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Mechanisms of dyspnoea relief and improved exercise endurance after furosemide inhalation in COPD

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Ventilatory and perceptual responses to inhaled furosemide during exercise in COPD.

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ABSTRACT

This study examined the effects of inhaled furosemide on the ventilatory and perceptual response to high intensity, constant-load cycle exercise in chronic obstructive pulmonary disease (COPD).

Methods: In a randomized, double-blind, placebo-controlled, cross-over study, 20 patients with COPD (forced expiratory volume in 1 sec 45 ± 15 % predicted; mean ± SD) received either nebulized furosemide 40 mg or placebo on two separate days. Thirty minutes after each treatment, patients performed pulmonary function tests and a symptom-limited cycle exercise test at 75% of their maximum incremental work-rate. Post-dose changes in spirometry, plethysmographic lung volumes, dynamic operating lung volumes, ventilation, breathing pattern, cardiovascular function, dyspnoea intensity and exercise endurance time were compared between-treatments.

Results: After treatment with furosemide compared with placebo, dyspnoea intensity at the highest equivalent exercise time (i.e., isotime for each patient) decreased by 0.9 ± 1.0 Borg units ($p<0.01$), with attendant improvement in exercise endurance time by 1.65 ± 0.63 min ($p<0.05$). These improvements were associated with increases in dynamic inspiratory capacity, tidal volume and mean tidal expiratory flow rates at isotime ($p<0.01$). The eight patients who improved exercise endurance time by >1 min had greater changes in operating lung volumes ($p<0.05$), submaximal oxygen pulse ($p<0.05$) and oxygen uptake ($p=0.05$) compared with those who did not.

Conclusion: Our results suggest that alleviation of exertional dyspnoea after single dose furosemide inhalation in COPD is multifactorial but that improvements in dynamic ventilatory mechanics are contributory in some individuals.

INTRODUCTION

Perceived respiratory difficulty (dyspnoea) and activity limitation are the dominant symptoms of chronic obstructive pulmonary disease (COPD) and contribute importantly to perceived poor health-related quality of life in this population.[1] Traditionally, efforts to alleviate dyspnoea have largely focused on improving dynamic ventilatory mechanics, reducing ventilatory demand, or both of these in combination.[2-8] However, recent studies have indicated that it may be possible to reduce the perception of exertional dyspnoea and improve exercise tolerance by altering the activity of vagal pulmonary afferents. In this regard, several published studies have found that inhalation of nebulized furosemide, a powerful loop diuretic known to modulate the activity of sensory afferents in the lungs and airways of laboratory animals,[9,10] alleviates the sensation of dyspnoea provoked by different respiratory stimuli applied experimentally in healthy humans.[11-13] Ong *et al*[14] showed that inhaled furosemide reduced dyspnoea intensity at a standardized time during constant work rate cycle exercise but not during incremental exercise in patients with COPD. A more recent study by Laveneziana *et al*[15] also found no effect on respiratory sensation during incremental exercise with simulated expiratory flow-limitation in healthy humans. While the study by Ong *et al* suggested that altered vagal afferent activity in response to topical furosemide directly and independently influenced the intensity and quality of dyspnoea in COPD, these authors did not conclusively rule out the possible simultaneous effects of inhaled furosemide on ventilatory demand and/or dynamic ventilatory mechanics. In particular, it remains unclear whether inhalation of furosemide, by altering vagal pulmonary afferent activity and reflexly reducing cholinergic tone of airway smooth muscle, can improve dynamic airway function (i.e., reduce dynamic hyperinflation) and in this manner contribute to exertional dyspnoea relief. It is also possible that inhaled furosemide may affect the cardiovascular response to exercise by either reducing dynamic pulmonary hyperinflation or by direct diuretic effects.

Therefore, the purpose of this study was to determine if reductions in exertional dyspnoea ratings following inhalation of furosemide was associated with improvements in ventilatory mechanics, ventilatory demand and/or cardiovascular function, or was independent of these mechanisms. Our hypothesis was that improvement in exertional dyspnoea and exercise endurance in response to treatment with inhaled furosemide will be associated with a bronchodilator action of this medication. In a randomized, double-blind, placebo-controlled crossover study, we therefore compared the effects of inhaled furosemide and placebo on dyspnoea intensity, airway function, ventilation, breathing pattern, dynamic operating lung volumes, pulmonary gas exchange, cardiovascular function and exercise endurance during high-intensity constant-load exercise in COPD.

METHODS

Subjects

Subjects included clinically stable patients with COPD who were > 40 yrs of age with a cigarette smoking history ≥ 20 pack-years, forced expiratory volume in 1 second (FEV_1) ≤ 70% predicted, FEV_1/forced vital capacity (FVC) < 70%, and a modified Baseline Dyspnoea Index focal score ≤ 6 [16]. Patients were excluded in the presence of significant diseases other than COPD that could contribute to dyspnoea and exercise limitation; a history of asthma, atopy, nasal polyps;

exercise-induced arterial blood O_2 desaturation to < 80% on room air; or drug allergy to sulfa.

Study design

This randomised, double-blind, placebo-controlled, cross-over study was approved by the local university/hospital research ethics committee. After giving written, informed consent patients completed: *(1)* an initial screening visit to determine eligibility for the study; *(2)* a second visit designed to familiarise patients with all tests that would be performed during subsequent treatment visits; and *(3)* two treatment visits, randomised to order, conducted 2-10 days apart. Visit 1 included a thorough medical history, clinical assessment, chronic dyspnoea evaluation, complete pulmonary function testing and a symptom-limited incremental cycle exercise test. Visit 2 included pulmonary function tests and a constant-load exercise test. After randomisation of treatments (visits 3 and 4), baseline pulmonary function tests were performed before patients inhaled a 4 ml solution containing either furosemide 40 mg (10 mg/ml) or placebo (0.9% saline), administered by means of a jet nebuliser (Parimaster compressor with Pari LC Jet+ nebuliser; PARI Respiratory Equipment Inc., Richmond, VA, USA) with subjects breathing spontaneously for 15-min using a facemask. Approximately 30-min after inhalation of either furosemide or placebo, subjects performed pulmonary function tests followed by a symptom-limited constant-load cycle exercise test at 75% of their maximal incremental work rate. Before each visit, subjects were asked to withdraw from short-acting β_2-agonists (4 hours), short-acting anticholinergics (6 hours), long-acting β_2-agonists (12 hours), long-acting anticholinergics (24 hours), short-acting theophyllines (24 hours) and long-acting theophyllines (48 hours). Subjects avoided caffeine and heavy meals for at least 4 hours prior to testing and avoided alcohol and major physical exertion entirely on visit days. All visits were conducted at the same time of day for each subject.

Pulmonary function testing

Pulmonary function tests, including routine spirometry and constant-volume body plethysmography, were conducted in accordance with recommended techniques [17,18] by use of automated equipment (Vmax 229d with Autobox 6200 D_L; SensorMedics, Yorba Linda, CA). Measurements were expressed as percentages of predicted normal values [19-21]; predicted normal inspiratory capacity (IC) was calculated as predicted total lung capacity (TLC) minus predicted FRC.

Cardiopulmonary exercise testing

Symptom-limited exercise tests were conducted on an electronically braked cycle ergometer (Ergoline 800S; SensorMedics) by use of a cardiopulmonary exercise testing system (Vmax229d; SensorMedics) as previously described.[5-7] Incremental exercise testing was performed at Visit 1 and consisted of a steady-state resting period of at least 3 minutes, followed by 1 minute of unloaded pedaling with subsequent increases in work rate in stepwise increments of 10 W each minute, starting at 10 W. Maximal work rate (W_{max}) was defined as the highest work rate that the subject was able to maintain for at least 30 seconds. Constant-load exercise tests consisted of a steady-state resting period, a 1-min period of unloaded pedaling, and then an immediate stepwise increase in work rate to 75% W_{max} (rounded up to the nearest 5 W) which was maintained until the point of symptom-limitation; endurance time was defined as the duration of loaded pedaling.

Measurements were collected at rest and during exercise while subjects breathed through a mouthpiece and a low-resistance flow transducer with nasal passages occluded by a noseclip.

Measurements included the following: cardiopulmonary and breathing pattern parameters [minute ventilation (V'_E), oxygen uptake ($V'O_2$), carbon dioxide production ($V'CO_2$), end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$), tidal volume (V_T), breathing frequency (*F*), inspiratory (T_I) and expiratory time (T_E), inspiratory duty cycle (T_I/T_{TOT}), and mean inspiratory (V_T/T_I) and expiratory flow (V_T/T_E)] were collected on a breath-by-breath basis and compared with predicted normal values [22]; oxygen saturation (SpO_2) by pulse oximetry; heart rate by 12-lead electrocardiography; blood pressure by auscultation of the right brachial artery using a sphygmomanometer with an arm cuff; intensity of dyspnoea (breathing discomfort) and leg discomfort was assessed using the 10-point Borg scale [23] at rest, during the last 30-seconds of every 1-minute interval during exercise, and at end-exercise; operating lung volumes [end-inspiratory (EILV) and end-expiratory (EELV) lung volumes] were derived from IC manoeuvres [7,24] performed at rest, within the last 30-second period of each 2-minute interval during exercise, and at end-exercise; and reason(s) for stopping exercise was recorded.

All breath-by-breath measurements were averaged in 30-second intervals throughout each test stage, i.e., rest, exercise and recovery. Cardiorespiratory measurements collected over the first 30-second period of every second minute during exercise were linked with symptom ratings and IC measurements collected in the latter 30 seconds of the respective minute (to avoid contamination of averaged breath-by-breath data surrounding IC manoeuvres). Three main time points were used for evaluation of exercise parameters, i.e., pre-exercise rest, isotime and peak exercise. Pre-exercise rest was defined as the steady-state period after at least 3 minutes of breathing on the mouthpiece while seated at rest before the start of exercise: cardiorespiratory parameters were averaged over the last 30 seconds of this period and IC measurements for this period were collected during breathing on the same circuit immediately after completion of the quiet breathing period. *Isotime* was defined as the highest equivalent exercise time achieved during each of the constant-load tests performed by a given subject, rounded down to the nearest whole minute. *Peak* exercise was defined as the last 30 seconds of loaded pedaling: cardiorespiratory parameters were averaged over this time period, and IC measurements and Borg ratings of dyspnoea and leg discomfort were collected immediately at the end of this period.

Statistical analysis

The sample size of 20 subjects provides the power (80%) to detect a significant difference in dyspnoea intensity measured at a standardized time during exercise based on a relevant difference in Borg dyspnoea ratings of ± 1 Borg unit, a SD based on values established in our laboratory for a similar group of 105 patients with COPD,[24] α=0.05 and a two-tailed test of significance. Treatment responses were compared by paired *t*-tests with appropriate Bonferroni adjustments for multiple comparisons. Reasons for stopping exercise were analysed using Fisher's exact test. Pearson correlations were used to establish associations between standardized (isotime) measurements of exertional dyspnoea intensity and relevant independent variables; forward stepwise multiple regression analysis was carried out with significant variables and relevant covariates. A $p<0.05$ significance level was used for all analyses. Results are reported as means ± SD unless otherwise specified.

A post hoc subgroup analysis was conducted after examination of the data showed that eight subjects had a clear furosemide-induced improvement in exercise endurance time by >1 min (*Responders*) compared with the remaining subjects who had no improvement in exercise endurance time (*Non-responders*). Within- and between-group comparisons were made using a two-way repeated measures analysis of variance.

RESULTS

Twenty subjects with moderate to severe COPD and significant chronic activity-related dyspnoea completed the study (Table 1); an additional subject with severe disease was withdrawn from the study due to worsening of COPD symptoms. Computed tomography scans of the chest were available for all subjects allowing examination of the presence of emphysema: 10 subjects had moderate to severe emphysema, the majority (9) of these with an upper lobe predominance; the remaining 10 subjects only had minimal airspace dilatation.

Table 1. Subject characteristics

Male : Female, n	11 : 9
Age, years	61.4 ± 8.3
Height, cm	168.0 ± 9.6
Weight, kg	79.9 ± 20.9
Body mass index, kg/m^2	28.4 ± 7.9
Smoking history, pack-years	47.7 ± 20.2
Duration of COPD, years	9.4 ± 7.8
Modified Baseline Dyspnoea Index, focal score	5.8 ± 0.7
FEV_1, L (% predicted)	1.16 ± 0.36 (45 ± 15)
FEV_1/FVC, % (% predicted)	42 ± 10 (59 ± 14)
FVC, L (% predicted)	2.78 ± 0.63 (75 ± 14)
IC, L (% predicted)	2.19 ± 0.53 (82 ± 24)
FRC, L (% predicted)	4.34 ± 1.60 (134 ± 37)
RV, L (% predicted)	3.45 ± 1.44 (162 ± 59)
sRaw, $cmH_2O \cdot s$ (% predicted)	28.1 ± 13.7 (667 ± 312)
DL_{CO}, ml/min/mmHg (% predicted)	15.1 ± 4.2 (70 ± 17)

Values are means ± SD.
FEV_1, forced expired volume in one second; FVC, forced vital capacity; IC, inspiratory capacity; FRC, plethysmographic functional residual capacity; RV, residual volume; TLC, total lung capacity; sRaw, specific airway resistance; DL_{CO}, diffusing capacity of the lung for carbon monoxide.

Treatment order was balanced such that nine (45%) were randomized to receive treatment with furosemide first. There were no pre-dose differences in spirometric parameters or plethysmographic lung volumes between placebo and furosemide treatment visits, other than a significantly (p=0.033) higher peak expiratory flow rate (PEFR) on the furosemide treatment day (Table 2). However, this difference in PEFR was maintained after furosemide and resulted in no significant treatment response compared with placebo.

Table 2. Pulmonary function tests

	Placebo		Furosemide	
	Pre-dose	**ΔPost-dose**	**Pre-dose**	**ΔPost-dose**
FEV_1, L	1.16 ± 0.37	-0.02 ± 0.11	1.19 ± 0.37	0.02 ± 0.08
FEV_1/FVC, %	42 ± 9	-0.9 ± 2.2	42 ± 9	-0.0 ± 4.7
FVC, L	2.79 ± 0.65	0.01 ± 0.19	2.84 ± 0.73	0.06 ± 0.29
PEFR, L/s	3.95 ± 0.97	-0.03 ± 0.35	4.17 ± 1.17 *	-0.04 ± 0.30
$FEF_{25-75\%}$, L/s	0.36 ± 0.12	-0.03 ± 0.07	0.36 ± 0.15	-0.01 ± 0.06
IC, L	2.18 ± 0.54	0.01 ± 0.11	2.23 ± 0.56	0.08 ± 0.18 †
SVC, L	3.07 ± 0.81	-0.00 ± 0.20	3.08 ± 0.74	0.13 ± 0.18 † ‡
FRC, L	4.35 ± 1.61	-0.00 ± 0.26	4.34 ± 1.57	-0.08 ± 0.14 †
RV, L	3.45 ± 1.44	0.01 ± 0.38	3.49 ± 1.31	-0.13 ± 0.20 †
TLC, L	6.53 ± 1.62	0.01 ± 0.23	6.57 ± 1.57	-0.00 ± 0.18
Raw, cmH_2O/L/s	5.4 ± 2.3	0.3 ± 0.7	5.2 ± 2.3	-0.2 ± 0.7

Values are mean ± SD.
* p<0.05 pre-treatment difference;
† p<0.05 post- *vs* pre-dose difference within treatment;
‡ p<0.05 difference between acute within-drug treatment responses (post- minus pre-drug).
ΔPost-dose, change from pre- to post-dose; FEV_1, forced expired volume in one second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; $FEF_{25-75\%}$, mean forced expiratory flow between 25% and 75% of FVC; $FEF_{25\%}$, forced expiratory flow when 25% of FVC has been expired; $FEF_{50\%}$, forced expiratory flow at 50% of FVC; $FEF_{75\%}$, forced expiratory flow at 75% of FVC; IC, inspiratory capacity; SVC, slow vital capacity; FRC, plethysmographic functional residual capacity; RV, residual volume; TLC, total lung capacity; Raw, airway resistance.

Post-treatment changes in resting pulmonary function are shown in Table 2. There were no significant pre- to post-dose differences in response to treatment with placebo. After furosemide: measurements of forced spirometry did not change; TLC did not change; slow vital capacity (SVC) and inspiratory capacity (IC) increased; and FRC and residual volume (RV) decreased. Despite these within-treatment differences, the only significant treatment response after furosemide compared to placebo was in the pre- to post-dose change in SVC (mean difference 0.13 L, 95% CI 0.05 to 0.21 L, p=0.002); the treatment response in RV (mean difference -0.14 L, 95% CI -0.30 to 0.01 L, p=0.060) reflected that of SVC since TLC did not change. The increase in SVC (decrease in RV) after furosemide may be explained, in part, by the prolongation of total expiratory time by an average of 1.7 seconds (p=0.08) during the vital capacity manoeuvre (r=0.52, p=0.014).

Treatment responses to constant-load cycle exercise

Exercise endurance time increased by 1.65±2.9 min (16±21%) after treatment with furosemide compared with placebo (p=0.017 paired t-test; p=0.004 Wilcoxon signed rank test): endurance time increased by >1 minute in 8 subjects (mean increase of 4.4±2.2 minutes), changed by less than ±1 minute in 11 subjects (mean change of 0.2±1.3 minute), and decreased by 4 minutes (13%) in 1 subject who had a very long endurance time of 30 minutes after placebo. The distribution of reasons for stopping exercise was different after furosemide compared with placebo (p=0.029): fewer subjects stopped primarily because of dyspnoea (25% *vs* 35%, respectively) and more subjects stopped because of leg discomfort (50% *vs* 40%) or a combination of breathing and leg discomfort (25% *vs* 10%).

Dyspnoea intensity during exercise is shown in Figure 1. After treatment with furosemide compared with placebo, dyspnoea intensity decreased by 0.9±1.0 Borg units (p=0.006) at isotime (10.2±8.7 min) during exercise (Table 3): 15 subjects decreased by at least 1 Borg unit, 4 subjects did not change their dyspnoea rating; and 1 subject increased by 2 Borg units. Borg ratings of perceived breathing and leg discomfort were not significantly different at end-exercise (Table 3).

There were no statistically significant treatment differences in pre-exercise resting measurements of breathing pattern, pulmonary gas exchange or cardiovascular function. Measurements at the symptom-limited endpoint of exercise (peak) are shown in Table 3: there were small but significant increases in peak $V'O_2$ (p=0.012), $V'CO_2$ (p=0.013), V'_E (p=0.009) and V_T/T_E (p=0.029) after furosemide compared with placebo. Measurements at the highest equivalent isotime during exercise are shown in Table 3: there were small increases in V_T (p=0.038), IC (p=0.028) and V_T/T_E (p=0.032) after treatment with furosemide compared to placebo.

Mean cardiorespiratory responses to exercise after treatment with furosemide and placebo are shown in an ***online data supplement***. Breathing pattern was relatively deeper and slower with a small but consistent prolongation of T_I and no significant change in V_T/T_I. Dynamic IC increased by approximately 0.12 L at rest and throughout exercise after treatment with furosemide compared to placebo; however, exercise-induced changes in dynamic IC from rest were not significantly different across treatments (Table 3). Furosemide had no significant effect on dynamic IRV throughout exercise.

Table 3. Post-dose cardiorespiratory and perceptual responses at the highest equivalent isotime and at the symptom-limited peak of constant-work-rate exercise at 75% W_{max} (53±4 watts)

	Isotime		Peak	
	Placebo	**Furosemide**	**Placebo**	**Furosemide**
Time, min	10.2 ± 8.7	10.2 ± 8.7	10.9 ± 9.0	12.6 ± 10.0 *
Dyspnoea, Borg	4.9 ± 2.1	4.1 ± 2.2 †	5.2 ± 2.2	5.2 ± 2.6
Leg discomfort, Borg	5.6 ± 2.5	5.0 ± 2.6	6.3 ± 2.7	6.3 ± 2.4
HR, beats/minute	115 ± 21	115 ± 19	116 ± 19	116 ± 19
O_2 pulse, mL/beat	9.5 ± 3.1	9.9 ± 3.7	9.7 ± 3.2	10.2 ± 3.1 *
$V'O_2$, mL/kg/min	13.6 ± 3.8	14.1 ± 3.7	14.0 ± 3.7	14.8 ± 3.3 *
$V'CO_2$, mL/kg/min	13.2 ± 4.1	14.0 ± 4.2 *	13.9 ± 4.0	14.8 ± 3.7 *
V'_E, L/min	34.8 ± 10.2	36.2 ± 11.9	36.1 ± 10.3	37.9 ± 10.5 *
V_T, L	1.17 ± 0.30	1.24 ± 0.33 *	1.18 ± 0.30	1.24 ± 0.31
f_R, breaths/min	30.5 ± 7.8	29.6 ± 7.4	31.4 ± 8.1	31.2 ± 7.2
T_I, sec	0.79 ± 0.20	0.82 ± 0.19	0.78 ± 0.20	0.77 ± 0.18
T_E, sec	1.29 ± 0.32	1.30 ± 0.31	1.28 ± 0.34	1.27 ± 0.28
T_I/T_{TOT}, %	38.0 ± 4.1	38.4 ± 4.0 *	38.6 ± 5.4	38.4 ± 4.2
V_T/T_I, L/sec	1.53 ± 0.46	1.56 ± 0.48	1.58 ± 0.47	1.65 ± 0.42
V_T/T_E, L/sec	0.94 ± 0.29	1.00 ± 0.34 *	0.96 ± 0.29	1.01 ± 0.30 *
IC, L	1.78 ± 0.46	1.90 ± 0.45 *	1.76 ± 0.43	1.84 ± 0.47
ΔIC from rest, L	-0.61 ± 0.38	-0.60 ± 0.47	-0.61 ± 0.42	-0.65 ± 0.44
IRV, L	0.61 ± 0.30	0.66 ± 0.29	0.58 ± 0.27	0.61 ± 0.32
$P_{ET}CO_2$, mmHg	37.6 ± 3.7	38.4 ± 4.2	37.7 ± 4.2	38.0 ± 4.5
SpO_2, %	93.6 ±2.5	93.8 ± 2.9	93.6 ± 2.7	93.6 ± 3.0

Values are mean ± SD.
*p<0.05, †p<0.01 furosemide *versus* placebo at same stage of exercise.
HR, heart rate; $V'O_2$, oxygen uptake; $V'CO_2$, carbon dioxide production; V'_E, minute ventilation; V_T, tidal volume; VC, vital capacity; f_R, breathing frequency; T_I, inspiratory tine; T_E, expiratory time; T_{TOT}, total time; V_T/T_I, mean inspiratory tidal flow; V_T/T_E, mean expiratory tidal flow; T_I/T_{TOT}, inspiratory duty cycle; IC, inspiratory capacity; ΔIC, change in IC from rest (i.e., extent of dynamic hyperinflation); IRV, inspiratory reserve volume; TLC, total lung capacity; $P_{ET}CO_2$, partial pressure of end-tidal CO_2; SpO_2, oxygen saturation measured by pulse oximetry.

Subgroup analysis

Baseline pulmonary function showed that Responders (subjects with a furosemide-induced improvement in exercise endurance time >1 min) had significantly less severe expiratory airflow limitation and hyperinflation than Non-responders (subjects who did not improve exercise endurance time). Mean improvements in exercise endurance and dynamic ventilatory mechanics that were seen in the group as a whole were driven by the changes seen in the *Responder* subgroup (Figure 2). Additional cardiovascular responses to inhaled furosemide were uncovered by the subgroup analysis: although heart rate and blood pressure responses to exercise did not change, oxygen pulse increased significantly ($p<0.05$) at isotime during exercise and at peak exercise (Figure 3). There were 7 patients in the *Non-responder* subgroup who did not improve exercise endurance but who reduced dyspnea intensity at isotime during exercise by 1 Borg unit. In these patients, there were no changes in cardiovascular measurements or intensity of leg discomfort, and changes in dynamic respiratory mechanics were intermediate to those of the *Responders* but non-significant (i.e., isotime IC increased by a mean of 0.11 L). Additional information on the subgroup analysis is presented in an ***online data supplement***.

Correlates of improvement

Intra-subject reductions in dyspnoea at the highest equivalent isotime correlated with concurrent changes in: T_I ($r=-0.78$, $p<0.0005$), V_T%predicted VC ($r=-0.61$, $p=0.004$), and IC%predicted ($r=-0.46$, $p=0.040$). Covariates of this improvement included baseline PEFR ($r=-0.66$, $p=0.002$), sRaw ($r=0.64$, $p=0.002$), IC ($r=-0.49$, $p=0.029$), FEV_1 ($r=-0.49$, $p=0.030$) and FEV_1/FVC ($r=-0.45$, $p=0.049$), all expressed as %predicted. Using stepwise multiple regression analysis, 80% of the variance in the change in dyspnoea intensity at isotime was explained by the combination of isotime changes in T_I, V_T/T_E and V_T ($r^2=0.796$). Increased exercise endurance time correlated significantly with the decreased dyspnoea intensity at isotime ($r=-0.45$, $p=0.047$).

DISCUSSION

The main findings of this study are as follows: *(1)* single-dose inhalation of nebulized furosemide was associated with reductions in the intensity of exertional dyspnoea with attendant improvement in exercise endurance time; *(2)* inhaled furosemide was associated with minor but consistent changes in slow vital capacity at rest, together with small mean changes in IC and breathing pattern during high-intensity, constant-load exercise; *(3)* improvements in exertional dyspnoea (and exercise endurance) occurred in the absence of significant changes in ventilatory demand and pulmonary gas exchange during exercise and correlated with improved volume and timing components of breathing; and *(4)* subgroup analysis determined that individuals who improved exercise endurance the most after furosemide inhalation showed greater improvements in airway and cardiovascular function during exercise.

The patients in this study had moderate-to-severe COPD with severe ventilatory constraints during exercise and clinically significant chronic activity-related dyspnoea. Consistent with the results of a previous study in COPD by Ong *et al*,[14] we found that single-dose inhalation of nebulized furosemide was associated with a significant decrease in ratings of dyspnoea intensity at a standardized time during exercise by an average of 0.9 Borg units ($p<0.01$); dyspnoea intensity was reduced by at least 1 Borg unit in 15 of 20 patients at isotime

after treatment with inhaled furosemide compared with placebo. This is the first study, however, to demonstrate significant improvement in exercise endurance time (by an average of 1.65 min or 16%; $p<0.02$) after treatment with inhaled furosemide compared with placebo in this population. Improved endurance time correlated with reduced dyspnoea intensity ratings at isotime during exercise. Differences in our results with those of Ong *et al* likely reflect differences in study protocols. For example, incremental and constant-load cycle exercise tests were conducted on separate days in our study so as to avoid the potentially confounding effects of peripheral locomotor muscle fatigue on measures of exercise endurance.[25,26]

We considered the following potential mechanisms of dyspnoea relief during exercise after treatment with inhaled furosemide: *(1)* reduced intrinsic mechanical loading of the inspiratory muscles, secondary to improved airway function and reduced dynamic hyperinflation; *(2)* reduced ventilatory demand; *(3)* furosemide-induced alterations in vagal pulmonary afferent activity, independent of changes in dynamic ventilatory mechanics and/or cardiovascular function; and *(4)* any combination of the above.

An earlier study in COPD observed a very small but statistically significant bronchodilator effect after inhalation of furosemide as evidenced by a 0.05 L (~5%) change in FEV_1.[14] This is unlikely to be due to a direct effect of furosemide on airway smooth muscle as it has been shown to have no effect on airway contractility *in vitro*.[27,28] However, there is evidence that inhaled furosemide, by increasing slowly adapting receptor (SAR) activity [9] and decreasing rapidly adapting receptor (RAR) activity,[9,10] may cause reflex bronchodilation, secondary to reduced parasympathetic outflow and thus cholinergic tone of airway smooth muscle.[29-32] We observed no significant change in resting tests of airway function or plethysmographic lung volumes after treatment with inhaled furosemide compared to placebo. This finding is similar to previous studies in healthy [12] and asthmatic subjects [33]. Interestingly, furosemide inhalation was associated with a significant increase in SVC by an average of 0.13 L. This may be explained, at least in part, by consistent increases in expiratory time during the vital capacity manoeuvre (by an average of 1.7 seconds). Inspection of maximal flow-volume loops and expiratory volume-time plots pre- and post-treatment showed that these were essentially superimposed for each subject. We postulate that this increased ability to tolerate a more prolonged expiration during spirometry may be associated with altered vagal mechanoreceptor input from the airways as previously described.[9,10,13,29,31,34] At isotime during exercise, dynamic IC was increased significantly by an average of 0.12 L after furosemide inhalation with no difference in dynamic IRV. Rest-to-peak exercise changes in IC, which reflect the rate of dynamic lung hyperinflation during exercise, were similar across treatments. These small mean changes in exercise IC were approximately 50% of the change previously seen with a variety of inhaled bronchodilators.[4,5,7] Small but significant increases in mean expiratory flow (with an unchanged T_E) in the setting of a reduced EELV were measured after furosemide suggesting some attenuation of expiratory flow limitation as an explanation of the increased IC.

Nishino *et al*[13] previously found that V'_E was significantly reduced in healthy subjects during mechanical loading of the respiratory system at rest after inhaled furosemide compared with placebo, suggesting that furosemide may reduce central ventilatory drive and that this may explain, at least in part, the observed dyspnoea relief. We could find no evidence of reduced ventilatory demand at rest or during exercise when patients were randomized to treatment with inhaled furosemide. In fact, ventilation tended to increase after furosemide ($p=0.066$), mainly due to an increase in V_T, and did so in the absence of a concomitant increase in dyspnoea ratings.

Furthermore, there were no differences in the pulmonary gas exchange responses to exercise that would account for the observed dyspnoea relief. The increase in V_T at a standardized time during exercise following inhaled furosemide could be accommodated in these hyperinflated patients as a result of the simultaneous recruitment of IC (without further reducing dynamic IRV). Interestingly, we found that 80% of the variance in post-furosemide improvements in dyspnoea ratings (at isotime) was explained by concomitant increases in T_I, V_T/T_E and V_T. We speculate that these changes in the volume and timing components of breathing could represent the effects of inhaled furosemide on dynamic ventilatory mechanics (increased V_T/T_E and reduced EELV).

A sub-analysis which compared responses in the group of patients who improved exercise endurance (n=8) while receiving furosemide with those who did not, revealed that the former had significantly greater treatment effects on dynamic ventilatory mechanics and on oxygen transport as indicated by consistent improvements in submaximal O_2 pulse, $V'O_2$ and $V'CO_2$. Those who showed the largest improvements in exercise endurance and dynamic airway function during exercise had less severe COPD at baseline but had similar flow and volume responses to furosemide at rest as the non-responders. To the extent that pulmonary vagal reflexes become more attenuated as severity of COPD progresses the relatively reduced bronchodilation effect in those with the worst disease may reflect this phenomenon.[35,36]

The improvements of submaximal metabolic parameters and O_2 pulse, together with significantly reduced perceived leg discomfort, point to possible improvements in cardiovascular performance. It is unclear whether this improvement relates to improved cardiopulmonary interactions as a result of mechanical unloading of the ventilatory muscles (as previously described with bronchodilator therapy)[37] or to a direct "systemic' effect of furosemide on cardiac function. Moosavi *et al*[12] demonstrated that inhaled furosemide (40 mg) relieved the sensation of experimentally-induced dyspnoea ("air hunger") for ~1 hour after its administration in healthy subjects at rest; however, clear evidence of a systemic diuretic effect was only apparent >1 hour after treatment. Inhaled furosemide, whose salutary effects on exertional dyspnoea were evident within an hour of dosing, had no demonstrable effect on heart rate or blood pressure responses at rest or during exercise in our study. It seems unlikely, therefore, that improvement in O_2 pulse (an imprecise surrogate for stroke volume) and $V'O_2$ uptake at isotime during exercise was the result of a systemic diuretic effect. Nonetheless, this possibility could not be definitively ruled out in this study.

The question arises whether the relief of exertional dyspnoea observed in this study is due to a local effect of inhaled furosemide on vagal sensory afferent discharge independent of reflexic anticholinergic bronchodilation, as has previously been postulated in both animal and human studies.[9-14] The possibility of such direct vagal sensory influences would be supported by the finding of consistent improvements of dyspnoea at a standardized exercise stimulus with concomitant changes in breathing pattern (i.e., T_I prolongation) in the absence of any change in dynamic ventilatory mechanics, metabolic or cardiovascular function.[38-40] Such was not the case in the present study. However, our results do indicate that perceptual responses to inhaled furosemide were variable across patients and that those who benefited most were more like to demonstrate the greatest bronchodilator effect during exercise. Those (n=7) who experienced exertional dyspnoea relief in the absence of improvement in exercise endurance time tended to have inconsistent, smaller improvements in dynamic IC but had no changes in cardiovascular responses or perceived leg discomfort during exercise. The relative importance of direct vagal effects on respiratory sensation (independent of any bronchodilator action) could not, therefore, be ascertained.

In conclusion, nebulized furosemide was associated with consistent and potentially clinically important improvements in dyspnoea intensity and exercise endurance in patients with advanced COPD. These acute improvements in exertional dyspnoea are multifactorial and could not be explained by alterations in ventilatory demand or pulmonary gas exchange. Improvements in airway function and dynamic ventilatory mechanics occurred in association with dyspnea relief after inhaled furosemide. This is supported by the strong correlation between dyspnoea reduction and changes in the volume and timing components of breathing during exercise. The magnitude of benefit of inhaled furosemide in this short term study is similar to that reported following acute administration of a number of established therapies in advanced COPD.[2-7] Therefore, this study supports the rationale for future scrutiny of inhaled furosemide as a therapeutic intervention for patients with COPD with incapacitating dyspnoea.

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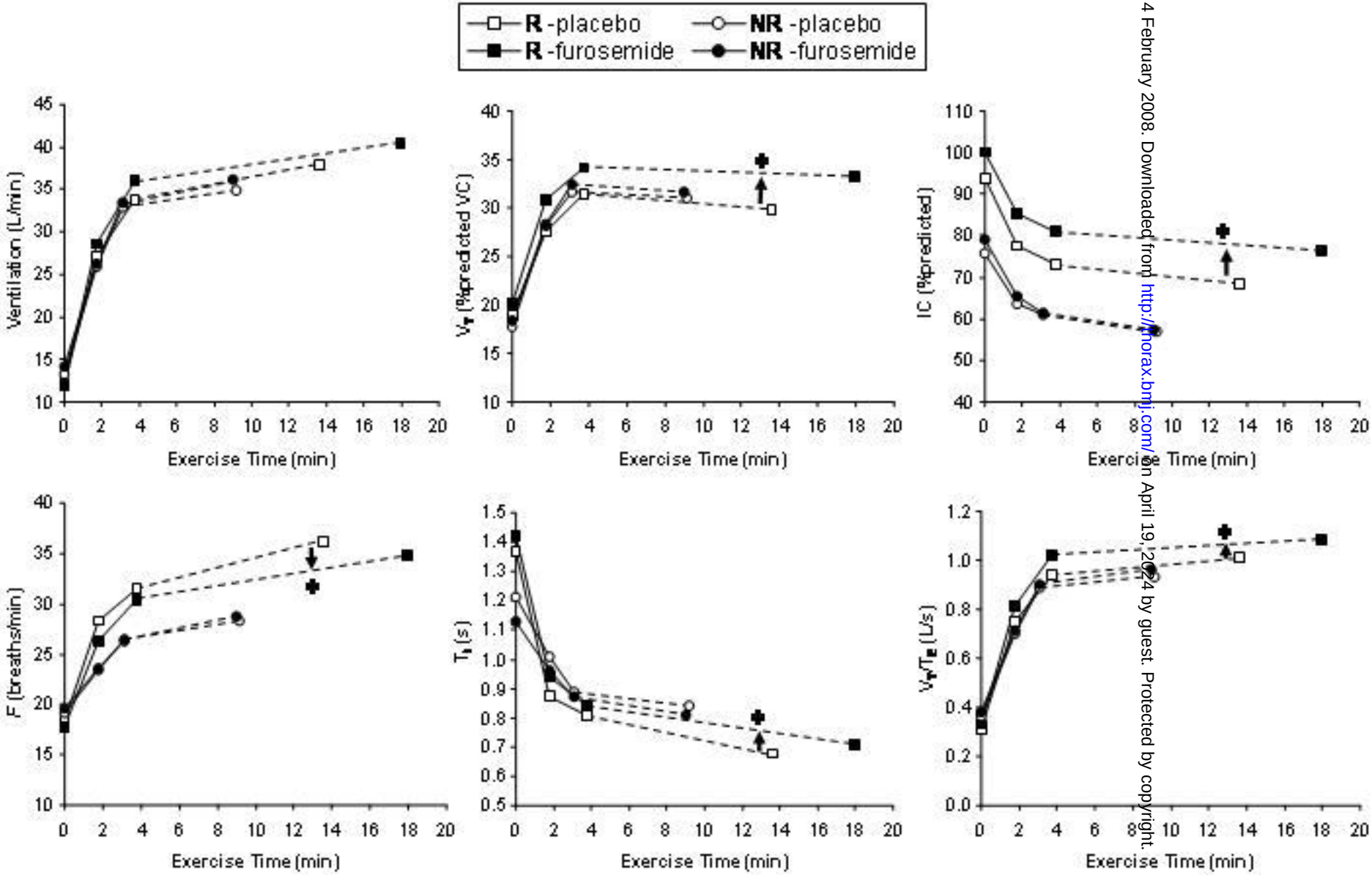
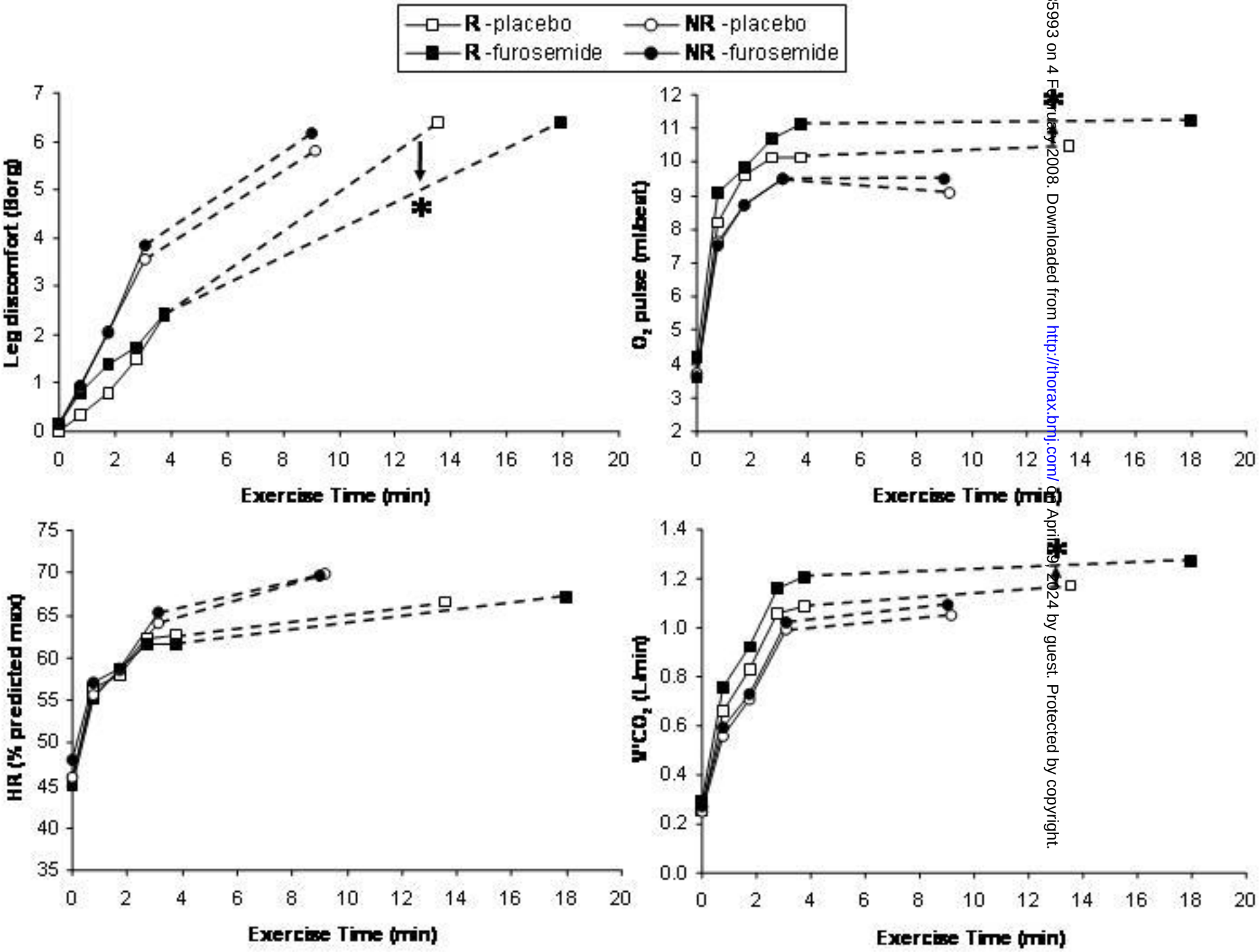
FIGURES

Figure 1. Mean±SE ratings (modified 10-point Borg scale) of dyspnoea intensity are shown during constant-load cycle exercise at 75% of peak work rate after inhalation of furosemide or placebo. Exertional dyspnoea intensity decreased significantly after furosemide compared with placebo at the highest equivalent isotime measurement (* $p<0.05$) but was not different between treatments when expressed against ventilation.

Figure 2. Ventilation, breathing pattern and operating lung volume measurements are shown during constant-load cycle exercise at 75% of peak work rate after inhalation of furosemide or placebo in the *Responder* (R) and *Non-responder* (NR) subgroups. V_T, tidal volume; VC, vital capacity; IC, inspiratory capacity; *F*, breathing frequency; T_I, inspiratory time; V_T/T_E, mean tidal expiratory flow. Data points are mean values at rest, at standardized time points early in exercise and at peak exercise. * $p<0.05$ placebo versus furosemide at the highest equivalent isotime (indicated by arrows) during exercise.

Figure 3. Intensity of leg discomfort, O_2 pulse, heart rate (HR) and carbon dioxide output ($V'CO_2$) are shown during constant-load cycle exercise at 75% of peak work rate after inhalation of furosemide or placebo in the *Responder* (R) and *Non-responder* (NR) subgroups. Data points are mean values at rest, at standardized time points early in exercise and at peak exercise. *$p<0.05$ placebo versus furosemide at the highest equivalent isotime (indicated by arrows) during exercise.





Mechanisms of dyspnoea relief and improved exercise endurance after furosemide inhalation in COPD.

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ONLINE DATA SUPPLEMENT

RESULTS

Exercise responses to furosemide

Cardiorespiratory responses to constant-load exercise after inhaled furosemide and placebo are shown for the group as a whole in Figures 1E and 2E.

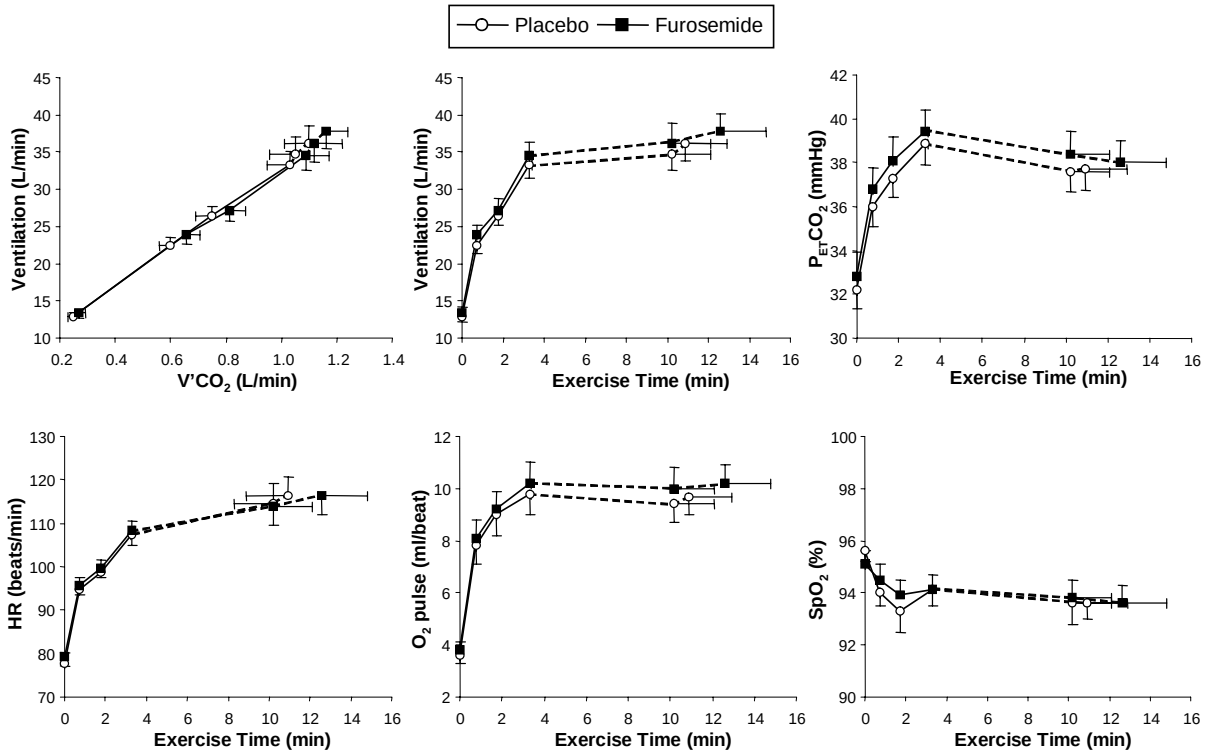

Figure 1E. Some standard measurements (mean±SE) of cardiorespiratory responses to constant-load cycle exercise at 75% of peak work rate are shown after inhalation of furosemide or placebo. $V'CO_2$, carbon dioxide output; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; HR, heart rate; O_2 pulse, oxygen pulse; SpO_2, oxygen saturation measured by pulse oximetry.

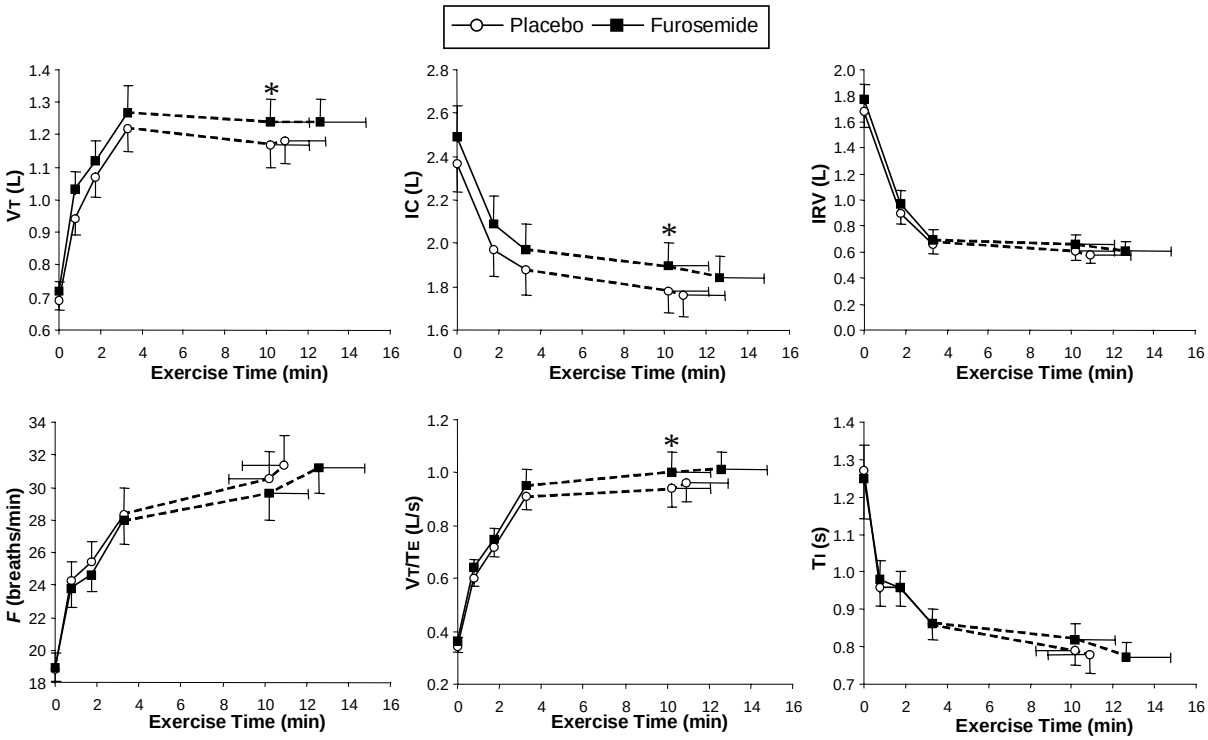

Figure 2E. Breathing pattern and operating lung volume measurements (mean±SE) are shown during during constant-load cycle exercise at 75% of peak work rate after inhalation of furosemide or placebo. * $p<0.05$ placebo versus furosemide at the highest equivalent isotime during exercise. V_T, tidal volume; IC, inspiratory capacity; IRV, inspiratory reserve volume; *F*, breathing frequency, V_T/T_E, mean tidal expiratory flow; T_I, inspiratory time.

Subgroup analysis

Based on the presence of a positive exercise response to inhaled furosemide, subjects were divided into: *1*) ***Responders***, furosemide-induced improvement in exercise endurance time >1 minute at 75% of peak work rate, and *2*) ***Non-responders***, less than 1 minute improvement in exercise endurance time. After inhaled furosemide compared with placebo, eight subjects improved exercise endurance time by more than 1 minute (4.4±2.2 min; mean±SD); all of these subjects also decreased dyspnea intensity (-1.8±0.7 Borg units, $p<0.0005$) and intensity of perceived leg discomfort (-1.9±1.7 Borg units, $p=0.013$) by at least 1 Borg unit at the highest equivalent isotime during exercise (Figure 3E). A significant ($p<0.005$) group × treatment interaction effect was found for each of these main outcome variables.

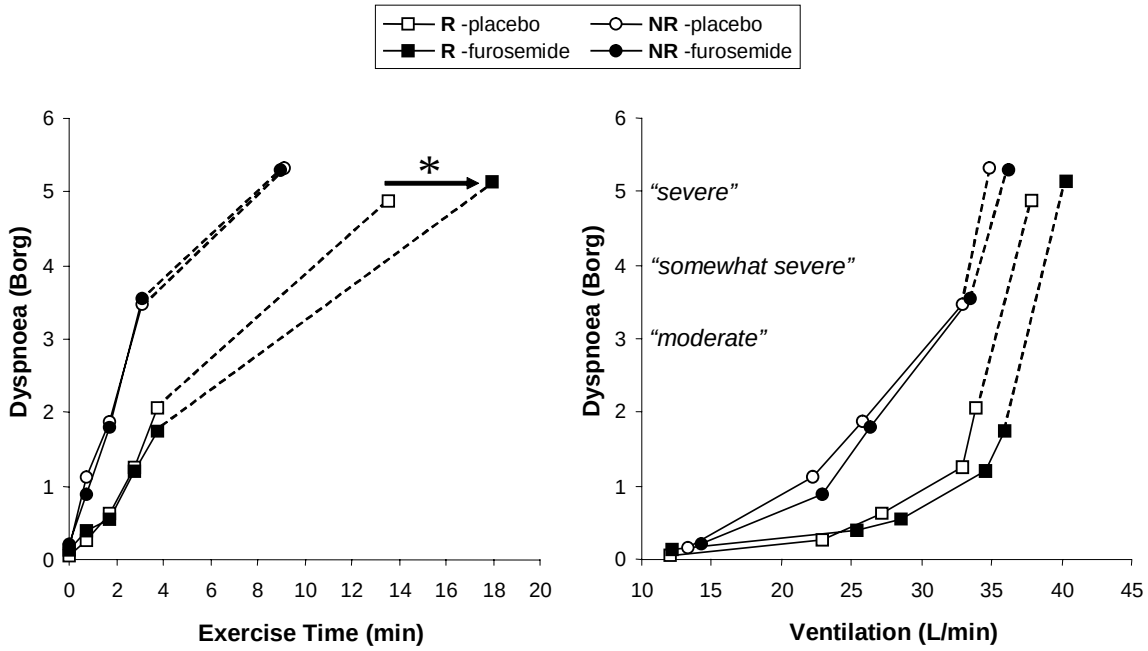

Figure 3E. Mean ratings (modified 10-point Borg scale) of dyspnoea intensity are shown during constant-load cycle exercise at 75% of peak work rate after inhalation of furosemide or placebo in the *Responder* (R) and *Non-responder* (NR) subgroups. Exercise endurance time and dyspnea/time slopes improved significantly after furosemide compared with placebo in R but not NR (*p<0.05); dyspnoea intensity when expressed against ventilation was not significantly different between treatments within each subgroup. NR had greater exertional dyspnea than R when expressed against exercise time or ventilation.

Baseline characteristics of each subgroup are shown in Table 1E. The *Responder* subgroup had significantly less severe expiratory airflow limitation and hyperinflation than the Non-responders. Examination of chest CT scans for the presence of emphysema showed that the distribution of emphysema was similar across subgroups: half of each group had moderate to severe emphysema, the majority with an upper lobe predominance. Although the peak incremental oxygen consumption ($V'O_2$) was similar across subgroups (Table 1E), subjects in the *Non-responder* subgroup had significantly worse exercise endurance and greater exertional dyspnoea intensity than the Responders during constant-work rate exercise at 75% of peak work rate (Figure 3E). There were no significant between-group differences in the magnitude of

change in resting spirometric and plethysmographic volume measurements after inhaled furosemide compared with placebo, i.e., no significant group × treatment interaction effects.

Table 1E. Baseline characteristics of the *Responder* and *Non-responder* subgroups

	Non-responders (n=12)	**Responders (n=8)**
Age, years	63 ± 9	60 ± 8
Body mass index, kg/m^2	26.1 ± 6.8	31.9 ± 8.4
Smoking history, pack-years	41 ± 12	58 ± 26 $^{p=0.05}$
Duration of COPD diagnosis, years	11 ± 8	7 ± 7
Predominance of moderate-severe emphysema, n (% of group)	6 (50%)	4 (50%)
Baseline Dyspnoea Index, focal score	5.7 ± 0.8	6.0 ± 0.5
Peak incremental VO_2, ml/kg/min	14.6 ± 4.8	13.9 ± 1.6
FEV_1, % predicted	39 ± 14	54 ± 13 *
FEV_1/FVC, %	38 ± 10	48 ± 7 *
$FEF_{25-75\%}$, % predicted	12 ± 6	14 ± 4
FVC, % predicted	71 ± 15	82 ± 12
IC, % predicted	71 ± 21	98 ± 19 *
FRC, % predicted	151 ± 38	109 ± 16 *
RV, % predicted	181 ± 68	133 ± 24 $^{p=0.07}$
TLC, % predicted	113 ± 19	104 ± 11
sRaw, % predicted	798 ± 326	471 ± 152 *
DL_{CO}, % predicted	74 ± 15	63 ± 19

Values are means ± SD. * p<0.05 *Responders* versus *Non-responders*.
FEV_1, forced expired volume in one second; FVC, forced vital capacity; $FEF_{25-75\%}$, forced expiratory flow between 25 and 75% of FVC; IC, inspiratory capacity; FRC, plethysmographic functional residual capacity; RV, residual volume; TLC, total lung capacity; sRaw, specific airway resistance; DL_{CO}, diffusing capacity of the lung for carbon monoxide; $V'O_2$, oxygen uptake.

At the highest equivalent isotime near end-exercise within the *Responder* subgroup, there were significant furosemide-induced improvements, all in the order of approximately 10% compared with placebo (Figure 4E): IC and V_T increased by 0.19±0.22 and 0.15±0.10 L, respectively (both $p<0.05$); *F* decreased by 2.2±1.4 breaths/min while T_I increased by 0.08±0.02 s (both $p<0.005$); V_T/T_E increased by 0.10±0.10 L/s ($p<0.05$); $V'CO_2$ increased by 0.14±0.13 L/min ($p<0.05$) and $V'O_2$ increased by a similar amount ($p=0.05$); and O_2 pulse increased by 1.2±1.4 mL/beat ($p<0.05$). There were no significant differences in isotime measurements during exercise within the *Non-responder* subgroup after inhalation of furosemide compared with placebo.

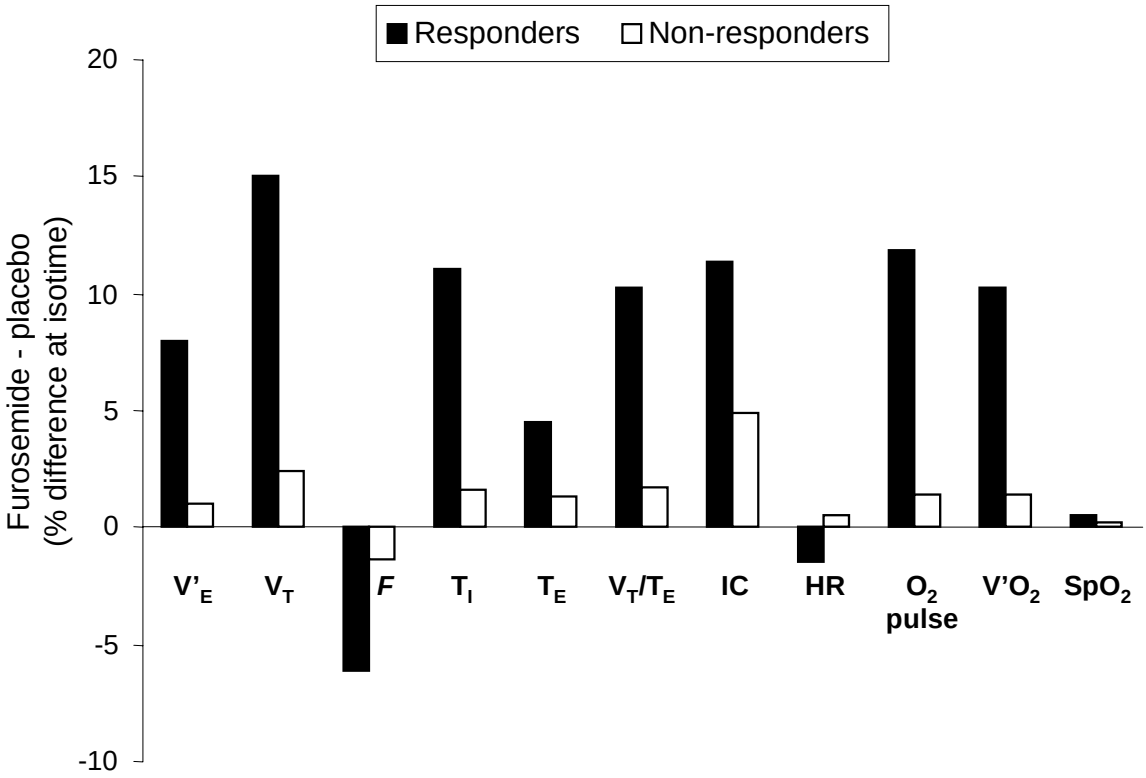

Figure 4E. Percentage differences between post-treatment measurements at isotime during exercise are shown for furosemide *Responders* and *Non-responders*. See text for abbreviations; T_E, expiratory time.