on proper recognition of airflow obstruction [11, 12], the interpretation of this functional pattern is of practical relevance.

This study was designed to investigate whether a careful assessment of respiratory symptoms, combined with tests sensitive to abnormalities of airway function, may help interpret the pattern of low  $FEV_1/VC$  ratio with normal  $FEV_1$ . For this purpose, 40 subjects presenting with an  $FEV_1/VC$  ratio below and an  $FEV_1$  above their lower limits of normality as from the European Respiratory Society (ERS) predicting equations [13] were studied. Symptoms were assessed by questionnaires and lung function by additional tests including measurement of lung volumes, single-breath diffusing capacity of the lung for carbon monoxide (DL,CO), bronchodilator response to salbutamol, methacholine (MCh) bronchial challenge and single breath nitrogen wash-out (SBN<sub>2</sub>W-O).

## **MATERIALS AND METHODS**

### ***Subjects***

Forty consecutive Caucasian subjects presenting with an FEV<sub>1</sub>/VC ratio below the lower limit of normality and FEV<sub>1</sub> within the ERS predicted normal range [13] were recruited from 1,386 workers (1,136 males and 250 females, 76% white-collars) referred to the Unit of Preventive and Occupational Medicine of San Martino University Hospital (Genoa, Italy) to be spirometrically tested for pre-employment or surveillance purposes. Of the remaining subjects, 1,269 showed a normal spirometry, 72 an obstructive and 5 a restrictive abnormality confirmed by lung volume measurements. None of the forty subjects suffered from known cardio-pulmonary or systemic diseases. Ten healthy volunteers with both FEV<sub>1</sub>/VC and FEV<sub>1</sub> within the normal range were recruited from the hospital staff to serve as control group. The study was approved by the institutional ethic committee of San Martino University Hospital and written informed consent was obtained prior to the study.

### ***Study design***

At first visit, the selected subjects completed two questionnaires (online supplementary data). One was administered by an occupational physician and focused on work-related air pollution [14] and habitual physical activity [15], the other was self-administered and focused on symptoms of atopic rhinitis, bronchial asthma [16, 17] and chronic obstructive pulmonary disease (COPD) [18]. A total score was computed for each disease by grading symptoms according to their temporal frequency. It ranged from 0 to 30 for asthma, 0 to 6 for COPD and 0 to 9 for atopic rhinitis. Control subjects (with normal FEV<sub>1</sub>/VC ratio and FEV<sub>1</sub>) showed a total score equal to zero for all the three sections of self-administered questionnaire. Subjects with a score  $\leq 2$  for all questionnaire sections were assigned to an asymptomatic group, subjects with a

score  $\geq 3$  for asthma to an asthma group, smokers  $\geq 20$  pack/years and a score  $\geq 3$  for COPD to a COPD group, subjects with scores  $\geq 3$  for rhinitis but  $< 3$  for asthma and COPD to a rhinitis group.

Absolute lung volumes and spirometry were measured before and after inhaling 400  $\mu\text{g}$  of salbutamol through a valved-holding chamber [19]. Approximately one week after the first visit, the subjects underwent a bronchial challenge with MCh.

### ***Lung function measurements***

Standard spirometry was obtained by a mass flowmeter (VIASYS-SensorMedics Inc., Yorba Linda, CA, USA) with numerical integration of the flow signal [19]. DL<sub>CO</sub> was measured (Vmax22D, VIASYS-SensorMedics Inc.) at least in duplicate [20].

Absolute lung volumes were measured by a transmural whole-body plethysmograph (V62J, VIASYS-SensorMedics Inc.) [21]. Following thoracic gas volume measurement, the subjects resumed regular breathing and performed a forced expiration from about 70% FVC (partial expiratory manoeuvre). Soon after and without disconnecting from the circuit, they took a fast deep breath to total lung capacity (TLC) and, without hesitation, performed a maximal forced expiratory manoeuvre of at least 6 s and until a flat volume-time plateau was achieved (maximal expiratory manoeuvre) [22, 23]. Then, without coming off the mouthpiece, they resumed tidal breathing and performed a slow inspiratory VC (IVC) manoeuvre. From this set of manoeuvres functional residual capacity, TLC, residual volume (RV), and maximal ( $\dot{V}_{\text{max}}$ ) and partial ( $\dot{V}_{\text{part}}$ ) forced expiratory flows at 40% control FVC were measured (fig.1) [24]. The reported value of FEV<sub>1</sub>/VC is the one calculated by using the largest of the technically acceptable IVCs or FVCs [19].

An SBN<sub>2</sub>W-O test [25] was performed by using a Vmax22D (VIASYS-SensorMedics Inc.). After at least 4 regular breaths, the subjects were asked to fully expire to RV and then to take an IVC of 100% O<sub>2</sub>. This was followed, without breath-hold, by a full expiration to RV at a rate of 0.30-0.50 L·s<sup>-1</sup>. Expiratory N<sub>2</sub> concentration was plotted against VC and the slope of N<sub>2</sub> alveolar plateau (phase III) calculated by drawing the best-fit line. The first departure from this straight line exceeding cardiogenic oscillations was taken as the onset of phase IV. The open capacity (OC) was calculated as the difference between TLC and the volume at which phase IV (closing capacity) began [26]. The slope of phase III and OC were measured at least in triplicate and the mean value retained for analysis. The results were expressed as percent of predicted [25].

### ***MCh challenge***

Aerosols of MCh chloride solutions (0.2, 1 and 6%) were delivered *via* a DeVilbiss 646 nebuliser attached to a KoKo (Rosenthal-French) breath-activated dosimeter (Ferraris, Louisville, CO, USA). Aerosols were inhaled during quiet tidal breathing in the sitting position [27]. Increasing doses of MCh from 40 to 4,800 µg were inhaled until a decrease of FEV<sub>1</sub> ≥ 20% from control was achieved. FVC, FEV<sub>1</sub>, V<sub>max</sub> and V<sub>part</sub> were measured only once at each step to avoid the effects of full lung inflation on airway caliber. The dose of MCh causing an FEV<sub>1</sub> decrease of 20% (PD<sub>20</sub>FEV<sub>1</sub>) was determined by interpolating between two adjacent points of the log dose-response curve. If the FEV<sub>1</sub> decrease was <20% of control, the last dose (4,800 µg) was retained as PD<sub>20</sub>FEV<sub>1</sub>. The extent of gas trapping during induced bronchoconstriction was estimated from slope and y-intercept of the simple regression analysis of all FVC values plotted against the corresponding FEV<sub>1</sub> values [28, 29]. The effects of deep inspiration (DI) during constriction were estimated by the slope and y-intercept of simple regression analysis of V<sub>max</sub>

values plotted against the corresponding  $V_{part}$  values [23]. As opposed to  $V_{max} / V_{part}$  ratio, the regression of  $V_{max}$  versus  $V_{part}$  is independent of thoracic gas compression volume [30].

### ***Statistical analysis***

PD<sub>20</sub>FEV<sub>1</sub> values were log-transformed before analysis. Data are presented as mean±SD. A generalized linear model was used for comparisons of the data between the 40 subjects with a low FEV<sub>1</sub>/VC ratio and normal FEV<sub>1</sub> and controls.

A mixed between-within-groups analysis of variance (ANOVA) with Duncan's *post hoc* comparisons was used to assess the significance of differences between categories of subjects with abnormally low FEV<sub>1</sub>/VC and controls. Chi-squared and Fisher's exact test were used in the analysis of categorical data. Values of  $p < 0.05$  were considered statistically significant. The computations were performed with SAS software package (version 8.2; SAS Institute Inc., Cary, NC).

## **RESULTS**

### ***Baseline condition***

The main anthropometric and life-style parameters were not significantly different between the whole group (n=40) with a low FEV<sub>1</sub>/VC ratio and normal FEV<sub>1</sub> and the control group. In the former, the % predicted FEV<sub>1</sub> and DL<sub>CO</sub> were slightly less than in controls (98±11% versus 113±9%;  $p < 0.001$  and 105±19% versus 118±11%;  $p = 0.048$ , respectively).

Based on questionnaires, only 7 subjects with a low FEV<sub>1</sub>/VC ratio and normal FEV<sub>1</sub> did not reach the pre-set minimum threshold of symptoms score to be considered abnormal and were included in the asymptomatic group (table 1). Of the remaining 33 subjects, 8 were assigned to rhinitis group, 12 to bronchial asthma group and 13 to COPD group. Of the 13 subjects assigned

to COPD group, 12 had also a score =3 for asthma but the COPD score was prevalent. Four subjects of the asthma group and 5 subjects of the COPD group had also positive rhinitis scores.

Anthropometric characteristics, life-style habits, work-related airborne irritants, or aerobic physical activity were similar between groups. The % predicted FEV<sub>1</sub> was slightly though significantly less (ranging from 102±7% to 97±12%; p=0.006) than in control group (113±9%) while all other spirometric parameters were not significantly different (p=0.61 and p=0.81 for IVC and FVC % of predicted, respectively) (table 2). The RV/TLC ratio in COPD group was slightly but significantly higher (0.34±0.06; p<0.001) than in any other group. The slope of phase III was in COPD significantly (p=0.001) higher (207±97%) than in other groups, whereas OC was less in both asthma and COPD (94±8% and 90±8%, respectively; p=0.011), thus suggesting a greater tendency for airway closure (fig. 2).

### ***Bronchodilator response***

On average, the FEV<sub>1</sub>, IVC, FVC and lung volumes remained unchanged after inhaling salbutamol in all groups. Exceptions were observed in one subject of the control and asymptomatic groups, two of the asthma group, and three of the COPD group, in whom the FEV<sub>1</sub> increased more than 12% and 200 mL of baseline. Interestingly, the FEV<sub>1</sub>/VC ratio was normalized after salbutamol in 4 subjects each of rhinitis, asthma, and COPD groups and in 2 of the asymptomatic group. Post-bronchodilator changes in  $\dot{V}_{max}$  were not significantly different among groups (p=0.91) whereas  $\dot{V}_{part}$  % showed a tendency (p=0.070) to increase more after salbutamol in rhinitis (56±29%) and asthma (64±52%) groups of subjects as compared to COPD (30±41%).



### ***MCh challenge***

At the second visit, the FEV<sub>1</sub>/VC at presentation was still below normal range in all subjects, thus confirming the repeatability of the parameter. All subjects of the asthma group had a cumulative PD<sub>20</sub>FEV<sub>1</sub> <800 µg (range 31-300 µg) consistent with airway hyperresponsiveness (fig. 3). This was also observed in 4 subjects with rhinitis and 10 with COPD. In the latter, the slope of FVC *versus* FEV<sub>1</sub> was significantly >1 (p=0.048) and steeper (1.24±0.40; p=0.002) than in any other groups (fig. 4), suggesting that all of the fall in FEV<sub>1</sub> was due to the decrease in FVC, *i.e.* to air trapping. Moreover, the y-intercept was lower (1.19±0.82; p=0.013) than in asymptomatic and rhinitis groups. Similarly, the y-intercept of  $\dot{V}_{max}$  *versus*  $\dot{V}_{part}$  in COPD was lower (0.28±0.14; p=0.006) than in control and rhinitis groups, suggesting a reduced bronchodilator effect of DI.

### **DISCUSSION**

The present study was conceived to investigate whether a low FEV<sub>1</sub>/VC ratio with an FEV<sub>1</sub> within the predicted normal range, may represent a physiological variant or an early sign of obstructive abnormalities. Our findings suggest that routine lung function tests are of little help to resolve this issue. In contrast, in most (33/40) of these individuals, the use of clinical questionnaires of symptoms with additional tests of airway mechanics such as bronchodilator and bronchoconstrictor responses and SBN<sub>2</sub>W-O, revealed abnormalities consistent with early airflow obstruction. In the remaining few subjects (7/40), who were classified as asymptomatic by respiratory questionnaires, the results of additional lung function tests were within normal ranges and indistinguishable from those of the control group, except for a borderline airway hyperresponsiveness to MCh in one subject with a symptoms score of zero.

In agreement with current guidelines [11, 12], an obstructive respiratory disease is diagnosed when symptoms are confirmed by appropriate functional tests. If, in theory, such statement is sound and represents the basis of our daily clinical practice, things may be not so straightforward when the disease is at its initial stage and/or the functional tests show borderline values. This is exactly the case of our study. In an attempt to shed light on this problem, we combined the results of clinical questionnaires with additional pulmonary function tests.

As for the choice of the functional tests, we used lung volumes, DL<sub>CO</sub> and the response to the bronchodilator and bronchoconstrictor agents. The latter was slightly modified to examine the response of the airways to DI, as repeatedly reported in bronchial asthma [31, 32] and COPD [22, 23, 33]. We also included the SBN<sub>2</sub>W-O by virtue of its high sensitivity to detect inhomogeneous distribution of ventilation [34].

The DL<sub>CO</sub> measurements did not reveal any significant differences between groups with low FEV<sub>1</sub>/VC ratio. The MCh challenge documented the presence of airway hyperresponsiveness in all subjects with a history of bronchospasm, thus confirming the results of the questionnaire. Surprisingly, however, we did not see significantly different responses to the DI in these asthmatics, as previously reported [31, 32]. In an attempt to explain such an unexpected finding, we postulate that at the transition from health to disease and with normal or near normal lung function, the bronchodilator effect of DI may still be preserved [31, 32]. In COPD, we observed a high rate of airway hyperresponsiveness and evidence of increased airway closure with gas trapping both at rest (increased RV/TLC and decreased OC) and after exposure to MCh (increased slope and decreased y-intercept of the FVC *versus* FEV<sub>1</sub> regression), as well as signs of impaired bronchodilation either with salbutamol (low  $\Delta$ FEV<sub>1</sub> and  $\Delta$ V<sub>part</sub> as % of control) or DI during the bronchial challenge (reduced y-intercept of the V<sub>max</sub> *versus* V<sub>part</sub> regression). With the assumption that these subjects were properly assigned to the COPD group, our findings

would suggest that increased airway closure with gas trapping and impaired response to large inflation or bronchodilator agents are already part of the early stages of the disease [22, 23].

Finally, we observed an increase in airway responsiveness in about half of the rhinitis group with no other functional alterations. This is consistent with the early stages of asthma being associated with an increased response to a constrictor agent without necessarily causing respiratory symptoms [35].

As for the group with a low FEV<sub>1</sub>/VC ratio but normal FEV<sub>1</sub> and without any history of respiratory diseases or symptoms, we did not observe any abnormalities of the functional tests, except one subject in whom the response to MCh and salbutamol was slightly abnormal. If on one hand, our examination does reasonably exclude the presence of early obstructive lung diseases in these subjects, on the other hand it does not help to explain the pattern. For instance, we could not see any differences from the control group as for gender, age, height, BMI, occupation and exercise activity. The pattern may have been caused by an asynchronous development of airways and air spaces during the early stages of life, as previously described as dysanaptic lung growth [6-8]. That is, in some individuals the lung parenchyma could increase during growth disproportionately to the airways as a result of various natural events or disease conditions occurring before definite maturation of the respiratory system.

As for the rhinitics with increased airway responsiveness, the functional pattern could have been caused by an imbalance between increased force generation capacity of the airway smooth muscle [35] and basal membrane thickness as a result of persistent allergen exposure [36]. Even though this is likely part of a remodeling process, there is no evidence suggesting that this might be a risk factor for exaggerated lung function decline. As for the rhinitics with normal response to MCh, the pattern could have been due to the same mechanisms discussed for the

asymptomatic subjects. In fact, 6 out of 8 subjects of rhinitis group may have been undergoing an excessive exercise-induced stress/strain imposed on the alveolar septa during growth [37].

More complicated appears to be the clinical interpretation of the reduced FEV<sub>1</sub>/VC ratio and normal FEV<sub>1</sub> in the asthma and COPD subjects. Under these conditions, the possibility exists that the decrease in FEV<sub>1</sub> was a sign of accelerated decline in lung function in subjects with initial spirometric values higher than normal. In smokers, CORBIN *et al.* [10] found a significant increase of TLC and VC due to loss of lung elastic recoil while FEV<sub>1</sub> only tended to decrease, thus resulting in a decrement of FEV<sub>1</sub>/VC preceding the decrease of FEV<sub>1</sub>. In the absence of pressure-volume measurements, we can only speculate that this mechanism might have contributed to our findings in the COPD group.

We recognize that our study has some limitations. First, we used the predicting equations published by the ERS [13, 38], mainly because they are still the most largely used in Europe [1]. The values of VC and FEV<sub>1</sub> obtained in our control group were mostly >100% of predicted, suggesting that the predicted values were somehow underestimated. Supposedly, the use of different reference equations might have resulted in an FEV<sub>1</sub> falling below normal range in some of our subjects, thus leading to a diagnosis of airflow obstruction *tout court*. When predicted values were re-calculated by using predicting equations recently derived from a large Caucasian American population [39] or a local population of northern Italy [40], in only one subject of the COPD the FEV<sub>1</sub> resulted to be slightly below the lower limits of normality (9 and 6%, respectively). Exclusion of this subject did not abolish the significance of differences between groups. Second, we used questionnaires that had already been validated [14-18] but a cutoff >2 for positive responses was arbitrarily chosen. This was done to seek for high specificity, but could have resulted in a low sensitivity. However, only one subject assigned to the asymptomatic group had a symptom score for asthma =2, but the disease was reasonably excluded by a lack of

response to MCh. Third, there were overlaps of either symptoms or lung function particularly between asthma and COPD groups, but this does not invalidate the conclusion that the low FEV<sub>1</sub>/VC ratio in these subjects may be a marker of airflow obstruction even though the FEV<sub>1</sub> is still normal.

In summary, in subjects with a reduction of FEV<sub>1</sub>/VC as the only spirometric abnormality, lung volumes measurement, reversibility or challenge tests, SBN<sub>2</sub>W-O and appropriate questionnaires may help assist in detecting an early obstructive abnormality. In the case of positive history for bronchial asthma and significant responses to bronchodilator and/or bronchoconstrictor agents or in heavy smokers with signs of airway closure at baseline or after induced bronchoconstriction, the pattern is highly suggestive of airflow obstruction. By contrast, in subjects with rhinitis or no respiratory symptoms and normal or slightly increased bronchodilator or bronchoconstrictor responses, the pattern is presumably due to either initial airway remodeling or dysanaptic lung growth.

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## LEGENDS

FIGURE 1. Representative tidal, partial, and maximal flow-volume curves at baseline in a subject with low FEV<sub>1</sub>/VC ratio and normal FEV<sub>1</sub>. Vertical dashed line is drawn at 40% forced vital capacity (FVC) to indicate the point where instantaneous maximal ( $\dot{V}_{\max}$ ) and partial ( $\dot{V}_{\text{part}}$ ) expiratory flows were measured.

FIGURE 2. Slope of phase III and open capacity (OC) as % of predicted of the single-breath nitrogen wash-out. Values are mean  $\pm$  SD. *Open columns* are control group, *dotted columns* asymptomatic, *light grey columns* rhinitis, *dark grey columns* asthma, and *black columns* COPD. \*: p=0.001 *versus* all other groups; †: p=0.011 *versus* rhinitis; ‡: p=0.011 *versus* control, asymptomatic, and rhinitis.

FIGURE 3. Mean values ( $\pm$  SD) of log-transformed dose of methacholine causing a 20% decrease of forced expiratory volume in one second (MCh PD<sub>20</sub>FEV<sub>1</sub>). Columns are as in fig. 2. \*: p<0.001 *versus* all other groups.

FIGURE 4. Mean linear regression analysis of absolute values (L) of forced vital capacity (FVC) *versus* FEV<sub>1</sub> and of instantaneous maximal ( $\dot{V}_{\max}$ ) and partial ( $\dot{V}_{\text{part}}$ ) flows (L·s<sup>-1</sup>) at 40% of control FVC at each step of methacholine challenge in the five groups. By regressing FVC against FEV<sub>1</sub> values, an increase of slope or a decrease of y-intercept suggests an enhanced gas trapping and *vice versa*. Similarly, an increase of slope or a decrease of y-intercept of  $\dot{V}_{\max}$  *versus*  $\dot{V}_{\text{part}}$  values suggests a reduced bronchodilator effect of deep inspiration. In each panel, the line of identity is shown as dotted grey. See text for statistical differences among groups.

**TABLE 1. Main anthropometric, life-style parameters and symptoms of the groups of subjects**

<b>Parameters</b>	<b>CTRL</b> (n = 10)	<b>Asymptomatic</b> (n = 7)	<b>Rhinitis</b> (n = 8)	<b>Asthma</b> (n = 12)	<b>COPD</b> (n = 13)
Age, yrs	45 ± 12	45 ± 17	34 ± 6	36 ± 14	57 ± 9
Gender, M/F	8/2	7/0	8/0	11/1	7/6
Height, cm	171 ± 8	175 ± 8	178 ± 5	172 ± 8	168 ± 12
BMI, kg·m <sup>-2</sup>	23 ± 2	25 ± 3	24 ± 3	24 ± 3	26 ± 3
Smoking habit, pack/years	3 ± 4	3 ± 4	4 ± 5	1 ± 4	35 ± 9*
Work-related airborne irritants (score = 2)	0	2	2	2	2
Aerobic physical activity (score = 2)	8	2	6	7	3
Symptoms score range					
Rhinitis	0-0	0-2	4-9	1-8	0-9
Bronchial asthma	0-0	0-2	0-3	4-18	3-18
COPD	0-0	0-0	0-1	0-1	4-6

CTRL: control group; M/F: male/female; BMI: body mass index. Comparisons were made using the ANOVA or chi-squared (exact Fisher's test). Values are expressed as means ± SD or absolute numbers of subjects for work-related airborne irritants, aerobic physical activity, and range of symptoms score. \*: p<0.001 *versus* all other groups.

TABLE 2. Main spirometric and volumetric data of the groups of subjects

Parameters	CTRL	Asymptomatic	Rhinitis	Asthma	COPD	ANOVA (p-value)
IVC, % pred	118 ± 13	120 ± 16	118 ± 13	122 ± 14	126 ± 14	0.61
FVC, % pred	118 ± 15	122 ± 19	120 ± 7	124 ± 16	126 ± 14	0.81
$t_{FE}$ , s	8.5 ± 2.1	9.7 ± 3.4	9.1 ± 1.7	9.0 ± 1.9	12 ± 4	0.080
FEV <sub>1</sub> , % pred	113 ± 9*†‡§	98 ± 14*	102 ± 7†	100 ± 12‡	97 ± 12§	0.006
ΔFEV <sub>1</sub> , %	4 ± 7	8 ± 4	6 ± 3	11 ± 6	8 ± 7	0.082
FEV <sub>1</sub> /VC	0.77 ± 0.05*†‡§	0.64 ± 0.05*	0.67 ± 0.02†	0.63 ± 0.04‡	0.60 ± 0.05§	<0.001
$\dot{V}_{max}$ , L·s <sup>-1</sup>	1.80 ± 0.58	1.70 ± 0.60*	2.31 ± 0.60*†	2.02 ± 0.67	1.03 ± 0.28†	<0.001
Δ $\dot{V}_{max}$ , %	31 ± 50	32 ± 8	19 ± 9	26 ± 18	28 ± 18	0.91
$\dot{V}_{part}$ , L·s <sup>-1</sup>	2.44 ± 0.85	1.95 ± 0.65	2.43 ± 2.34	2.09 ± 0.76	1.60 ± 0.70	0.15
Δ $\dot{V}_{part}$ , %	37 ± 39	37 ± 50	56 ± 29	64 ± 52	30 ± 41	0.070
TLC, % pred	108 ± 10	112 ± 11	112 ± 9	115 ± 13	116 ± 7	0.38
FRC, % pred	98 ± 17	105 ± 23	109 ± 21	107 ± 17	109 ± 18	0.68
RV, % pred	91 ± 18	100 ± 12	94 ± 21	100 ± 29	111 ± 18	0.24
RV/TLC	0.26 ± 0.05*	0.27 ± 0.07†	0.22 ± 0.05‡	0.24 ± 0.06§	0.34 ± 0.06*†‡§	<0.001
DLCO, % pred	118 ± 11	104 ± 9	106 ± 15	112 ± 28	99 ± 14.6	0.14
$V_{A, sb}/V_{A, plethys}$	0.99 ± 0.09	0.92 ± 0.06	0.96 ± 0.07	0.93 ± 0.07	0.92 ± 0.07	0.096

CTRL: control group; IVC: slow inspiratory vital capacity; FVC: forced vital capacity;  $t_{FE}$ : forced expiratory time; FEV<sub>1</sub>: forced expiratory volume in one second;  $\dot{V}_{max}$  and  $\dot{V}_{part}$ , maximal and partial forced expiratory flows measured at the same absolute lung volume at 40% of baseline FVC;  $\Delta FEV_{1, \Delta \dot{V}_{max}}$  and  $\Delta \dot{V}_{part}$ , %: percent change from baseline of the relevant parameter 30 min after inhaling 400  $\mu$ g of salbutamol; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; DL<sub>CO</sub>: single-breath diffusing capacity of the lung for carbon monoxide;  $V_{A, sb}/V_{A, plethys}$ : single-breath alveolar volume/plethysmographic alveolar volume ratio. Values are expressed as means  $\pm$  SD. \*†‡§: Set of symbols indicate statistically significant differences between groups by Duncan's *post hoc* analysis. Statistical details are present in the RESULT section.

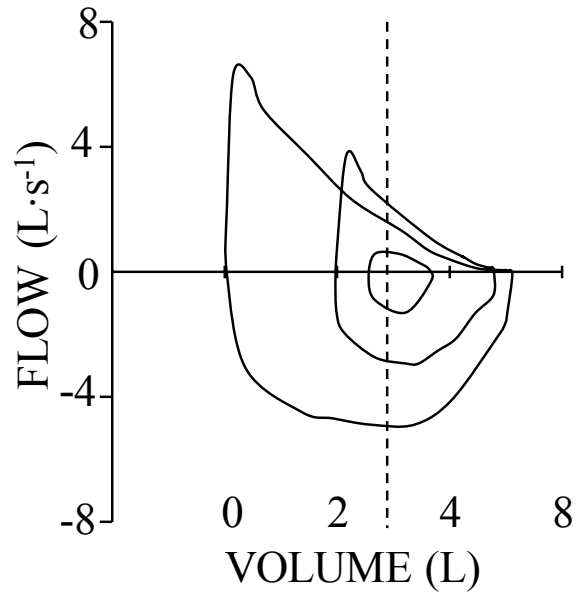


FIGURE 1

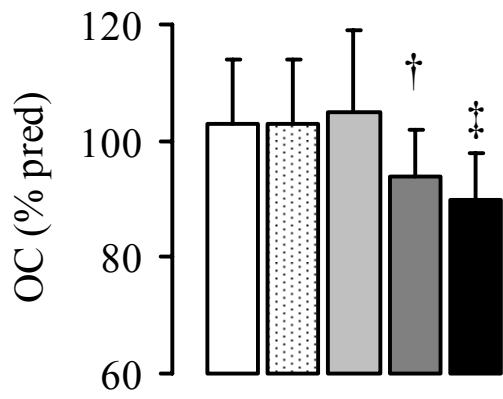
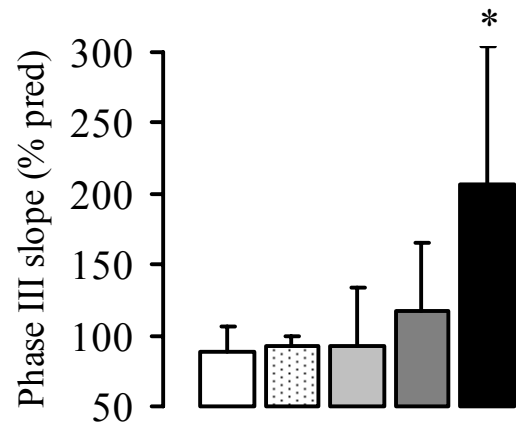


FIGURE 2

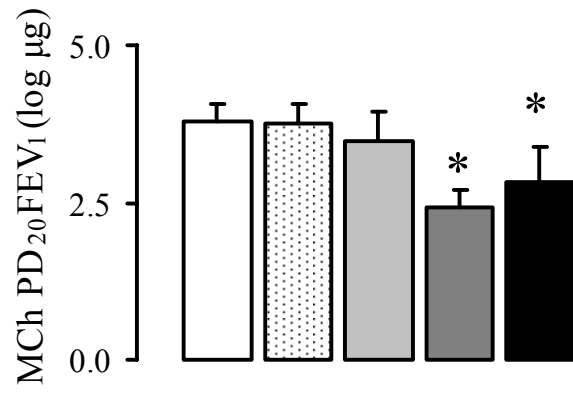


FIGURE 3



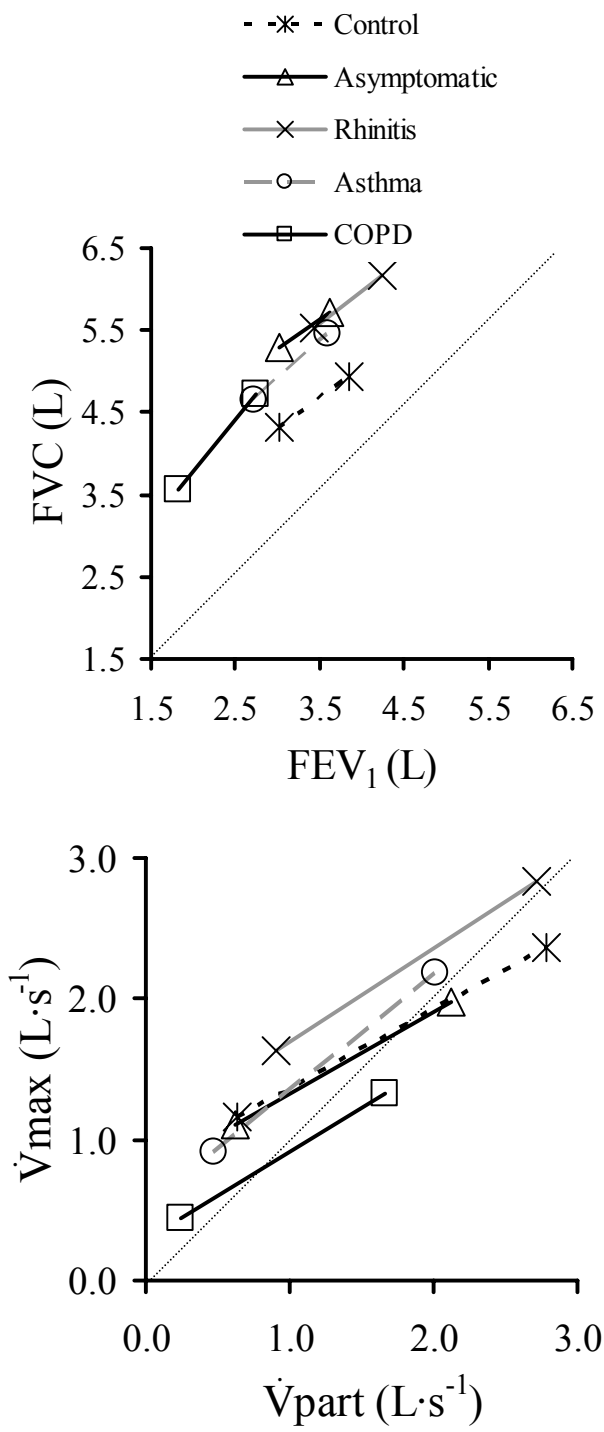


FIGURE 4