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Effect of tiotropium on spontaneous expiratory flow–volume curves during exercise in GOLD 1-2 COPD

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ABSTRACT

This substudy of a large, randomized, controlled trial (NCT01072396) examined tiotropium (18µg qd) effects on dynamic hyperinflation during constant work rate treadmill exercise. Areas-under-the-spontaneous expiratory flow-volume (SEFV)-curves were compared in 20 COPD patients and 16 age-matched untreated controls, using rectangular area ratio (RAR) between peak intrabreath and end-expiratory flow.

Seven patients exhibited SEFV curve concavity with RAR≤0.5 (RAR_{low}) in ≥1 test without tiotropium; (mean±SD FEV_1: 1.60±0.59 L; 63.4±14.0 %predicted). In RAR_{low} patients, tiotropium increased end-exercise inspiratory capacity (IC, 2.10±0.05 vs. 1.89±0.05 L, tiotropium vs. placebo; p=0.045) and RAR (0.57±0.02 vs. 0.53±0.02; p<0.001). Patients without SEFV curve concavity with RAR>0.5 (n=13; RAR_{high}), had higher screening FEV_1 (2.15±0.47 L; 79.6±10.1 %predicted) versus RAR_{low} patients and no difference in end-exercise IC and RAR between tiotropium and placebo (IC: 2.24±0.03 vs. 2.17±0.03 L; RAR: 0.63±0.005 vs. 0.62±0.005). RAR and %predicted IC at peak exercise were positively correlated in RAR_{low} patients (R^2=0.43, p=0.0002).

Tiotropium increased exercise RAR in GOLD 1-2 patients with SEFV curve concavity.

Keywords:

Constant work rate; Dynamic hyperinflation; Expiratory flow limitation; Inspiratory capacity; Rectangular area ratio; Treadmill exercise

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality and morbidity, affecting millions of people worldwide (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2017). Currently, the accepted classification standard for grading the spirometric severity of COPD is set out in the Global Initiative for Chronic Obstructive Lung Disease (GOLD) report (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2017), and is based on post-bronchodilator forced expiratory volume in 1 s (FEV_1). However, this spirometric variable correlates poorly with clinical variables such as exercise tolerance (Puente-Maestu et al., 2016), and may provide an oversimplified representation of COPD severity.

It has been well recognized that there is information embedded in the spontaneous flow–volume loop beyond that reflected in spirometric values, but the task of analyzing the geometry of flow–volume loop shapes in a meaningful manner remains difficult. Traditionally, expiratory flow limitation is said to occur when the tidal flow–volume loop encroaches on the maximal expiratory flow–volume curve (Johnson et al., 1999). During exercise in COPD, as hyperinflation develops, the elastic recoil decreases lengthening the expiratory time constants of these lung units, necessitates increased intrathoracic pressure to maintain flow. Greater expiratory pressures augment gas compression and dynamic airway compression, leading to a progressive fall in expiratory flow, which manifests as concavity in the spontaneous expiratory flow–volume (SEFV) curve (Lee et al., 2016; Ma et al., 2010; Varga et al., 2016). Expiratory flow limitation contributes to dynamic hyperinflation during exercise, whereby the end-expiratory lung volume progressively increases and inspiratory capacity (IC) and inspiratory reserve volume are reduced, and which is associated with dyspnea and exercise intolerance in COPD (O'Donnell et al., 2004). In case of these events, the following is our working hypothesis: i) the shape of the flow–volume loop becomes concave; ii) the concavity reflects increased airway resistance; iii) reduced elastic recoil in COPD (particularly in emphysema) increases expiratory time constants, which necessitates increased intrathoracic pressure to maintain flow; this should further worsen

the concavity of the flow–volume loop; iv) bronchodilation should improve this situation and, as such, it should reflect in analysis of the concavity of the flow–volume loop which could then be useful to evaluate the effects of bronchodilators. Breath-by-breath quantification of SEFV curve concavity has been used to describe progressive shape changes denoting expiratory flow limitation during incremental exercise in patients with COPD (Ma et al., 2010; Varga et al., 2016).

Tiotropium, a once-daily, long-acting muscarinic antagonist (LAMA) bronchodilator, approved for COPD management, is associated with significant improvement in lung hyperinflation and exercise tolerance in COPD patients with GOLD spirometric grades 2–4 (Maltais et al., 2005; O'Donnell et al., 2004; Verkindre et al., 2006). In GOLD 1 COPD patients, tiotropium significantly reduced dynamic hyperinflation, but this did not translate into significant exercise tolerance improvement (Casaburi et al., 2014). The authors postulated this lack of improvement may be due to exercise limitation from leg muscle fatigue rather than from dyspnea. Another possibility is that there is a heterogeneous response to tiotropium within GOLD 1 patients. The differences in response to tiotropium between patients with GOLD 1 and 2 COPD may represent fundamental differences in the nature and extent of abnormalities of peripheral airway dynamics and propensity to dynamic hyperinflation, which are directly explored in this current study. While the GOLD classification system broadly categorizes patients with COPD based on their FEV_1 and FEV_1/forced vital capacity (FVC), there is growing evidence that considerable clinical heterogeneity exists within groups with similar spirometric values (Gagnon et al., 2015).

The aim of this study was to examine how tiotropium versus placebo influences the shape of the SEFV curve during constant work rate (CWR) treadmill exercise in patients with mild and moderate COPD (GOLD grades 1 and 2) and determine the association of any treatment-related change in the SEFV curve with change in dynamic hyperinflation. We employed the strategy of dividing the patient group between those who did or did not

demonstrate flow-volume concavity during exercise. We reasoned that those who demonstrated flow-volume concavity would be more likely to respond to bronchodilation with reduction in dynamic hyperinflation and improvement in exercise tolerance.

2. Materials and methods

2.1 Study design

This substudy constituted an exploratory analysis of a large multicenter trial (ClinicalTrials.gov identifier: NCT01072396). The main study was conducted over 22-weeks, and was a multicenter (11 US and four Canadian sites), randomized, double-blind, two-period, crossover study (Fig. 1), which assessed the effects of once-daily tiotropium vs. placebo on dynamic hyperinflation and exercise tolerance in patients with symptomatic GOLD 1 and 2 COPD (Casaburi et al., 2014; O'Donnell et al., 2014). Patients who completed a 2-week Characterization Phase (Visits 1–3) (O'Donnell et al., 2014) and were eligible for further study participation were entered into the Treatment Phase (Visits 4–6) (Casaburi et al., 2014). Patients were randomized 1:1 to 6 weeks of 18 µg tiotropium or placebo (oral inhalation capsule) administered once daily via a HandiHaler® (Boehringer Ingelheim, Ingelheim, Germany). Following a 4-week washout, patients switched over treatments. After completion of the last 6-week treatment period patients were followed up for 30 days (Visit 7). Patients who discontinued, were followed up for 30 days after the final dose of study medication. In addition, a reference group of healthy, age-matched, control participants were enrolled in the Characterization Phase.

The main study and the substudy were approved for all participating institutions by local or central Independent Review Boards and were conducted in accordance with the principles of the Declaration of Helsinki (October 1996) and the International Conference on Harmonisation Tripartite Guidelines for Good Clinical Practice. Written informed consent was obtained from all participants before entering the study.

2.2 Study participants

The patients in this substudy were those recruited at two sites each in the US and Canada that were equipped for data collection for the substudy: the patients were not subjected to any further selection criteria. Study participants were males and females, aged ≥40 years, body mass index 18–35 kg/m^2, current or ex-smokers (smoking history ≥10 pack-years) with a post-bronchodilator FEV_1/FVC <70%, and FEV_1 ≥50% predicted (Quanjer et al., 1993) at Visit 1. Patients were categorized according to the 2014 GOLD spirometric classification scheme (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2014): GOLD grade 1 (post-bronchodilator FEV_1/FVC <0.7 and FEV_1 ≥80% of predicted) and GOLD grade 2 (post-bronchodilator FEV_1/FVC <0.7 and 50% ≤FEV_1 <80% predicted). Patients were required to demonstrate dynamic hyperinflation during exercise manifested by a >100 mL IC decrease during exercise from mean resting values in two out of three baseline exercise tests (Visits 1 through Visit 3; Fig. 1). Participants were symptomatic, as determined by a Baseline Dyspnea Index focal score ≤9 (Mahler et al., 1984) and/or daily cough with production of sputum for 3 months/year during at least two consecutive years. Patients with significant disease other than COPD were excluded if it was likely that the study results, patient welfare, or ability of patients to participate in the study would be affected. Healthy, age-matched control subjects, with normal lung function and minimal smoking history (i.e., no cigarettes in the preceding 2 years and <1-pack year smoking history) participated in the Characterization Phase of the study only (Visits 1–3).

2.3 Study procedures

During screening (–30 to –14 days from the start of treatment) participants provided a medical history and demographic data, and pre-exercise pulmonary function assessed by spirometry. Spirometry values were obtained 60 min before the exercise tests and 80 min after

dosing (placebo or tiotropium). All spirometry was carried out according to American Thoracic Society/European Respiratory Society recommendations (Miller et al., 2005). The predicted values of Quanjer et al. (Quanjer et al., 1993) were used for FVC and FEV_1. A symptom-limited treadmill incremental exercise test (IET) was performed on Visit 1 using a linearized protocol (Porszasz et al., 2003). To achieve an optimal exercise time between 8 and 12 min, for patients with COPD, the IET was carried out using a 10 W/min protocol; which was repeated at 15 W/min if peak work rate was ≥150 W. For the control subjects, a 15 W/min protocol was used, which was repeated at 20 W/min if the peak work rate was ≥200 W. Results of the IET were used to set the treadmill speed and inclination for subsequent CWR tests to produce a work rate that was equivalent to 80% peak work rate in the IET. The target exercise duration for the CWR tests was 4–10 min; if this target range was not met during a practice CWR test at Visit 2, work rate adjustments were made for subsequent visits. One further adjustment was permitted, and subjects were excluded if the target duration was still not met. CWR tests were performed at Visits 2–6, with Visit 2 used for participant familiarization. Visits 3 and Visit 5 were baseline tests for the corresponding therapeutic intervals. CWR testing was performed before study drug administration on Visits 3 and 5, and after study drug administration on Visits 4 and 6. During exercise tests, minute ventilation ($\dot{V}_E$) was measured breath by breath using a Vmax® Spectra, Version 12.2 (SensorMedics Corp., Yorba Linda, CA, USA) and was compared with indirectly estimated maximum voluntary ventilation (MVV), which was calculated as $FEV_1 \times 40$. IC was measured at rest, during warm-up (level walking at 0.8 mph for 3 min), and every 2 min during the exercise test and recovery; all ICs were manually checked for accuracy and, if needed, were adjusted to the stable end-expiratory lung volume. The IC was expressed as % predicted values as calculated from the predicted total lung capacity minus functional residual capacity (Stocks and Quanjer, 1995).

2.4 Analysis of tidal flow

Spontaneous tidal flow was digitized using 16-bit A/D conversion at 100 Hz (National Instruments, Austin, TX, USA). Progressive expiratory flow limitation was quantified by comparing, on a breath-by-breath basis, the area under the SEFV curve relative to a rectangle spanning peak intrabreath and end-expiratory flow (the rectangular area ratio [RAR]) (Ma et al., 2010). For a visual representation of the derivation of the RAR see Fig. 2 (Varga et al., 2016). Customized procedures were executed using IGOR software (WaveMetrics Inc., Lake Oswego, OR, USA) for geometric analysis of flow–volume curves. The breath-by-breath output of the analysis is presented from two representative subjects along with the instantaneous flow in Fig. 3. The black lines in this figure are the result of an exponential smoothing; the corresponding RAR values were taken from this smooth line. As shown in Fig. 3B, the RAR initially was around 0.6, it then gradually decreased during exercise and falling to below 0.5 towards end-exercise; this suggests that there is a developing concavity in the expiratory flow–volume curve. Based on the SEFV curve shape, the patients with COPD were categorized into two groups according to whether or not they exhibited concavity in the expiratory limb of the flow–volume curve during any of the screening incremental or baseline CWR tests (IET in Visit 1 or during Visits 2, 3, or 5 CWR tests); the RAR_{high} group included COPD patients with RAR >0.5 and the RAR_{low} group included COPD patients with RAR ≤0.5. The actual values of RAR were taken from the curve resulting from an exponential smoothing, as detailed in the legend to Fig. 3. RAR was calculated in the control participants in the same way, as a comparator for the RAR_{high} and RAR_{low} COPD patients.

2.5 Statistical analysis

Comparison of baseline characteristics was performed between the RAR_{high} and RAR_{low} groups by unpaired Student t-tests, or one-way ANOVA with post hoc comparisons when appropriate. Most of the analyses, involving two treatment conditions and exercise levels were carried out by two-way repeated measures ANOVA with post hoc Holm–Šídák multiple

comparisons. In those instances when the groups, treatment conditions, and exercise levels were simultaneously compared, we used three-way ANOVA. In these, the least square means and the standard error of least square means are reported. For the statistical analyses and graphical presentations SigmaPlot Version 13 (Systat Software, Inc., San Jose, CA) was used.

3. Results

3.1 Baseline characteristics

All participants' tests were completed between March 2010 and August 2011. The baseline demographic characteristics of the study participants are shown in Table 1. Of the 20 patients with COPD enrolled in the study, 7 patients exhibited concavity (RAR_{low} group) during at least one of the baseline exercise tests (Visit 3 and Visit 5). These patients were mainly GOLD 2 COPD (6 out of 7 patients [86%]) and had worse obstruction compared with patients without concavity (RAR_{high} group) on the SEFV curve of whom 31% (4 out of 13 patients) were categorized as GOLD 2 COPD.

3.2 Effect of placebo and tiotropium treatment on spirometry

At baseline, both the RAR_{high} and RAR_{low} groups showed significantly lower FEV_1 and FEV_1/FVC than the control group (both $p = 0.001$), and the RAR_{low} group had significantly worse spirometry data than the RAR_{high} group (Table 1). Compared with baseline, FEV_1 tended to be lower in both RAR groups after placebo treatment; however, this decrease did not reach statistical significance in either group. Conversely, after tiotropium FEV_1 increased significantly in both RAR groups compared with placebo (RAR_{high}, $p = 0.004$; RAR_{low}, $p = 0.038$; Table 1).

3.3 Initial characterization of SEFV curve and IC

During the baseline CWR tests, as ventilation increased, RAR responded differently in RAR_{low} participants compared with RAR_{high} and control participants (Fig. 4A). In general, as

previously shown, (Ma et al., 2010; Varga et al., 2016) control subjects increase RAR during exercise. Similarly, RAR tended to rise in the RAR_{high} group as exercise progressed. In the RAR_{low} group, RAR was significantly lower at rest and warm up compared with the other two groups, and failed to increase during exercise. IC increased as a function of $\dot{V}_E$/MVV with exercise in control subjects. In both the RAR_{high} and RAR_{low} groups, IC decreased with exercise, with the RAR_{low} group having lower absolute IC values throughout all stages of exercise than the other two groups (three-way ANOVA $p < 0.001$; Fig. 4B), suggesting greater dynamic hyperinflation. It is worth noting that the fall in IC during exercise is similar in both groups; the main difference is resting IC, which is lower in the RAR_{low} group.

3.4 Effect of placebo and tiotropium treatment on dynamic hyperinflation during CWR exercise

In the RAR_{high} group, after both placebo and tiotropium treatment, IC significantly decreased during exercise (two-way repeated measures ANOVA $p < 0.001$ between exercise phases); however, there was no difference between the two treatment conditions; the IC at peak exercise was 2.17 ± 0.03 L vs. 2.24 ± 0.03 L for placebo vs. tiotropium (Fig. 5A). A similar decrease in IC during exercise was found in the RAR_{low} group; however, after tiotropium treatment compared with placebo, IC was higher at all CWR test phases, with post hoc tests showing significance at rest, and at all exercise phases and in the first minute of recovery (all $p < 0.05$); the IC at peak exercise was 1.89 ± 0.05 L vs. 2.10 ± 0.05 L ($p = 0.045$) for placebo vs. tiotropium (Fig. 5B). In the RAR_{low} group, but not in the RAR_{high} group, tiotropium was associated with less static hyperinflation.

3.5 Effect of placebo and tiotropium treatment on RAR during CWR exercise

As in the control subjects (Fig. 4), as exercise proceeded the expiratory limb of the spontaneous flow–volume curves became more convex in the RAR_{high} group after both placebo and tiotropium treatment, i.e., RAR increased (Fig. 6A; two-way repeated measures ANOVA $p <$

0.001 for exercise phases) without significant difference between the two treatment modalities. For the RAR_{high} group, RAR was 0.64 ± 0.011 and 0.65 ± 0.011 at peak exercise for placebo and tiotropium, respectively. As in the RAR_{high} group, there was a similar pattern during exercise in the RAR_{low} group; however, the increase in convexity with exercise did not reach significance. On the other hand, after tiotropium treatment, the RAR was significantly higher than after placebo treatment (two-way ANOVA main effect for treatment $p < 0.001$) overall, during exercise and recovery, although post hoc comparisons did not reveal significant differences at any point of exercise (Fig. 6B). In the RAR_{low} group at peak exercise RAR was 0.53 ± 0.02 and 0.57 ± 0.02 for placebo and tiotropium, respectively (Fig. 6B).

3.6 Correlation of dynamic hyperinflation and RAR at end-exercise in CWR exercise tests

In the RAR_{low} group, considering baseline, placebo and tiotropium tests, there was a significant association between RAR and % predicted IC at peak exercise (Stocks and Quanjer, 1995) ($R^2 = 0.43$, $p = 0.0002$); tests with higher IC also had higher RAR (Fig. 7). These findings confirm those reported by Varga et al. (Varga et al., 2016) in a more spirometrically severe COPD group. In contrast, the RAR_{high} group showed a weaker and negative correlation with IC % predicted ($R^2 = 0.22$, $p = 0.0004$) (data not shown).

3.7 Exercise endurance

Exercise endurance of RAR_{high} patients was similar between the baseline, after placebo, and after tiotropium treatment (495 ± 187 s, 484 ± 184 s, and 487 ± 203 s, respectively). In the RAR_{low} group, two way ANOVA showed that, with respect to baseline, the change in exercise tolerance after placebo was -18 ± 72 s and the change after tiotropium was 68 ± 109 s; this difference, however, did not achieve significance ($p= 0.151$). However, the difference in exercise tolerance after tiotropium (498 ± 227 s) differed significantly from that after placebo (318 ± 76 s), $p=0.034$, by paired t-test.

3.8 Ratings of Perceived Dyspnea

The isotime Borg ratings of the perceived dyspnea before and after each therapeutic arm was 3.9 ± 1.4 before vs 4.9 ± 3.3 after placebo, and 3.5 ± 2.0 before vs. 3.7 ± 1.8 after tiotropium in the RAR_{low} group. The same comparison in the RAR_{high} group was 5.9 ± 2.4 before and 6.3 ± 2.4 after placebo and 6.0 ± 2.1 before and 5.8 ± 2.5 after tiotropium. The changes follow the logical expectation, however none of these comparisons were statistically significant. This finding is congruent with the finding reported in the primary manuscript based on this trial (Casaburi et al., 2014).

4. Discussion

In healthy control subjects, the expiratory limb of the flow–volume curve is convex and becomes more convex on exercise. In contrast, we have observed in this study in GOLD 1 and 2 COPD patients, that there is a subset of patients in whom the expiratory limb of the flow–volume curve becomes less convex, and even in some cases (in 7 out of 20 patients) becomes concave on exercise. This might reflect increased airway resistance, which contributes to causing dynamic hyperinflation. In the RAR_{low} group (i.e., those patients with concavity in the expiratory limb of the flow–volume curve during exercise), the main effect of tiotropium treatment appears to have been on resting hyperinflation: at rest (pre-exercise), IC was higher in the tiotropium group compared with the placebo group, and this difference between treatments was maintained throughout exercise and in the first minute of recovery. This difference was seen, however, only in patients who demonstrate concavity during exercise. In the RAR_{high} group, IC at rest and over the exercise period was similar between treatment groups, indicating they did not respond to tiotropium therapy and there were similar RARs during rest and exercise.

Among tests, end-exercise RAR and IC showed a significant positive correlation. This is the first report showing that the shape of SEFV curve responds to bronchodilator therapy in COPD patients who demonstrate concavity during exercise, and that this response is associated with less dynamic hyperinflation. Patients with concavity in the SEFV curve have significantly lower resting FEV_1 than those without.

LAMAs are currently recommended as first-line therapy for moderate-to-severe COPD (Group B, C, and D) and as second line therapy for mild COPD (Group A) as an alternative to short-acting bronchodilators (Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2017). However, spirometry alone is inadequate in distinguishing which patients may benefit from LAMA therapy. Previous studies have shown that graphical analysis of SEFV curve can be useful in identifying progressive expiratory flow limitation (Lee et al., 2016; Ma et al., 2010) and correlates better with clinical parameters, such as the 6-minute walk test distance, than do traditional spirometric values (Lee et al., 2016). This study is the first to investigate whether the SEFV curve changes with interventions that are designed to improve expiratory flow.

The aim of this study was to utilize geometric analysis of expiratory flow–volume curves to discriminate between subgroups of GOLD 1 and 2 COPD patients who may have different airway dynamics. This study showed that a subgroup of COPD patients who exhibited some concavity during exercise formed a distinct phenotype that was associated with more dynamic hyperinflation, as shown by a smaller IC at rest and similarly smaller IC was maintained during exercise. Although, as compared with placebo treatment, FEV_1 increased in response to tiotropium in all COPD patients, the change versus baseline was only significant in the RAR_{low} group. This subgroup of patients with some concavity in SEFV curve at baseline had a statistically significant increase in RAR in response to tiotropium, while the subgroup of patients who did not develop SEFV curve concavity at baseline had no RAR response to tiotropium. This is consistent with previous reports of heterogeneity within groups of COPD patients with similar spirometry and reaffirms the potential benefits of an individualized approach to patient therapy

(Gagnon et al., 2015). The method of measuring RAR change is especially attractive due to its ability to characterize the profile of the expiratory flow–volume curve during spontaneous breathing without the need for additional ventilatory maneuvers.

It is worth also noting that the exercise endurance of the RAR_{high} patients was virtually insensitive to any treatment or lack thereof (495 ± 187 s, 484 ± 184 s, and 487 ± 203 s during baseline, after placebo, and after tiotropium treatment, respectively). On the other hand, RAR_{low} patients decreased exercise endurance after placebo (338 ± 81 to 318 ± 76 s, paired t-test, not significant), and increased endurance after tiotropium (498 ± 227 s, paired t-test vs. placebo $p = 0.034$). The difference (180 s) exceeds the minimal clinically important difference (105 s in training-related trials and 60 s for bronchodilator trials) established for CWR exercise tests (Puente-Maestu et al., 2016). In the parent study (Casaburi et al., 2014), tiotropium both reduced hyperinflation and increased exercise endurance in GOLD 2 COPD patients, but only hyperinflation was reduced in GOLD 1 COPD patients who had no change in exercise endurance. In this substudy, neither the IC nor exercise endurance increased in the RAR_{high} group, which was comprised mainly of GOLD 1 patients (9 out of 13). These results, suggest that better airway dynamics (higher RAR) may be responsible for reducing dynamic hyperinflation and increasing exercise tolerance.

The initial group selection for this study was based on RAR above or below 0.5 in at least one of the baseline exercise tests. However, it appears that control RAR is closer to 0.6 at rest and increases during exercise. Therefore, a resting or exercise RAR in the SEFV curve that is below that found in normal controls is likely sufficient to distinguish patients at risk for dynamic hyperinflation and exercise intolerance, and who will respond positively in these variables to bronchodilator treatment. Many COPD patients, as in this study, have an RAR >0.5 at rest, and while, in some, this tends to decrease with exercise, it may not fall below 0.5 before the patient reaches peak exercise. Nevertheless, an SEFV curve during expiration that is less convex than in normal subjects might indicate expiratory flow limitation. Therefore, irrespective of whether

the value of RAR falls below 0.5, the comparison between those who increase versus those who decrease the RAR during exercise seems to be a reasonable focus of future studies.

A major limitation of this substudy is only having data for a portion of subjects tested in the parent study; hence, it was not powered for these analyses and this limits the generalizability of our findings. Despite this and the modest sample size, it was possible to observe statistically significant changes in RAR in response to placebo and tiotropium treatments. We believe these data help us to better understand the differences in responses to tiotropium in GOLD 1 and GOLD 2 groups in the main study whereby patients with concavity in the SEFV curve, rather than simply those with low FEV_1, are more likely to respond positively to LAMA therapy. Whether RAR of <0.5 is a useful demarcation point for predicting benefits of bronchodilator therapy (e.g., improved exercise tolerance, reduced hyperinflation) is not assessed. A separate study with a larger sample size is needed to understand if 0.5 or another RAR value would provide better discrimination.

In summary, this study showed that subgroups of patients with different airway dynamics exist within GOLD classification groups. The RAR method can be used non-invasively to identify patients with concavity in the SEFV curve who represent a small proportion of GOLD 1 patients (1 out of 10 [10%] in this study population) but most GOLD 2 patients (6 out of 10 [60%] in the group we studied). Patients with low RAR at rest and during exercise demonstrate a significant reduction in expiratory flow limitation after tiotropium therapy, as well as in dynamic hyperinflation that is associated with increase in exercise endurance. Although it is true that there was some difference between FEV_1 % predicted between the two groups, it appears that this type of analysis of the SEFV curves adds to the dynamics of airway function in that only those subjects improved their dynamic hyperinflation in response to tiotropium therapy who showed a decrease in RAR relative to the resting value. Future studies might seek to reproduce these findings in a larger population and in COPD patients with more severe obstruction.

Statement of interests

Janos Porszasz reports personal fees and non-financial support from Boehringer Ingelheim during the conduct of the study. In addition he has "Exercise test speed and grade modification" US #7628732 & US #7927251 patents issued. He currently has no other conflicts of interest to declare.

Nicolò Carraro, Robert Cao, Ashwani Gore, Shuyi Ma, Thomas Jiang, and Denis O'Donnell have no conflicts of interest to declare.

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Author contributions

All authors meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE).

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Figure legends

Fig. 1. Study design. In this analysis, the constant work rate exercise tests at Visit 3 and Visit 5 are considered as 'baseline' tests and Visit 4 and Visit 6 were either after placebo or tiotropium treatment phase. Reprinted with permission of the American Thoracic Society. Copyright © 2017 American Thoracic Society. Casaburi R, Maltais F, Porszasz J, Albers F, Deng Q, Iqbal A, Paden HA, O'Donnell DE. 2014. Effects of tiotropium on hyperinflation and treadmill exercise tolerance in mild to moderate chronic obstructive pulmonary disease. *Annals of the American Thoracic Society.* Volume 11(9), Pages 1351-61. *Annals of the American Thoracic Society* is an official journal of the American Thoracic Society.

Fig. 2. Derivation of the rectangular area ratio [RAR]. RAR, rectangular area ratio; $\dot{V}_{EE}$, end-expiratory flow rate; $\dot{V}_{max}$, intra-breath peak flow rate. Reprinted from *Respiratory Physiology & Neurobiology*, Volume 234, Varga J, Casaburi R, Ma S, Hecht A, Hsia D, Somfay A, Porszasz J. Relation of concavity in the expiratory flow–volume loop to dynamic hyperinflation during exercise in COPD, Pages 79-84, 2016©, with permission from Elsevier.

Fig. 3. Sample outputs from **A**: a subject showing no concavity and **B**: a subject showing concavity during exercise from IGOR procedures of breath-by-breath analysis. Traces from top to bottom: peak intrabreath flow rate (L/sec; $\dot{V}_{max}$), end-expiratory flow rate (L/sec; ($\dot{V}_{EE}$), rectangular area ratio (RAR), and instantaneous flow (L/sec); expiration upward, inspiration downward. The horizontal red line on the RAR panel represents RAR=0.5. The black lines are the result of an exponential smoothing; the corresponding RAR values were taken from this smooth line.

Fig. 4. A: Rectangular area ratio (RAR) and **B**: inspiratory capacity (IC) in control, RAR_{high} group and RAR_{low} group COPD patients during baseline constant work rate exercise tests as a function of ventilatory demand (minute ventilation/maximum voluntary ventilation [$\dot{V}_E$/MVV]). Data points are least square mean ± standard error of least square mean. For the control group data are from Visit 3; for COPD patients the points are the averages from Visit 3 and 5 (baseline

visits). In both panels, points from left to right: rest, warm-up, 4 and 2 min before peak exercise and peak exercise. **A**: RAR in controls, RAR_{high} group and RAR_{low} group COPD patients as a function of ventilatory demand ($\dot{V}_E$/MVV). Two-way repeated measures (RM) ANOVA $p < 0.001$, *: Pairwise multiple comparison by (Student–Neuman–Keuls test $p < 0.01$ for both controls and RAR_{high} group patients). **B**: Inspiratory capacity in controls, and RAR_{high} group and RAR_{low} group COPD patients as a function of ventilatory demand ($\dot{V}_E$/MVV). There are no statistical significant differences between groups by two-way RM ANOVA. MVV = forced expiratory volume in 1 s × 40.

Fig. 5. Change in inspiratory capacity (IC) in response to placebo or tiotropium treatment during constant work rate exercise in **A**: RAR_{high} group and **B**: RAR_{low} COPD patients. Data points are least square mean ± standard error of least square mean. In the RAR_{low} group, but not RAR_{high} group, the course of change in IC are significantly different between the two treatments (two-way repeated measures ANOVA $p < 0.01$); *: denote significant difference according to pairwise comparison (Holm–Šídák $p < 0.05$). Exercise phases: 1r: mean of three ICs during rest, w: warm up, p-4: 4 min before peak exercise, p-2: 2 min before peak exercise, r1: first minute into recovery.

Fig. 6. Rectangular area ratio (RAR) at rest and during exercise in response to placebo and tiotropium treatment during constant work rate exercise in the **A**: RAR_{high} group and **B**: RAR_{low} group COPD patients. Data points are least square mean ± standard error of least square mean. In the RAR_{low} group, but not the RAR_{high} group, the RAR in the two treatment conditions are significantly different (two-way ANOVA $p < 0.001$). Multiple comparison did not reveal significances between any of the points during exercise. Exercise phases: 1r: mean of three ICs during rest, w: warm up, p-4: 4 min before peak exercise, p-2: 2 min before peak exercise, r1: first minute into recovery.

Fig. 7. Correlation between inspiratory capacity (IC) % predicted and the rectangular area ratio (RAR) at peak exercise in the 7 RAR_{low} patients. Data points are from all tests in these subjects,

including baseline and after both placebo and tiotropium treatment phase. (R^2 = 0.43, p = 0.0002, y0 = –59, a = 260, SEE = 15.4). Predicted inspiratory capacity was calculated as total lung capacity predicted – functional residual capacity predicted. For these reference values those of the Official Statement of the European Respiratory Society (Stocks and Quanjer, 1995) were used.

Table 1 Subject characteristics.

	Control (n = 16)	RAR_{high} group (RAR >0.5 in baseline studies) (n = 13)	RAR_{low} group (RAR ≤0.5 in baseline studies) (n = 7)	One-way ANOVA p-value
Males/females	8/8	6/7	2/5	
GOLD 1/GOLD 2, n	0/0	9/4	1/6	
Age, years	60.4 ± 8.1	61.0 ± 7.0	60.1 ± 8.2	
FEV_1, L (at baseline)	2.78 ± 0.65	2.15 ± 0.47[a]	1.60 ± 0.59[a]	0.001
FEV_1, % predicted[f]	103.2 ± 17.9	79.6 ± 10.1[a]	63.4 ± 14.0[a,d]	0.001
FVC, L (at baseline)	3.39 ± 0.81	3.62 ± 0.85	3.06 ± 0.88	0.365
FEV_1/FVC	82.3 ± 4.2	59.9 ± 5.6[a]	51.7 ± 7.4[a,d]	0.001
FEV_1, L post placebo		2.03 ± 0.45	1.49 ± 0.70[e]	
FEV_1, L post tiotropium		2.22 ± 0.54[b]	1.64 ± 0.71[c]	

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RAR, rectangular area ratio.

[a] $p < 0.05$ vs. control (Holm–Šídák post hoc test).

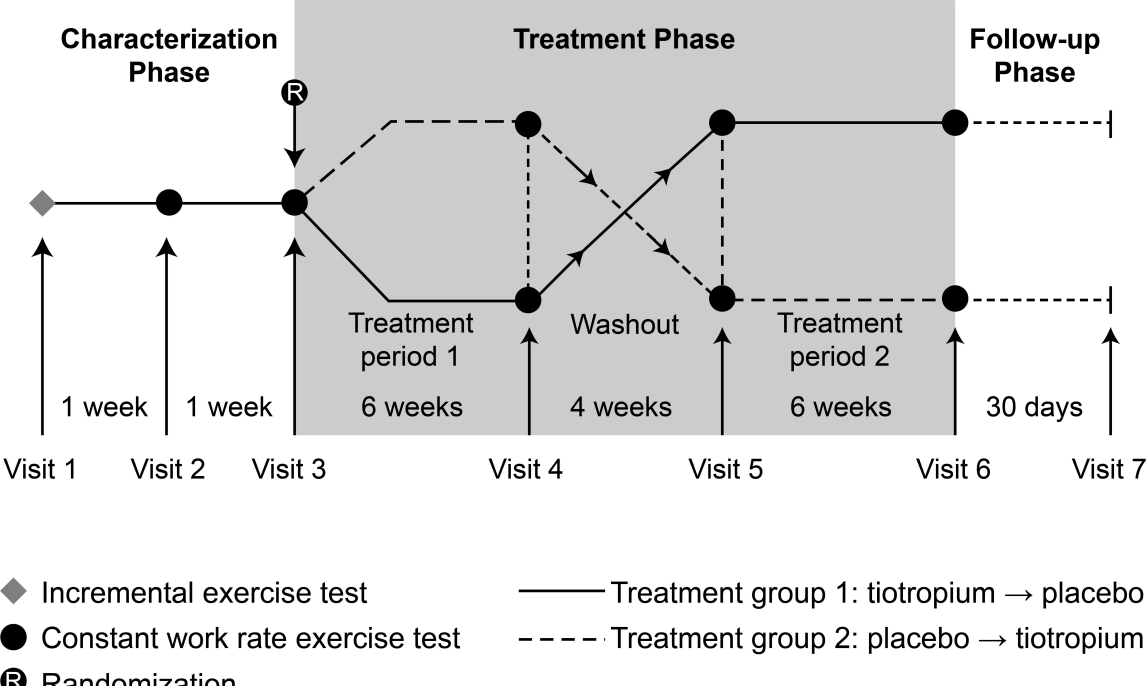
[b] $p = 0.004$ vs. placebo (paired two-tailed t-test).

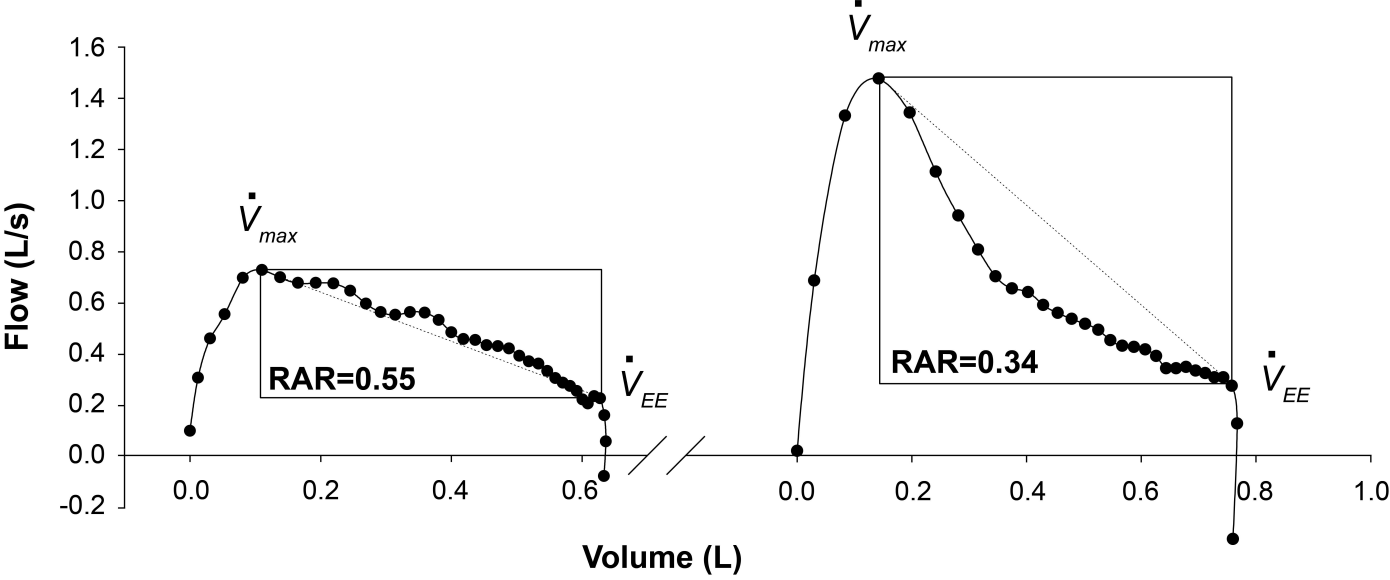
[c] $p = 0.038$ vs. placebo (paired two-tailed t-test).

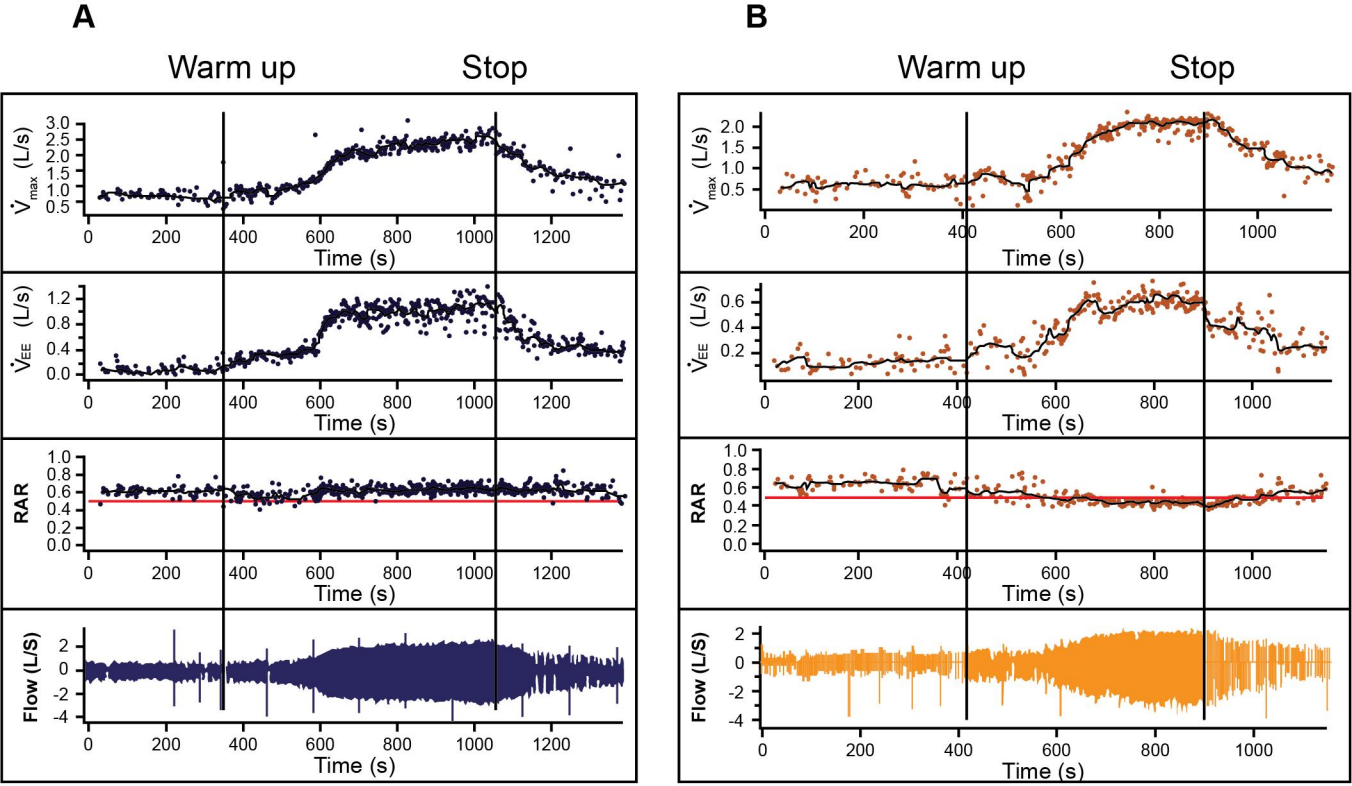
[d] $p = 0.003$ vs. RAR_{high} (Holm–Šídák post hoc test).

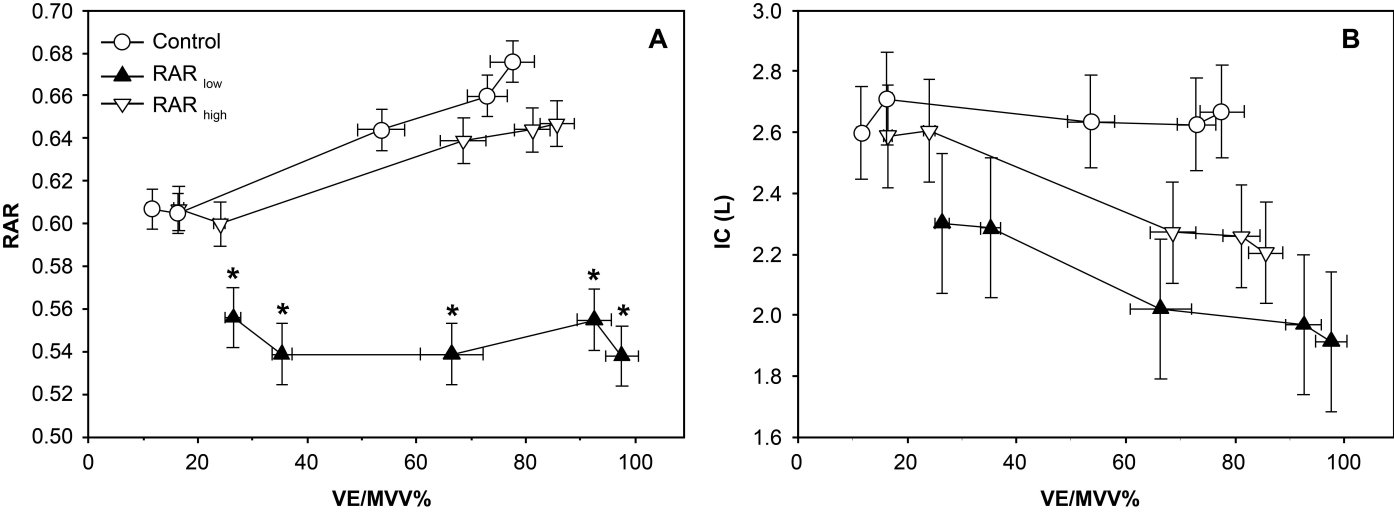
[e] $p = 0.047$ Student unpaired two-tailed t-test (RAR_{high} vs. RAR_{low}).

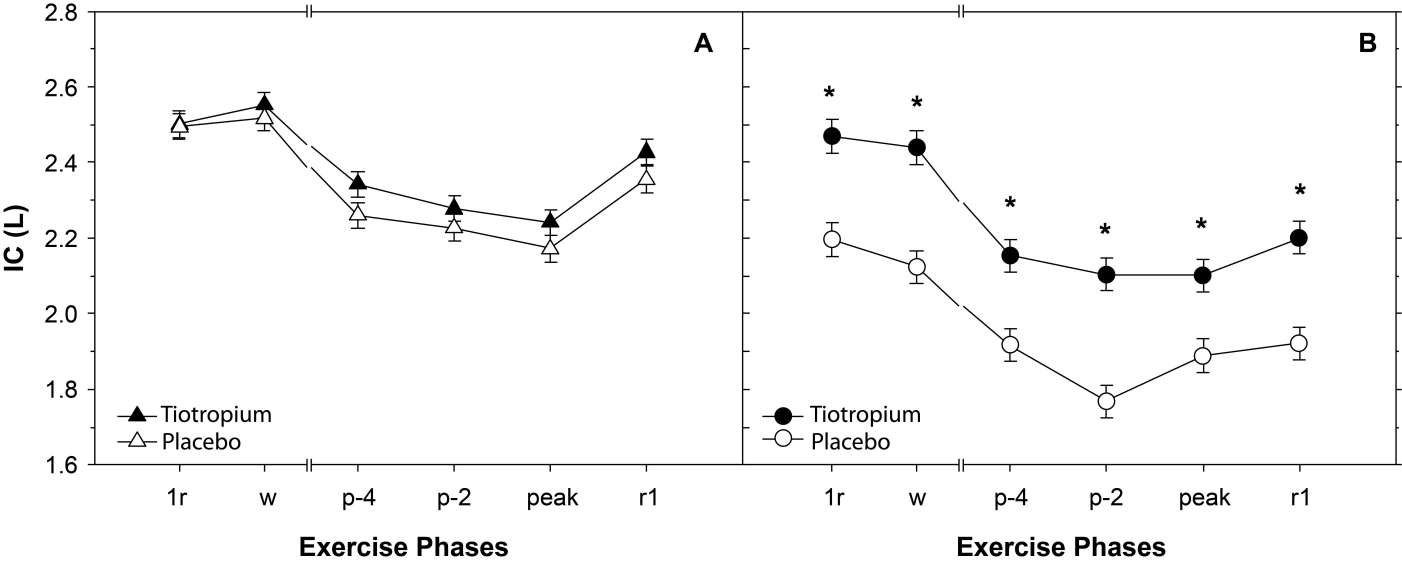
[f] Predicted values for FVC and FEV_1 are from Quanjer et al.

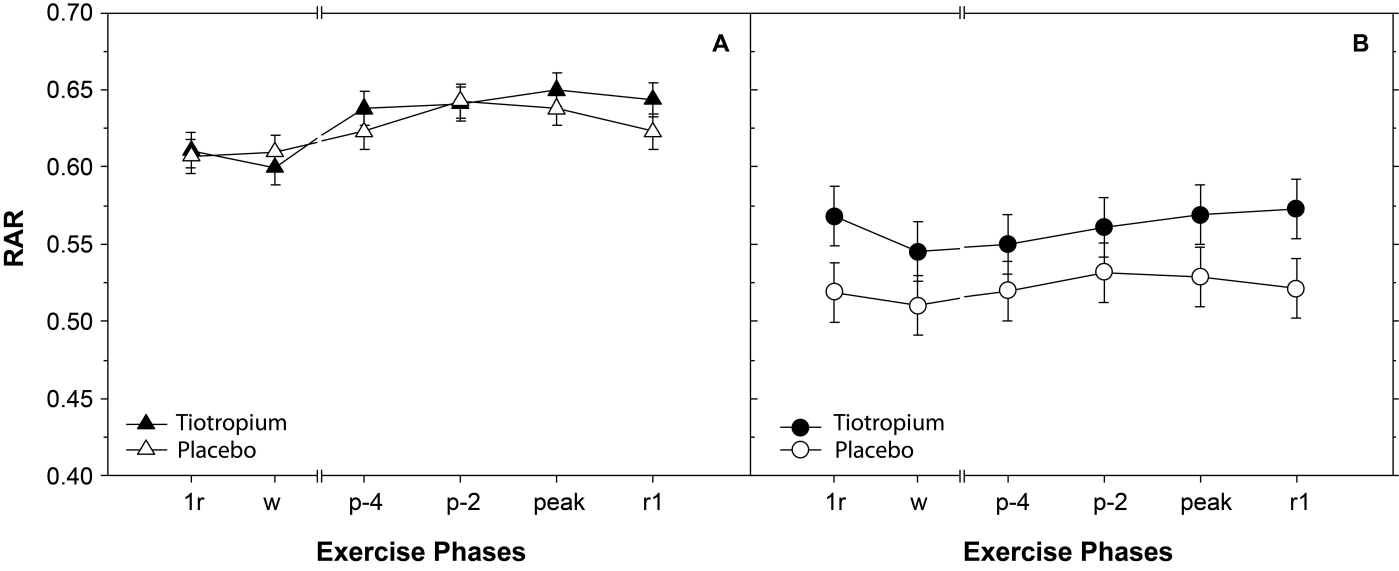

Characterization Phase
Treatment Phase
Follow-up Phase
R
Treatment period 1
Washout
Treatment period 2
1 week
1 week
6 weeks
4 weeks
6 weeks
30 days
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
Visit 7
Incremental exercise test
Constant work rate exercise test
Randomization
Treatment group 1: tiotropium → placebo
Treatment group 2: placebo → tiotropium

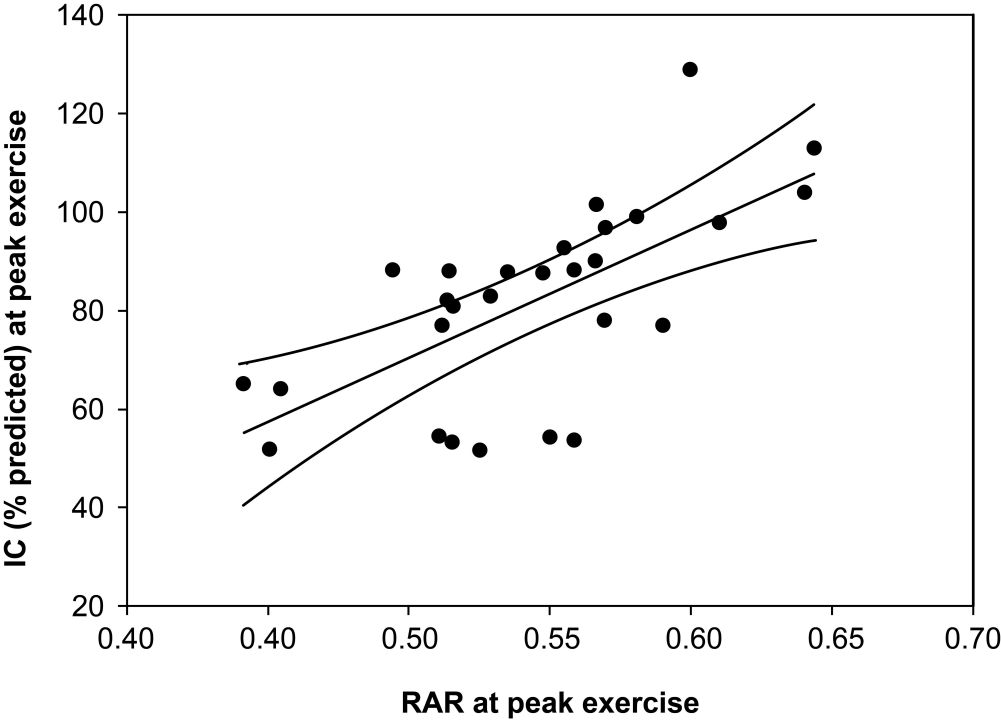

Flow (L/s)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
$\dot{V}_{max}$
RAR=0.55
$\dot{V}_{EE}$
0.0
0.2
0.4
0.6
$\dot{V}_{max}$
RAR=0.34
$\dot{V}_{EE}$
0.0
0.2
0.4
0.6
0.8
1.0
Volume (L)


A
Warm up
Stop
$\dot{V}_{max}$ (L/s)
3.0
2.5
2.0
1.5
1.0
0.5
0
200
400
600
800
1000
1200
Time (s)
$\dot{V}_{EE}$ (L/s)
1.2
0.8
0.4
0.0
RAR
1.0
0.8
0.6
0.4
0.2
0.0
Flow (L/S)
2
0
-2
-4
B
Warm up
Stop
$\dot{V}_{max}$ (L/s)
2.0
1.5
1.0
0.5
$\dot{V}_{EE}$ (L/s)
0.6
0.4
0.2
RAR
1.0
0.8
0.6
0.4
0.2
0.0
Flow (L/S)
2
0
-2
-4
0
200
400
600
800
1000
Time (s)


A
Control
RAR low
RAR high
RAR
VE/MVV%
0.70
0.68
0.66
0.64
0.62
0.60
0.58
0.56
0.54
0.52
0.50
0
20
40
60
80
100
*
B
IC (L)
VE/MVV%
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6


A
B
IC (L)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
Tiotropium
Placebo
1r
w
p-4
p-2
peak
r1
Exercise Phases
*


A
B
RAR
0.70
0.65
0.60
0.55
0.50
0.45
0.40
Tiotropium
Placebo
1r
w
p-4
p-2
peak
r1
Exercise Phases


140
120
100
80
60
40
20
IC (% predicted) at peak exercise
0.40
0.40
0.50
0.55
0.60
0.65
0.70
RAR at peak exercise