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Normal Limits For Oscillometric Bronchodilator Responses and Relationships With Clinical Factors

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ABSTRACT

Introduction: We aimed to determine normal thresholds for positive bronchodilator responses for oscillometry in an Australian general population sample aged ≥40 years, to guide clinical interpretation. We also examined relationships between bronchodilator responses and: respiratory symptoms, asthma diagnosis, smoking and baseline lung function.

Methods: Subjects recruited from Sydney, Melbourne and Busselton, Australia underwent measurements of spirometry, resistance (Rrs_6) and reactance (Xrs_6) at 6Hz, before and after inhalation of salbutamol 200μg. Respiratory symptoms and/or medication use, asthma diagnosis and smoking were recorded. Threshold bronchodilator responses were defined as the 5th percentile of decrease in Rrs_6 and 95th percentile increase in Xrs_6 in a healthy subgroup.

Results: Of 1318 participants, 1145 (570 female) were analysed. The lower threshold for ΔRrs_6 was -1.38cmH_2O.s.L^{-1} (-30.0% or -1.42 Z-scores) and upper threshold for ΔXrs_6 was 0.57cmH_2O.s.L^{-1} (1.36 Z-scores). Respiratory symptoms and/or medication use, asthma diagnosis and smoking all predicted bronchodilator response, as did baseline oscillometry and spirometry. When categorised into clinically relevant groups according to those predictors, ΔXrs_6 was more sensitive than spirometry in smokers without current asthma or COPD, approximately 20% having a positive response. Using absolute or Z-score change provided similar prevalences of responsiveness, except in COPD in whom responsiveness measured by absolute change was twice that for Z-score.

Discussion: This study describes normative thresholds for bronchodilator responses in oscillometry parameters, including intra-breath parameters, as determined by absolute, relative and Z-score changes. Positive bronchodilator response by oscillometry correlated with clinical factors and baseline function, which may inform clinical interpretation of oscillometry.

INTRODUCTION

Reversibility of airflow obstruction in response to a bronchodilator is a hallmark of asthma and is commonly seen in patients with chronic obstructive pulmonary disease (COPD)[1]. In asthma, bronchodilator responsiveness is useful for diagnosis and is associated with a greater risk of future adverse events[2]. In COPD, bronchodilator responsiveness is only modestly repeatable[1, 3] and correlates with: FEV1 improvement following oral corticosteroids[4]; with response to inhaled corticosteroid/long acting bronchodilator combination treatment (albeit inconsistently)[5, 6]; and with lower risk of hospitalisation and mortality in severe COPD[7]. Although spirometry is the gold standard for bronchodilator testing, it likely misses clinically important responses. For example, reduced hyperinflation to short-acting bronchodilator is greater with more severe COPD, whereas the FEV_1 response is less[8]. Respiratory system impedance measured by oscillometry (also known as forced oscillation technique) is a sensitive way to measure bronchodilator responses. There are currently insufficient data on oscillometric responses to bronchodilator in healthy subjects[9] and consequently, little data on its prevalence in disease.

Oscillometry involves low amplitude pressure oscillations applied to the mouth during tidal breathing. This is an important physiological difference compared to spirometry in which the forced manoeuvre of spirometry may induce airway collapse and expiratory flow limitation, or complete closure. This likely explains the poor correlation between spirometric and oscillometric bronchodilator responses[10]. Respiratory system resistance (Rrs) reflects airway calibre, while reactance (Xrs) reflects predominantly the oscillatory stiffness of respiratory system. Oscillatory stiffness is thus a dynamic stiffness (as opposed to static), and as such, is sensitive to heterogeneously distributed airway narrowing and closure[11-13]. The literature suggests that Rrs and Xrs are more sensitive than spirometry in detecting bronchodilator responses in asthma[14-16],

smokers without COPD[17] and in COPD[18], which also correlate with changes in ventilation distribution seen on lung imaging[19, 20]. However, further data on cut-off values are needed to inform clinical interpretation.

There is a single published study that includes sufficient participants to allow the reliable estimation of cut-off values. This study involved 5 different devices used in healthy populations across 4 countries[21]. While this facilitated applicability of the derived cut-offs, there were some measurement differences between devices, which complicates the interpretation of the values. Thus, we aimed to examine bronchodilator responses using a single device to derive cut-off values for bronchodilator responsiveness, and determine its relationships with respiratory symptoms, asthma diagnosis, smoking and spirometric airflow obstruction, in a general population sample over the age of 40 years. We hypothesised that oscillometry would be more sensitive than spirometry in detecting bronchodilator responses, and that bronchodilator responsiveness measured by oscillometry was related to symptoms, asthma diagnosis, smoking and spirometric airflow obstruction. This study was a retrospective analysis of the data obtained in the Australian arm of the Burden of Obstructive Lung Diseases Study (BOLD), which was part of a multi-centre study of the prevalence of spirometrically determined airflow obstruction[22, 23]. Parts of this analysis have been previously published in abstract form[24] and oscillometry data from the Busselton site have been published[25].

METHODS

Study population

Subjects 40 years or older were recruited randomly from three cities across Australia (Sydney in New South Wales, Melbourne in Victoria, and Busselton in Western Australia). Details on how the study populations were sampled are in the On Line supplement (OLS). Study operations and testing methods were consistent across sites, which were part of the global Burden of Obstructive Lung Disease (BOLD) project[23], with added local tests and questionnaires. The study was approved by the Human Research Ethics Committee of the University of Sydney (ref. no. 12-2006/9724). All subjects gave informed written consent.

Study design

This was a cross-sectional study of oscillometric bronchodilator responses in the BOLD cohort in which the limits of responsiveness in healthy subjects were defined, then applied to the remainder of the cohort to determine the prevalence of increased bronchodilator responsiveness and its relationships with symptoms, Doctor diagnosis of asthma and smoking. All subjects underwent oscillometry and spirometry measurements, in that order, at baseline and 15 minutes after the administration of 200μg salbutamol administered by metered dose inhaler through a spacer. Participants withheld all respiratory medications on the day of testing; short-acting bronchodilator inhalers for ≥6 hours before testing. Respiratory symptoms, medication use, and smoking history were obtained using the BOLD core questionnaire.

To define cut-off values we identified a healthy group ($Health_{Asym}$) as those with no respiratory symptoms or inhaled medications use in the past year, no doctor diagnosis ever of either asthma or

COPD ever, currently not smoking and having less than 10 pack/years past smoking. Spirometry was not used to define this group since we wanted to be consistent with definitions used in studies of normal spirometry, e.g. GLI.. Normative equations for oscillometry parameters were developed from this healthy group, based on previously published methodology[25], from which Z-scores for bronchodilator responsiveness were calculated.

Oscillometry

The oscillometry device was built in-house and has been described previously[26]. A detailed description is presented in the OLS. In brief, a multi-frequency pressure oscillation (6, 11 and 19Hz) was imposed at the mouth. After establishing stable tidal breathing, a single 60second recording was acquired with subjects supporting their own cheeks. Only the impedance parameters at 6 Hz were analysed in this study. Quality control procedures were applied as previously described[27]. Resistance was expressed as the mean across the entire recording (Rrs_6), and also separately for inspiration only (Rrs_6(insp)). Similarly, reactance was expressed as the mean (Xrs_6) and Xrs_6(insp). Mean Xrs_6(insp) – mean expiratory Xrs_6 was calculated as an index of expiratory flow limitation (EFLi)[28]. Z-scores were calculated for each pre- and post-bronchodilator measurement, for each participant, based on age- and sex-specific expected mean and standard deviation values in the $Healthy_{Asym}$ group.

Spirometry

Spirometry was performed according to ATS/ERS Taskforce criteria[29], using an EasyOne Plus hand-held spirometer (NDD Medical Technologies, Andover, MA, USA). All spirograms were reviewed by one study investigator (DPJ) who assigned a standardised quality score. The highest recorded FEV_1 and FVC from acceptable trials were used in the analysis. Prediction equations of the Global Lung Initiative[30] were used.

Bronchodilator responses

The bronchodilator responses (Δ) were calculated as post-bronchodilator values – baseline (pre-bronchodilator) values, and expressed as absolute change, proportional (%) change from baseline, and as changes in Z-scores (derived from the $Health_{Asym}$ group). Since very extreme values of relative ΔXrs occur, even for transformed data, absolute and Z-score changes only were used. A negative ΔRrs_6 indicated decrease (the expected response) hence, the lower limit of normal (LLN) was defined as the 5^{th} percentile of bronchodilator response in the $Health_{Asym}$ group. A positive ΔXrs_6 indicated an increase (the expected response) hence, the upper limit of normal (ULN) was defined as the 95^{th} percentile. Positive bronchodilator responsiveness in either FEV_1 or FVC was defined as an increase of ≥12% and ≥200 ml.

Statistical analyses

The data were analysed using SPSS software (IBM Armonk NY, V21). Paired T-tests and the Wilcoxon-signed rank test were used to compare baseline and post-bronchodilator lung function as appropriate. Natural logarithm and exponential transformations were used to normalise Rrs_6 and Xrs_6 distributions, respectively, in the healthy group. Multiple linear regressions were used to define normative equations as performed previously[25]. Spearman correlations were used to evaluate the relationships between bronchodilator responses and: potential anthropometric predictors (age, sex, height, BMI), baseline lung function, and clinical predictors (respiratory symptoms, asthma diagnosis, smoking history).

RESULTS

From 1318 subjects, 163 were excluded due to incomplete data, 10 had highly disparate Rrs_6 and Xrs_6 values indicating artefact, leaving 1145(86.9%) with complete questionnaire and technically satisfactory pre- and post- bronchodilator FOT and spirometry data. The anthropometric characteristics of the entire cohort are described in Table 1. The anthropometric characteristics of the healthy subgroup of the entire cohort are also shown. A smoking history of ≥10 pack/years was reported by 27.9%(320/1145), while 10.4%(119/1145) had obstructed baseline spirometry and 7.1%(81/1145) had obstructed post-bronchodilator spirometry. Positive spirometric bronchodilator responses occurred in 6.6%(75/1145). The post-bronchodilator spirometry and all oscillometric parameters, were all significantly different compared with baseline (see Figure 2).

Bronchodilator responses in healthy subjects

There were 577 subjects in the $Healthy_{Asym}$ group. Their baseline and post-bronchodilator spirometry and oscillometry parameters are shown in Table E1. This shows that there were minimal but statistically significant bronchodilator associated changes in FEV_1, FVC, FEV_1/FVC ratio, and all oscillometry parameters. The normative equations for oscillometry that were derived from this group to determine Z-scores, are in Table E2 and the normative thresholds for bronchodilator responses in Table 2. The LLN of ΔRrs parameters and ULN for ΔXrs parameters, are provided in Table 2. The bronchodilator responses in all other subjects (Remainder – see Table 2), were significantly different for all oscillometry and spirometry parameters, compared with the $Healthy_{Asym}$ group.

Predictors of bronchodilator responses

The anthropometric (sex, height and BMI), clinical (symptoms, asthma diagnosis, smoking history) and baseline lung function (Rrs_6, Xrs_6 and spirometry Z-scores) predictors of bronchodilator responses, for the entire cohort are shown in Table E3 in the OLS. Males had larger ΔXrs_6 Z-score and ΔFVC, but changes were very small and clinically insignificant. Higher BMI was associated with larger ΔRrs_6, ΔRrs_6(insp), all ΔXrs_6 parameters, and ΔFVC. Asthma diagnosis, and respiratory symptoms were associated with larger bronchodilator responses in all but one of the spirometry and oscillometry parameters (ΔEFLi). Smoking history was associated with larger bronchodilator responses in spirometry in all oscillometry parameters. Greater impairment of baseline oscillometry and spirometry parameters predicted greater bronchodilator responses in all parameters except for FEV_1/FVC not predicting ΔFVC (see Table E3).

In the $Healthy_{Asym}$ group, anthropometric parameters were not predictive of bronchodilator responses (see Tables E4 and E5 of the OLS). However, baseline Rrs_6 and Xrs_6 Z-scores predicted all ΔRrs_6 and ΔXrs_6 parameters (except ΔEFLi and ΔRrs_6(insp) (%) in males). Baseline FEV_1 and FEV_1/FVC Z-scores predicted ΔFEV1 and all ΔRrs_6 parameters in females, while only FEV_1/FVC Z-scores were predictive in males.

Clinical context of bronchodilator responses

Given the above associations with asthma diagnosis, symptoms and/or medication use, smoking history and airflow obstruction, the prevalence of bronchodilator responses were determined for clinical groups based on those parameters, to provide clinical context. Consequently, five mutually exclusive groups were defined: 1) symptomatic non-smokers ($Healthy_{Symp}$ – defined as no asthma diagnosis but reported respiratory symptoms in the last year and <10 pack/years smoking); 2) asymptomatic smokers ($Smokers_{Asym}$ – no respiratory symptoms in the last year, no asthma diagnosis but ≥10 pack/years smoking history and FEV_1/FVC ratio Z-score ≥LLN; 3) symptomatic

smokers (Smokers$_{Symp}$ – same as Smokers$_{Asym}$ but reported respiratory symptoms); 4) non-smokers with current asthma defined as any past asthma diagnosis and reporting respiratory symptoms and/or respiratory medication use in the last year, and <10 pack/years smoking); 5) smokers with fixed airflow obstruction ('COPD' – ≥10 pack/years smoking history and post-bronchodilator FEV_1/FVC ratio Z-score < -1.645). Therefore COPD subjects could have a Dr diagnosis of asthma (present in 20/46 subjects) and Asthma subjects could have abnormal FEV_1/FVC ratio (32/122 and 20/122 had reduced Z-scores pre- and post-bronchodilator, respectively). The definition of these groups is shown in Figure 1.

The anthropometric characteristics of each group are shown in Table E6, their median bronchodilator changes are shown in Table E7, and prevalence of abnormal baseline function in Table E8. The percentage of positive bronchodilator responsiveness in each group are shown in Table 3 and in Figure 2. The proportions of positive bronchodilator responsiveness were compared with that in the Healthy$_{Asym}$ group: The Healthy$_{Symp}$ group were similar; Smokers$_{Asym}$ had more positive bronchodilator responsiveness in ΔXrs_6 (absolute change) and ΔEFLi, but not in spirometry; Smokers$_{Symp}$, had more positive bronchodilator responses in all of the ΔXrs_6 parameters and in ΔFEV_1. In the Asthma and COPD groups, bronchodilator responsiveness was increased for almost all parameters (except ΔRrs_6 (%) and ΔRrs_6 Z-score in COPD). The Asthma and Smokers$_{Symp}$ groups appeared similar in terms of responsiveness in both oscillometry and spirometry. Notably 109/122 (89.3%) of asthmatics reported wheeze in the last 12 months. Bronchodilator responsiveness was greatest in COPD, in ΔXrs_6 expressed as absolute change or ΔEFLi where approximately half exhibited positive responses. Positive ΔFEV_1 responsiveness were also demonstrated in about a third of this group.

Except in the COPD group, the prevalence of responsiveness was similar, for each of the ΔRrs_6 and ΔXrs_6 parameters. In COPD, absolute changes resulted in more positive responders than either

relative or Z-score changes ($p<0.01$, Chi-square), likely due to their more severe baseline obstruction. Also, responsiveness was generally more frequent for Xrs_6 than for Rrs_6 parameters in smoking and disease groups, particularly in COPD (Figure 2 and Table 3). Bronchodilator responsiveness is more prevalent for ΔXrs_6 parameters, compared to ΔFEV_1, in the symptomatic and smoking groups, but not in the Asthma or COPD groups (see Table 4).

DISCUSSION

In summary, we have defined lower and upper limits of bronchodilator responsiveness for Rrs_6 and Xrs_6 parameters, respectively from a healthy, community cohort, which included intra-breath parameters and changes in Z-scores, to allow us to compare bronchodilator responsiveness measured by oscillometry to responsiveness measured by spirometry. Our thresholds for bronchodilator responsiveness measured by oscillometry were almost identical to published values[21] and oscillometry was more sensitive than spirometry, in symptomatic non-smokers and in both symptomatic and asymptomatic smokers who had normal spirometry We also found bronchodilator responsiveness was related to asthma diagnosis, respiratory symptoms, smoking history and baseline airway function (both spirometry and oscillometry). In five clinical groups defined according to those factors, oscillometric bronchodilator responsiveness quantified as absolute, percentage and Z-score changes, produced similar outcomes, except in COPD where responses measured as absolute changes were about twice as frequent as measured by relative or Z-score change. This was likely due to the dependence of bronchodilator responsiveness on baseline values.

Only one of several published studies on bronchodilator responses in healthy subjects [21, 31-33], had sufficient numbers to reliably define upper and lower limits of bronchodilator responses[9, 21], although not for intra-breath parameters and not expressed as changes in Z-scores that we produced. These normal limits were derived from pooled measurements from five different devices, in a

slightly younger population than the present study. Despite these differences, our lower limit of bronchodilator response for Rrs_6 (-1.38 $cmH_2O.s.L^{-1}$ or -30.0%) and upper limit for Xrs_6 (0.57 cmH_20L/s) are practically identical to what is reported in the study of Oostveen et al. (Rrs_6 -1.28 $cmH_2O.s.L^{-1}$ or -31.5% and Xrs_6 0.47 $cmH_2O.s.L^{-1}$). This suggests that these cut-points based on absolute and relative change in mean Rrs_6 and absolute change in Xrs_6 are fairly robust given their consistency across different populations and devices. Although increasing BMI predicted lower bronchodilator response in Rrs_6 in healthy males, it was marginal (see Table E5) and did not predict responses in females. Correction for BMI was therefore not done in determining normal responsiveness.

Bronchodilator responsiveness of all oscillometry parameters (and for ΔFEV_1 and ΔFVC) correlated with baseline values for Rrs_6, Xrs_6 and spirometry. This suggests that bronchodilator responses should be expressed as relative change [34]. However, we did not use relative change for Xrs_6 parameters because of the very large values and highly skewed distribution that resulted from values that were close to zero. However, use of the absolute, relative or Z-score changes in Rrs_6 or Xrs_6 did not affect the prevalence of bronchodilator responders in groups other than COPD, thus these cut-offs could be used interchangeably. In the COPD group, when ΔXrs_6 was expressed as absolute change, positive bronchodilator responsiveness was about twice that responsiveness expressed as Z-score change. This is likely due to the strong dependence of ΔXrs_6, expressed as absolute change, on baseline values. This may be explained by Xrs_6 being sensitive to airway closure and heterogeneous severe narrowing that is common in COPD[11-13, 35, 36]. Even small decreases in bronchoconstriction could alleviate airway severe narrowing and closure, but would have a large effect in improving Xrs_6. Thus, bronchodilator responses should arguably be expressed as either relative or Z-score change, given their dependence on baseline function. However, it is also possible that the high prevalence of bronchodilator responsiveness measured by ΔXrs_6 as absolute change, may be clinically meaningful; this requires further study.

Showing the prevalence of bronchodilator responsiveness by groups was to provide clinical context. Disease or diagnostic labels are arbitrary and our primary aim was not to investigate disease or diagnostic label differences. Nevertheless, there were some clinically interesting observations. The Asthma group was large (10.7%) and despite being a community sample with normal baseline function, oscillometric bronchodilator responsiveness was common in nearly 20%. In the COPD group, bronchodilator responsiveness was present in nearly half according to either ΔXrs_6 or spirometry. This could be related to asthma diagnoses in 20/46 in addition to their smoking; and arguably, they may justifiably have an asthma/COPD overlap label. Although BMI predicted ΔXrs responses in the whole cohort (Table E3), it may be explained by its association with symptoms and smoking since $Healthy_{Symp}$ and both smoking groups were heavier than the reference group (Tables E4 and E5, OLS). Despite their normal spirometry, 18.2% of asymptomatic smokers and 31.3% of symptomatic smokers had abnormal baseline oscillometry (defined as abnormality in either Rrs_6 or Xrs_6). Furthermore, their bronchodilator responsiveness in Xrs_6 parameters (up to 20%) was about twice that of spirometry. There may be value in exploring potential clinical correlations in symptomatic smokers, given the evidence of their increased morbidity [37].

Because of the greater bronchodilator responsiveness in oscillometry in the COPD and Asthma groups, in particular in Xrs, the concordance between the bronchodilator responses measured by oscillometry and spirometry, was only fair ($\kappa = 0.21 – 0.40$) while being poor or slight in the other groups. This is consistent with published studies[10, 21, 38, 39]. In COPD, this difference could possibly be due to oscillometry being more sensitive to smoking-related lung damage, than spirometry. The basis of the differences is unknown but may reflect the different manoeuvres used during spirometry and oscillometry measurements. In a tertiary asthma clinic study[15], κ between spirometry and Xrs_5 was 0.45, perhaps due to a wider range of lung function impairment. However, oscillometric bronchodilator responses related more strongly to asthma control than spirometry[15].

Discordant bronchodilator responsiveness between oscillometry and spirometry could be clinically important, i.e. complementary information, but this also needs further study.

A potential limitation of this study was the participant ages of 40 years or older, which meant that these findings could not be applied to younger subjects. The oscillometry device was a proprietary device and therefore, not used clinically elsewhere and our use of 6Hz and 19Hz is non-standard (usually 5Hz and 19Hz). Theoretically any differences in measurements associated with different frequencies are likely to be insignificant. We have also compared our device with 3 other commercial oscillometry devices and showed that while Rrs_6 was comparable between devices, there was greater variability in Xrs_6[40]. These findings are consistent with other studies comparing those same devices[21, 41] but it should also be noted that there are greater disparities in Xrs measurements between other oscillometry devices[41]. Nevertheless, any small differences in measurements between devices are unlikely to affect bronchodilator responses given the within session changes being measured. The COPD group was small (46/1145) and approximately half also had an asthma diagnosis. Given the many COPD phenotypes, our findings would not be generalisable to COPD or to other smoking-related airways diseases.

In conclusion, we have defined normative values for bronchodilator responses for oscillometry parameters, in a large, well-characterised healthy population sample, of 40 years and older. These thresholds are potentially useful to inform interpretation of oscillometry in airways disease. Bronchodilator responsiveness was associated with respiratory symptoms, asthma diagnosis and smoking history. ΔXrs_6 may be a particularly sensitive measure of airway dysfunction in smokers with normal spirometry. For the purposes of clinical interpretation, given the strong dependence of bronchodilator response on baseline oscillometric or spirometric function, it may be more appropriate to express responses as either relative or Z-score change. The clinical significance of oscillometric bronchodilator responsiveness, particularly in relation to disease phenotypes and

treatable traits, needs further study, given the fair concordance with spirometry suggests potential complementarity.

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TABLES

Table 1. Anthropometric characteristics of the entire cohort and the healthy subgroup reporting no respiratory symptoms.

	[§]**Entire cohort (n=1145)**	**Healthy$_{Asymp}$ (n=577)**
Age (years)	61.3 ±12.4	60.6 ±12.5
Male:female	570:575	244:333
Height (m)	1.68 ±0.09	1.67 ±0.09
Smoking (pack/years)	0.0 (0.0, 12.0)	0 (0.0, 0.75)
BMI (kg/m^2)	27.8 ±4.6	26.8 ±4.0
FEV1 (Z-score)	-0.24 ±1.03	0.1 ±0.9
FEV1/FVC (Z-score)	-0.46 ±0.96	-0.3 ±0.8
Rrs_6 ($cmH_2O.s.L^{-1}$)	3.29 (2.65 – 4.15)	3.10 (2.53, 3.78)
Xrs_6 ($cmH_2O.s.L^{-1}$)	-0.45 (-0.83, -0.22)	-0.39 (-0.66, -0.18)
EFLi ($cmH_2O.s.L^{-1}$)	-0.12 (-0.27, 0.89)	-0.15 (-0.27, -0.02)

[§]The Entire cohort (n=1145) includes Healthy$_{Asymp}$ subjects.
Mean ±SD or median (IQR).

Table 2. Bronchodilator responses for Rrs, Xrs and spirometry parameters in the healthy, asymptomatic group and remainder.

Parameter	HealthyAsym (n=577)		Remainder (n=568)
ΔRrs6 (cmH2O.s.L-1)	-0.23 (-0.57 – 0.04)	LLN = -1.38	-0.36 (-0.81 – -0.01)
ΔRrs6 (%)	-6.4 (-17.2 – 1.3)	LLN = -30.0	-11.3 (-20.4 – -0.2)
ΔRrs6 (Z-score)	-0.31 (-0.73 – 0.03)	LLN = -1.42	-1.1 (-2.4 – -0.3)
ΔRrs6(insp) (cmH2O.s.L-1)	-0.31 (-0.67 – -0.04)	LLN = -1.38	-0.41 (-0.93 – -0.11)
ΔRrs6(insp) %	-8.5 (-20.7 – 1.3)	LLN = -42.9	-12.7 (-25.6 – -0.2)
ΔXrs6 (cmH2O.s.L-1)	0.09 ±0.26	ULN = 0.57	0.28 ±0.57
ΔXrs6 (Z-score)	0.25 ±0.67	ULN = 1.36	0.5 ±0.8
ΔXrs6(insp) (cmH2O.s.L-1)	0.12 ±0.23	ULN = 0.53	0.22 ±0.40
ΔEFLi (cmH2O.s.L-1)	0.039 ±0.281	LLN = -0.37	-0.099 ±0.592
ΔFEV1 (ml)	76 ±119	-	106 ±135
ΔFVC (ml)	34 ±176	-	39 ±208

Mean ±SD or median (IQR). Responses for Rrs are defined as the 5th percentiles, and ULNs for Xrs are defined as the 95th percentiles. Healthy$_{Asymp}$ = subjects who were asymptomatic, did not use respiratory medications had not history of asthma or COPD diagnosis. Remainder = subjects not in the Healthy$_{Asymp}$ group. Healthy$_{Asymp}$ compared with Remainder; $p \leq 0.002$ for all Rrs_6 parameters (Mann Whitney) and $p < 0.001$ for all Xrs_6 parameters (T-test). Δ = bronchodilator response (post – pre-bronchodilator values), expressed as either absolute change, percentage change (%) or change in Z-score (Z-score).

Table 3. Percentage of positive bronchodilator responses for Rrs, Xrs and spirometry parameters, for each of the clinical groups.

	$Healthy_{Asym}$	**$Healthy_{Symp}$**	**$Smokers_{Asym}$**	**$Smokers_{Symp}$**	**Asthma**	**COPD**
	n=577	**n=126**	**n=159**	**n=115**	**n=122**	**n=46**
ΔRrs_6 (abs)	4.9	4.0	8.2	7.8	12.3*	17.4**
ΔRrs_6 (%)	5.0	6.3	8.8	11.3	13.9*	10.9
ΔRrs_6 (Z-score)	4.5	6.3	9.4	12.2*	13.1*	10.9
ΔRrs_{6insp} (abs)	4.9	7.1	8.2	9.6	15.6*	21.7*
ΔRrs_{6insp} (%)	4.9	6.3	8.8	11.3	13.9*	10.9
ΔXrs_6 (abs)	4.9	7.9	11.9*	17.4*	19.7*	47.8*****
ΔXrs_6 (Z-score)	5.0	6.3	7.5	17.4*	14.8*	26.1***
ΔXrs_{6insp} (abs)	4.7	7.9	7.5	20.0*†	18.9*	37.0***
$\Delta EFLi$ (abs)	4.9	9.5	13.2*	19.1*	13.9*	45.7*****
$\Delta FEV1$ (%)	2.3	0.8	1.9	9.6**	17.2***	30.4****
ΔFVC (%)	1.4	0.8	0.6	5.2	9.8***	30.4*****

*significant difference in proportions compared with $Healthy_{Asymp}$ only (Z test with Bonferroni correction, $p<0.05$).
**significantly different from $Healthy_{Asymp}$ and $Healthy_{Symp}$ ($p<0.05$).
***significantly different from $Healthy_{Asymp}$, $Healthy_{Symp}$ and $Smokers_{Asym}$ ($p<0.05$).
**** significantly different from $Healthy_{Asymp}$, $Healthy_{Symp}$, $Smokers_{Asym}$ and Asthma ($p<0.05$).
*****significant difference in proportions compared with all other groups ($p<0.05$).
†also significantly different compared with $Smokers_{Asym}$ ($p<0.05$).
Δ = bronchodilator response (post – pre-bronchodilator values), expressed as either absolute change (abs), percentage change (%) or change in Z-score (Z-score).

Table 4. Unadjusted p-values from comparisons of the proportion of bronchodilator responsiveness within groups, measured by ΔXrs_6 parameters versus ΔFEV_1, in healthy but symptomatic, smoking, asthmatic and COPD groups. Comparisons were by McNemar tests.

	$Healthy_{Symp}$	**$Smokers_{Asym}$**	**$Smokers_{Symp}$**	**Asthma**	**COPD**
	n=126	**n=159**	**n=115**	**n=122**	**n=46**
ΔXrs_6 (abs)	0.012*	0.001*	0.108	0.678	0.077
ΔXrs_6 (Z-score)	0.039	0.035	0.078	0.648	0.774
ΔXrs_{6insp} (abs)	0.012*	0.035	0.023*	0.824	0.549
$\Delta EFLi$ (abs)	0.003*	0.0003*	0.043	0.556	0.118

* Significant p-values after Benjamini Hochberg adjustment for multiple comparisons, using α = 0.10 (10% false discovery rate).

Figure Legends

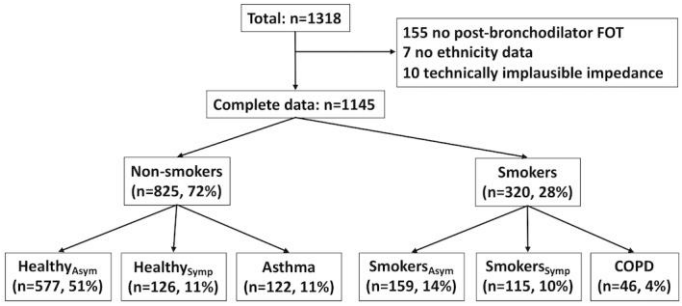

Figure 1: Disposition into clinical groups. See Results section for definition of groups.

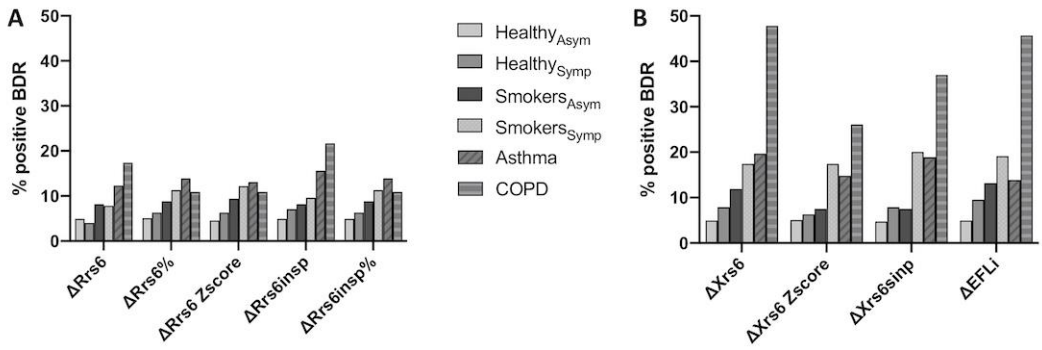

Figure 2: The BD-induced changes in Rrs (A) and Xrs parameters (B), for each of the clinically defined groups. ΔRrs_6 and ΔRrs_6 = post – pre-bronchodilator change in resistance and reactance, respectively, expressed as absolute values, percentage change, change in Z-score. ΔEFLi = change in expiratory flow limitation index.

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Online Supplement

Normal Limits For Oscillometric Bronchodilator Responses and Relationships With Clinical Factors

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Methods

Study population

The Sydney site was representative of the general population in Sydney based on gender distribution, age, employment status, and weekly average earnings. The Melbourne community had previously been studied as part of the European Community Respiratory Health Survey (ECRHS), and represented another Australian large city metropolitan site. Busselton is a small, coastal city that had research infrastructure from long-established general community cross-sectional and longitudinal population health studies [1]. Subjects at the Sydney and Melbourne sites were drawn from gender stratified random samples from the local divisions of the Commonwealth Electoral Roll. Subjects at the Busselton site were drawn from a random sample from the Commonwealth Electoral Roll for Busselton in the same manner as the aforementioned studies[1].

Oscillometry

A multiple-frequency oscillation (6, 11 and 19 Hz) was applied to the mouth, on one side of a flow splitter, while subjects breathed through the other side, which had a resistance mesh. Flow was measured using a 0–400 $L.min^{-1}$ pneumotachometer (Rudolph triple- screen pneumotach Series R4830B, Hans Rudolph Inc., Kansas City, MO, USA). Differential pressure was measured using a $\pm$2.5 cm H_2O solid-state pressure transducer (Sursense DC001NDC4, Honeywell Sensing and Control, Golden Valley, MN, USA) and mouth pressure was measured using a similar transducer ($\pm$12.5 cm H_2O, Sursense DC005NDC4). An antibacterial filter was used (resistance of 0.4 $cmH_2O.s.L^{-1}$ (SureGard® RJVKB2, Bird Healthcare, Port Melbourne, VIC, Australia). The pressure and flow signals were low-pass filtered at 25 Hz and then filtered using a bandwidth of 2 Hz centred around 6 Hz. All subsequent filtering and processing was performed digitally by custom software written in Matlab (The Mathworks Inc., MA, USA). Fast Fourier transforms were used to

calculate the Rrs and Xrs components of impedance from 1/6 second segments, at 0.1 second moving windows to provide 10 measurements per second. Daily impedance verifications were performed using a resistance tube of known impedance.

Subjects supported their cheeks with their hands, to minimise upper airway artefact. After verbal instruction and establishment of stable tidal breathing, a single one-minute measurement was acquired. Quality control of FOT measurements was performed after data acquisition using an automated computer algorithm based on analysis of complete breaths that has been previously described (Robinson et al., 2011). Complete breaths were considered acceptable on the basis of (1) no negative resistance points, (2) no spikes in admittance versus time that would indicate leak, (3) tidal volume within 1.5 standard deviations (SD) of the mean for all breaths, (4) expiratory flow-volume curve deviation within 1.5 SD of mean, defined in terms of sum of squared distances from the median flow value for all breaths at each volume point and (5) deviation in the Rrs-flow profile within 1.5 SD of mean, defined in terms of sum of squared distances from the median resistance value for all breaths at each flow point. A minimum of 5 acceptable breaths was used to determine average FOT parameters for each recording.

Predictors of bronchodilator response in healthy subjects

Anthropometric predictors of bronchodilator responsiveness measured by spirometry and by oscillometry were examined in the $Healthy_{Asym}$ group. The candidate anthropometric predictors that were examined were age, sex, height, weight, BMI. In addition, baseline FEV1 Z-scores and FEV1/FVC Z-scores, baseline Rrs_6 and Xrs_6, also expressed as their Z-scores, were included as predictors for spirometry bronchodilator responses.

Sex was found to be a significant predictor and so correlations were examined separately for females and males. Table E4 and E5 show univariate correlations for females and males, respectively. In females BMI was weakly, positively associated with the bronchodilator responses in EFLi only. In males, BMI was associated with the bronchodilator responses in Rrs_6 expressed as absolute and percentage change, and Rrs_6(insp), only. All Rrs_6, Xrs_6 and spirometry indices predicted the bronchodilator responses in each other. As expected, the relationships were strongest for baseline parameters predicting their own change with bronchodilator.

In summary, anthropometric factors have weak and limited influence bronchodilator responsiveness, whereas baseline lung function consistently and in some cases, strongly predict bronchodilator responses, in both females and males.

Proportion of abnormal oscillometry parameters in each of the 5 clinical groups

The proportions of subjects who had abnormal pre-bronchodilator Rrs_6, Xrs_6, EFLi, FEV_1 and FEV_1/FVC ratio within each of the clinical groups, are shown in Table E7. Note that both smokers groups were defined by having normal post-bronchodilator FEV_1/FVC Z-scores according to the GLI equations[2]. Table E7 shows however, that there was a small percentage who had abnormal FEV_1/FVC Z-scores.

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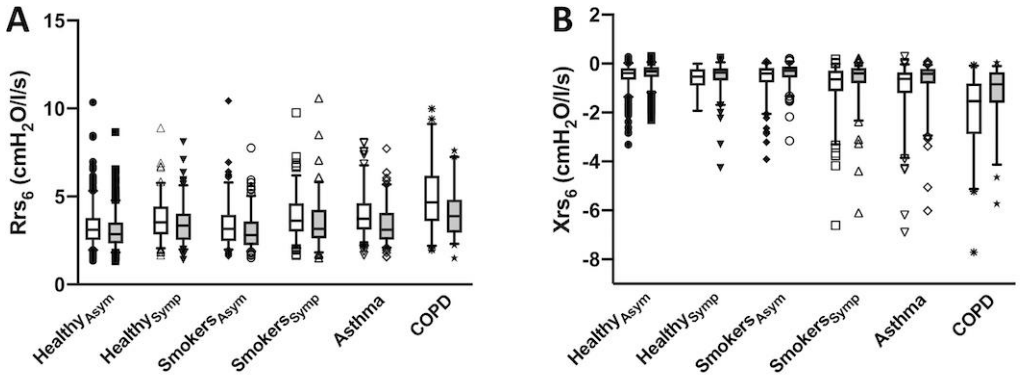

Figure E1. Rrs6 and Xrs6 pre- and post- bronchodilator inhalation, for the 6 clinical groups. Clear boxes are pre-bronchodilator and shaded boxes are post-bronchodilator values.

Table E1. Pre-bronchodilator and post-bronchodilator spirometry and impedance.

	Pre-BD	**Post-BD**	**p**
FEV1 (L)*	2.93 ±0.83	3.00 ±0.84	<0.0001
FVC (L)*	3.82 ±1.02	3.79 ±1.01	<0.0001
FEV1/FVC*	0.77 ±0.06	0.79 ±0.06	<0.0001
Rrs ($cmH_2O/L/s$)	3.10 (2.53 – 3.78)	2.86 (2.33 – 3.52)	<0.0001
Xrs ($cmH_2O/L/s$)	-0.39 (-0.66 – -0.18)	-0.32 (-0.32 – -0.14)	<0.0001
Rrs_{insp} ($cmH_2O/L/s$)	2.92 (2.43 – 3.56)	2.61 (1.28 – 3.15)	<0.0001
Xrs_{insp} ($cmH_2O/L/s$)	-0.48 (-0.77 – -0.25)	-0.37 (-0.59 – -0.19)	<0.0001
EFLi ($cmH_2O/L/s$)	-0.15 (-0.27, -0.02)	-0.12 (-0.22 – -0.03)	0.001

* Mean ±SD. The remainder or median (interquartile range). p is significance value for paired T-tests.

Table E2. Predicted values for impedance at 6Hz derived from the healthy, asymptomatic group (n=587).

Respiratory system resistance (Rrs_6) parameters		**RSD**	**Adusted R^2, p value**
Males	$Ln(Rrs_6) = 0.011 * Weight – 0.016 * Height + 2.912$	0.245	0.22, p<0.001
	$Ln(Rrs_6(insp)) = 0.010 * Weight – 0.016 * Height + 2.874$	0.242	0.21, p<0.001
Females	$Ln(Rrs_6) = 0.009 * Weight – 0.013 * Height + 2.714$	0.265	0.15, p<0.001
	$Ln(Rrs_6(insp)) = 0.009 * Weight – 0.013 * Height + 2.616$	0.258	0.15, p<0.001
Respiratory system reactance (Xrs_6) parameters			
Males	$Exp(Xrs_6) = 0.010 * Height – 0.018 * BMI – 0.002 * age – 0.283$	0.202	0.19, p<0.001
	$Exp(Xrs_6(insp)) = 0.010 * Height – 0.011 * BMI – 0.002 * age – 0.554$	0.191	0.14, p<0.001
	$EFLi = 0.009 * Weight – 0.009 * Height + 0.821$	0.329	0.08, p<0.001
Females	$Exp(Xrs_6) = 0.003 * Height – 0.012 * BMI – 0.004 * age + 0.660$	0.196	0.16, p<0.001
	$Exp(Xrs_6(insp)) = 0.005 * Height – 0.009 * BMI – 0.003 * age + 0.213$	0.181	0.13, p<0.001
	$EFLi = 0.006 * age + 0.006 * Weight – 0.898$	0.352	0.06, p<0.001

Age in years, Weight in Kg, Height in cm, BMI in kg/m^2, Ln: natural logarithm, Exp: raised to the exponent e, RSD standard deviation of residuals.

Table E3. Univariate correlations between anthropometric characteristics, symptoms, asthma diagnosis, smoking history, baseline lung function, and; bronchodilator responses in oscillometry and spirometry parameters. Analysis involves the entire cohort (n=1145).

	Age	Height	BMI	Asthma diagnosis	Symptoms [#]	Smoking	FEV1 Z-score	FEV1/FVC Z-score	Rrs_6 Z-score	Xrs_6 Z-score
ΔRrs_6 (abs)	0.07 (0.10)	-0.12 (0.66)	**-0.10 (0.001)**	**0.13 (0.003)**	**0.14 (0.001)**	**-0.11 (0.001)**	**0.23 (<0.001)**	**0.25 (<0.001)**	**-0.50 (<0.001)**	**0.26 (<0.001)**
ΔRrs_6 (%)	0.08 (0.10)	-0.08 (0.07)	-0.04 (0.24)	**0.10 (0.001)**	**0.13 (0.002)**	**-0.09 (0.002)**	**0.19 (<0.001)**	**0.24 (<0.001)**	**-0.40 (<0.001)**	**0.20 (<0.001)**
ΔRrs_6 (Z-score)	-0.05 (0.12)	-0.03 (0.25)	-0.04 (0.20)	**0.10 (0.001)**	**0.13 (<0.001)**	**-0.09 (0.001)**	**0.19 (<0.001)**	**0.23 (<0.001)**	**-0.40 (<0.001)**	**0.21 (<0.001)**
ΔRrs_6(insp) (abs)	0.07 (0.09)	-0.02 (0.65)	**-0.11 (<0.001)**	**0.13 (<0.001)**	**0.15 (<0.001)**	**-0.08 (0.004)**	**0.26 (<0.001)**	**0.27 (<0.001)**	**-0.51 (<0.001)**	**0.27 (<0.001)**
ΔRrs_6(insp) (%)	0.08 (0.06)	-0.08 (0.07)	-0.04 (0.24)	**0.10 (0.001)**	**0.13 (0.002)**	**-0.09 (0.002)**	**0.19 (<0.001)**	**0.24 (<0.001)**	**-0.40 (<0.001)**	**0.20 (<0.001)**
ΔXrs_6 (abs)	0.07 (0.12)	0.02 (0.72)	**0.16 (<0.001)**	**-0.17 (<0.001)**	**0.15 (<0.001)**	**0.15 (<0.001)**	**-0.29 (<0.001)**	**-0.21 (<0.001)**	**0.39 (<0.001)**	**-0.51 (<0.001)**
ΔXrs_6 (Z-score)	-0.02 (0.65)	**0.12 (0.004)**	**0.11 (<0.001)**	**-0.11 (<0.001)**	**0.11 (0.008)**	**0.12 (<0.001)**	**-0.22 (<0.001)**	**-0.17 (<0.001)**	**0.30 (<0.001)**	**-0.40 (<0.001)**
ΔXrs_6(insp) (abs)	0.00 (0.99)	0.05 (0.22)	**0.12 (<0.001)**	**-0.17 (<0.001)**	**-0.16 (<0.001)**	**-0.08 (0.008)**	**-0.28 (<0.001)**	**-0.20 (<0.001)**	**0.38 (<0.001)**	**-0.45 (<0.001)**
$\Delta EFLi$ (abs)	**-0.14 (0.01)**	0.01 (0.78)	**-0.11 (<0.001)**	0.03 (0.29)	**0.09 (0.03)**	**-0.14 (<0.001)**	**0.10 0.002**	**0.07 0.01**	**-0.10 0.001**	**0.20 (<0.001)**
$\Delta FEV1$ (%)	0.06 (0.15)	0.00 (0.98)	-0.01 (0.71)	**-0.22 (<0.001)**	**-0.25 (<0.001)**	**-0.07 (0.01)**	**-0.41 (<0.001)**	**-0.37 (<0.001)**	**0.21 (<0.001)**	**-0.18 (<0.001)**
ΔFVC (%)	**0.15 (<0.001)**	0.01 (0.86)	**0.10 (0.01)**	**-0.13 (0.001)**	**-0.22 (<0.001)**	**0.12 (<0.001)**	**-0.30 (<0.001)**	-0.06 0.05	**0.17 (<0.001)**	**-0.20 (<0.001)**

Values are Spearman correlations and (p vales).
[#]highest Rho for any symptoms.
Bold values indicate $p<0.05$.

Table E4. Univariate Spearman correlations between bronchodilator responses (measured by spirometry and oscillometry) and; anthropometric features and baseline lung function, in females from the Healthy$_{Asym}$ group (n=333). The significant correlations (p<0.05) are in bold.

FEMALE	Age	Height	BMI	Rrs_6 (Z-score)	Xrs_6 (Z-score)	EFLi	FEV1 (Z-score	FEV1/FVC Z-score
ΔRrs_6 (abs)	0.04 (0.53)	0.02 (0.66)	-0.08 (0.14)	**-0.46 (<0.001)**	**0.25 (<0.001)**	-0.07 (0.23)	**0.18 (<0.001)**	**0.19 (<0.001)**
ΔRrs_6 (%)	0.05 (0.34)	-0.019 (0.73)	-0.01 (0.89)	**-0.35 (<0.001)**	**0.17 (<0.001)**	-0.02 (0.67)	**0.14 (0.01)**	**0.19 (<0.001)**
ΔRrs_6 (Z-score)	0.05 (0.34)	-0.019 (0.73)	-0.01 (0.89)	**-0.35 (<0.001)**	**0.17 0.002**	-0.02 (0.67)	**0.14 (0.01)**	**0.19 (0.001)**
ΔRrs_6(insp) (abs)	0.10 (0.07)	-0.04 (0.44)	-0.07 (0.20)	**-0.50 (<0.001)**	**0.28 (<0.001)**	-0.03 (0.54)	**0.21 (<0.001)**	**0.23 (<0.001)**
ΔRrs_6(insp) (%)	0.05 (0.34)	-0.02 (0.73)	-0.01 (0.89)	**-0.35 (<0.001)**	**0.17 0.002**	-0.02 (0.67)	**0.14 (0.01)**	**0.19 (0.001)**
ΔXrs_6 (abs)	-0.11 (0.05)	0.03 (0.59)	0.07 (0.20)	**0.31 (<0.001)**	**-0.41 (<0.001)**	0.03 (0.59)	**-0.11 (0.04)**	-0.07 (0.21)
ΔXrs_6 (Z-score)	**0.14 (0.01)**	0.063 (0.25)	0.03 (0.58)	**0.26 (<0.001**	**-0.34 <0.001**	0.00 (0.98)	-0.1 (0.08)	-0.09 (0.12)
ΔXrs_6(insp) (abs)	-0.10 (0.06)	0.06 (0.27)	0.04 (0.47)	**0.36 (<0.001)**	**-0.39 (<0.001)**	**-0.16 (0.003)**	**-0.13 0.02)**	-0.06 (0.28)
ΔEFLi (abs)	-0.04 (0.44)	0.089 (0.11)	**-0.12 (0.03)**	0.04 0.51	0.05 0.35	**-0.41 (<0.001)**	0.04 (0.48)	0.03 (0.63)
ΔFEV1 (%)	0.07 (0.19)	0.04 (0.53)	0.05 (0.40)	**0.20 (<0.001)**	-0.05 0.38	0.10 (0.07)	**-0.37 (<0.001)**	**-0.3 (<0.001)**
ΔFVC (%)	0.14 (0.01)	0.01 (0.89)	0.06 (0.26)	**-0.12 0.03**	-0.04 0.52	0.09 (0.12)	**-0.21 (<0.001)**	0.07 (0.20)

Values are Spearman correlations and (p vales).

Bold values indicate p<0.05.

Table E5. Univariate Spearman correlations between bronchodilator responses (measured by spirometry and oscillometry) and; anthropometric features and baseline lung function, in males from the Healthy$_{Asym}$ group (n=244). The significant correlations (p<0.05) are in bold.

Male	Age	Height	BMI	Rrs_6 (Z-score)	Xrs_6 (Z-score)	EFLi	FEV1 (Z-score	FEV1/FVC Z-score
ΔRrs_6 (abs)	-0.01 (0.90)	0.03 (0.68)	**-0.16 (0.02)**	**-0.34 (<0.001)**	**-0.34 (<0.001)**	0.06 (0.33)	0.07 (0.28)	**0.15 (0.02)**
ΔRrs_6 (%)	-0.02 (0.74)	0.03 (0.69)	**-0.17 (0.01)**	**-0.28 (<0.001)**	**-0.28 (<0.001)**	0.09 (0.16)	0.06 (0.34)	**-0.16 (0.01)**
ΔRrs_6 (Z-score)	0.05 (0.34)	-0.02 (0.73)	-0.01 (0.89)	**-0.35 (<0.001)**	**-0.35 (<0.001)**	0.09 (0.16)	0.06 (0.34)	**0.19 (0.01)**
ΔRrs_6(insp) (abs)	-0.04 (0.57)	0.04 (0.53)	**-0.17 (0.01)**	**-0.43 (<0.001)**	**0.18 (0.003)**	0.04 (0.54)	**0.17 (0.008)**	**0.20 (0.002)**
ΔRrs_6(insp) (%)	-0.02 (0.74)	0.03 (0.69)	-0.07 (0.30)	**-0.30 (<0.001)**	0.11 (0.09)	0.09 (0.16)	0.06 (0.34)	**0.16 (0.01)**
ΔXrs_6 (abs)	0.04 (0.51)	-0.1 (0.11)	0.02 (0.82)	**0.26 (<0.001)**	**0.26 (<0.001)**	-0.06 (0.35)	-0.09 (0.15)	-0.1 (0.11)
ΔXrs_6 (Z-score)	0.04 (0.51)	-0.1 (0.13)	0.01 (0.85)	**0.23 (<0.001)**	**0.23 (<0.001)**	-0.07 (0.28)	-0.09 (0.18)	-0.11 (0.09)
ΔXrs_6(insp) (abs)	0.06 (0.35)	-0.11 (0.10)	0.11 (0.09)	**0.32 (<0.001)**	**-0.44 (<0.001)**	**-0.21 (0.001)**	**-0.19 (0.003)**	-0.07 (0.27)
ΔEFLi (abs)	0.04 (0.57)	0.24 (0.71)	0.01 (0.86)	-0.02 (0.74)	-0.02 (0.74)	**-0.41 (<0.001)**	-0.08 (0.20)	0.07 (0.29)
ΔFEV1 (%)	0.04 (0.52)	-0.04 (0.51)	0.08 (0.22)	0.00 (0.96)	-0.003 (0.96)	0.00 (0.95)	**-0.25 (<0.001)**	**-0.27 (<0.001)**
ΔFVC (%)	0.09 (0.16)	0.00 (0.98)	-0.04 (0.54)	-0.05 (0.45)	0.01 (0.90)	0.09 (0.14)	-0.09 (0.18)	0.06 (0.37)

Table E6: Anthropometric data of the clinical groups

	$Healthy_{Asymp}$	$Healthy_{Symp}$	$Smokers_{Asym}$	$Smokers_{Sym}$	Asthma	COPD
n (male%)	577 (42%)	126 (52%)	159 (65%)	115 (65%)	122 (43%)	46 (63%)
Age (years)	60.7 ±12.6	62.0 ±12.8	62.4 ±12.3	62.2 ±11.5	60.4 ±12.5	65.8 ±11.4
Height (cm)	167 ±0.09	168 ±10.2	170.1 ±9.1	169.8 ±9.2	168.2 ±9.1	169.8 ±8.3
BMI ($kg.m^{-2}$)	26.9 ±4.0	29.1 ±5.3*	28.1 ±4.5*	29.9 ±4.6*#	28.4 ±5.2	27.0 ±3.9
Smoking (pack/years)	1.1 ±2.2	1.1 ±2.3	24.7 ±15.1	30.6 ±19.7	1.3 ±2.5	40.6 ±31.8
Rrs_6 ($cmH_2O.s.L^{-1}$)	3.12 (2.54,3.85)	3.53* (2.84,4.43)	3.16 (2.47,3.96)	3.62* (3.00,4.61)	3.73* (3.11,4.61)	4.67* (4.67,6.18)
Rrs_6 (z-score)	-0.1 (-0.7,0.6)	-0.1 (-0.5,0.9)	0.0 (-0.7,0.9)	0.4* (-0.5,1.1)	0.5* (-0.2,1.3)	1.7* (0.8,2.4)
Xrs_6 ($cmH_2O.s.L^{-1}$)	-0.39 (-0.67,-0.19)	-0.53* (-0.91,-0.23)	-0.41 (-0.77,-0.18)	-0.64* (-1.13,-0.29)	-0.64* (-1.21,-0.36)	-1.53* (-2.89,-0.81)
Xrs_6 (z-score)	0.4 (-0.2,1.1)	0.2 (-0.8,0.9)	-0.2 (-0.8,1.0)	-0.3* (-1.3,0.7)	-0.3* (-1.4,0.6)	-2.0* (-2.8,-0.4)
FEV1 (Litres)	2.92 ±0.83	2.75 ±0.85*	2.97 ±0.75	2.74 ±0.80*	2.58 ±0.84*	1.95 ±0.66*
FEV1 (z-score)	0.1 ±0.9	-0.4 ±1.0*	-0.1 ±0.8*	-0.6 ±0.9*	-0.7 ±1.2*	-2.0 ±1.0*
FVC (Litres)	3.8 ±1.02	3.61 ±1.03	3.92 ±0.93	3.70 ±1.02	3.57 ±0.99*	3.43 ±0.95*
FVC (z-score)	0.2 ±0.9	-0.2 ±0.9*	0.0 ±0.8*	-0.3 ±1.0*	-0.3 ±1.0*	-0.5 ±1.2*
FEV_1/FVC	0.77 ±0.06	0.76 ±0.07	0.77 ±0.06	0.74 ±0.06*	0.71 ±0.10*	0.56 ±0.08*
FEV_1/FVC (z-score)	-0.3 ±0.8	-0.3 ±0.9	-0.3 ±0.7	-0.5 ±0.7*	-0.8 ±1.2*	-2.5 ±0.7*

Mean ±SD or median (IQR).

* significant differences compared with the Non-Smokers group (Kruskal-Wallis $p<0.05$, with Bonferroni correction).

$Smokers_{Symp}$ have BMIs greater than the asthma and COPD groups.

Differences in smoking history was not tested.

Table E7. Bronchodilator responses for the 5 clinical groups.

	$Healthy_{Symp}$ N=126	$Smokers_{Asym}$ N=159	$Smokers_{Sym}$ N=115	Asthma N=122	COPD N=46
ΔRrs_6 ($cmH_2O.s.L^{-1}$)	-0.28 (-0.79 – 0.08) (4.0%)	-0.32 (-0.67 – 0.02) (8.2%)	-0.32 (-0.79 – 0.07) (7.8%)	-0.51 (-0.99 – -0.09) (12.3%)	-0.71 (-1.21 – -0.23) (17.4%)
ΔRrs_6 (%)	-8.5 (-18.6 – 3.2) (6.3%)	-10.6 (-18.6 – 0.78) (8.8%)	-9.2 (-19.5 – 1.4) (11.3%)	-13.4 (-22.9 – -3.4) (13.9%)	-14.5 (-25.3 – -5.9) (10.9%)
ΔRrs_6 (z-score)	-1.1 (-2.1 – -0.2) (6.3%)	-1.2 (-2.2 – -0.5) (9.4%)	-1.6 (-3.1 – -0.5) (12.2%)	-0.9 (-2.3 – -0.2) (13.1%)	-1.3 (-2.4 – -0.56) (10.9%)
ΔRrs_6(insp) ($cmH_2O.s.L^{-1}$)	-0.41 (-0.73 – 0.01) (7.1%)	-0.32 (-0.72 – -0.08) (8.2%)	0.37 (-0.82 – -0.13) (9.6%)	-0.59 (-2.3 – -0.1) (15.6%)	-0.71 (-1.28 – -0.16) (21.7%)
ΔRrs_6(insp) %	-9.3 (-22.9 – 3.1) (6.3%)	-11.9 (-22.9 – 0.7) (8.8%)	-10.1 (-24.2 – 1.4) (11.3%)	-15.5 (-29.7 – -3.5) (13.9%)	-16.9 (-33.8 – -6.3) (10.9%)
ΔXrs_6 ($cmH2O.s.L^{-1}$)	0.15 ±0.49 (7.9%)	0.19 ±0.39 (11.9%)	0.27 ±0.52 (17.4%)	0.18 (0.02 – 0.43) (19.7%)	0.48 (0.06 – 1.34) (47.8%)
ΔXrs_6 (z-score)	0.29 ±0.69 (6.3%)	0.42 ±0.71 (7.5%)	0.51 ±0.86 (17.4%)	0.4 (0.1 – 0.9) (14.8%)	0.5 (0.1 – 1.4) (26.1%)
ΔXrs_6(insp) ($cmH_2O.s.L^{-1}$)	0.14 ±0.28 (7.9%)	0.12 ±0.29 (7.5%)	0.19 ±0.41 (20.0%)	0.24 (0.06 – 0.43) (18.9%)	0.41 (0.13 – 0.99) (37.0%)
ΔEFLi ($cmH_2O.s.L^{-1}$)	-0.017 ±0.641 (9.5%)	-0.033 (-0.17 – 0.105) (13.2%)	-0.142 ±0.536 (19.1%)	0.046 (-0.131 – 0.202) (13.9%)	-0.255 (-0.682 – 0.074) (45.7%)

Mean ±SD for normally distributed data, otherwise median (IQR).
Numbers in brackets are % with positive bronchodilator responses as defined in Table 2.

Table E8. Percentage of abnormal oscillometry parameters and spirometry, as defined by their Z-scores, for each of the clinical groups.

	$Healthy_{Asym}$ N=577	$Healthy_{Symp}$ N=126	$Smokers_{Asym}$ N=159	$Smokers_{Sym}$ N=115	Asthma N=122	COPD N=46
Rrs_6	5.4	7.1	7.5	13.0	18.0	52.0
Xrs_6	8.8	15.9	16.4	29.6	27.9	63.0
$EFLi^{\#}$	0	1.6	1.3	2.6	2.5	10.9
FEV_1	1.4	12.7	2.5	14.8	20.5	69.6
FEV_1/FVC	4.7	4.8	1.9	7.8	26.2	100

The Z-scores for oscillometry were calculated from the equations in Table E1 above. $^{\#}$For EFLi, a value of >2.8 $cmH_2O.s.L^{-1}$ was used to define the presence of expiratory flow limitation, as defined by Dellaca et al[3]. Note that the 95th percentile for EFLi in the $Healthy_{Asym}$ group was 2.00 $cmH_2O.s.L^{-1}$. For spirometry, abnormality was define by the GLI equations[2].