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Exercise-induced wheeze: Fraction of exhaled nitric oxide-directed management

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ABSTRACT

Background and objective: **Exercise-induced wheeze (EIW) is common. Several treatment options exist. Patients with low fraction of exhaled nitric oxide (F_ENO) are unlikely to be steroid-responsive and might benefit from non-steroidal therapies. We assessed: the efficacy of cromoglycate, formoterol and montelukast in patients with EIW and low F_ENO (<35 ppb) in a randomized cross-over trial, and the efficacy of inhaled corticosteroid in a high F_ENO (>35 ppb) group.**

Methods: **Patients had EIW and airway hyperresponsiveness (AHR) to mannitol and/or exercise. Those with low F_ENO ($n = 19$) received cromoglycate (20 mg inh. bd + before challenge tests), formoterol (12 μg inh. bd + before challenge tests) and montelukast (10 mg p.o. od), each for 2 weeks. Those with high F_ENO ($n = 20$) took inhaled fluticasone (500 μg) daily for 4 weeks. Primary end-points were: 50% reduction in maximum FEV_1 %fall (clinical protection) and decrease in AHR to mannitol.**

Results: **In patients with low F_ENO, cromoglycate, formoterol and montelukast significantly decreased AHR to mannitol in 63%, 61% and 47% of patients, respectively. In this group, the magnitude of exercise-induced bronchoconstriction (EIB) was significantly reduced with montelukast and formoterol; between-treatment differences were not significant. Of 6/19 with low F_ENO and EIB, protection occurred in 67% (cromoglycate), 83% (formoterol) and 50% (montelukast), respectively. In the high F_ENO group, AHR to mannitol and EIB decreased significantly with fluticasone ($P < 0.001$, $P = 0.005$, respectively), and protection occurred in 7/8 (88%) with EIB.**

Conclusions: **In patients with EIW and low F_ENO, the number of 'responders' to cromoglycate, formoterol and montelukast was similar. In a high F_ENO population the response to inhaled corticosteroid was highly significant and comparable to previous studies.**

SUMMARY AT A GLANCE

In exercise-induced wheeze treatment responses are heterogeneous. This may potentially be addressed by phenotyping prior to treatment. Patients were stratified using exhaled nitric oxide (F_ENO) into likely steroid responders (high F_ENO) and non-responders (low F_ENO). In a randomized cross-over trial, cromoglycate, formoterol and montelukast were equally effective in low F_ENO patients.

Key words: airway hyperresponsiveness, asthma, exercise-induced wheeze, exhaled nitric oxide, mannitol.

INTRODUCTION

Exercise-induced wheeze (EIW) is common. In a cross-sectional study of 8571 adolescents, 13% reported EIW in the preceding year.[1] However, whether EIW is associated with true exercise-induced bronchoconstriction (EIB) is difficult to establish. Only a minority of patients with EIW have evidence of EIB.[2] Furthermore, the sensitivity of exercise testing is low, and false negatives may occur if testing is not standardized.[3] The International Olympic Committee advocates eucapnic voluntary hyperventilation as the optimal test to confirm EIB.[4] The mannitol challenge has similar sensitivity and is more practical.[5]

In practice, a diagnosis of 'asthma' is often made in patients with EIW and empirical treatment commenced. This too is problematic. Clinical trials have focused on patients with objective evidence of EIB, the assumption being that outcomes can be generalized. Treatment options include inhaled β-agonists,[6–9] cromoglycate,[10–12] leukotriene receptor antagonists[13,14] or inhaled corticosteroid (ICS).[15–17] However, there are

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no guidelines as to which treatment to choose. The use of β-agonists is overshadowed by issues of tolerance with reduced duration of effect over time,[18,19] as well as safety when given as monotherapy.[20] Although the severity of EIB is reduced with ICS treatment,[15,21,22] the response is variable where the studied population is heterogeneous. In one study using budesonide, the prevalence of EIB was reduced by only a third and severity by only a half.[23]

Against this background, an approach based on pretreatment phenotype determination might provide benefits for patients with EIW. We sought to rationalize our management of EIW using exhaled nitric oxide as a phenotype biomarker. Increased fraction of exhaled nitric oxide (F_ENO) is a surrogate marker for eosinophilic airway inflammation.[24] Sputum eosinophilia correlates with EIB severity[25,26] and patients with eosinophilia show greater reduction in EIB with ICS than non-eosinophilic patients.[26] In addition, F_ENO correlates with the degree of EIB in asthmatics.[27] In one study, low F_ENO levels excluded EIB in patients with exercise-related symptoms.[2] Finally, F_ENO is a predictor of steroid response in steroid-naïve patients with non-specific respiratory symptoms,[28] indicating that patients with EIW and high F_ENO may benefit from ICS treatment. Conversely, patients with low F_ENO are less likely to have sputum eosinophilia or respond well to ICS treatment. The negative predictive values for low F_ENO levels are high.[28] Non-steroidal treatments may be an appropriate alternative in this subgroup.

Our aim was to assess the effectiveness of phenotype-stratified treatment for EIW. Patients with airway hyperresponsiveness (AHR) and low F_ENO underwent a blinded, randomized, cross-over trial of cromoglycate, formoterol and montelukast. Patients with AHR and high F_ENO were allocated to receive treatment with ICS.

METHODS

Patients

Patients aged 10–70 years were enrolled from among primary care referrals to either the pulmonary function laboratory or chest clinic for investigation of exercise-related cough, wheeze or dyspnoea as their predominant symptom. Exclusion criteria included cardiac disease, abnormal ECG, FEV_1 <60% predicted, severe hypertension (>200/100 mm Hg), and >10 pack year smoking history or smoking within the preceding 3 months.

Study design

Patients were stratified by F_ENO level; those with low F_ENO (<35 ppb) underwent the randomized trial of non-steroidal treatments, while those with high F_ENO (≥35 ppb) were received treatment with ICS (see Fig. 1). The cut-point of 35 ppb was 2 SD above the mean F_ENO in a healthy population.[29]

Phase 1

Patients gave written informed consent. Demographic data were obtained and a clinical examination

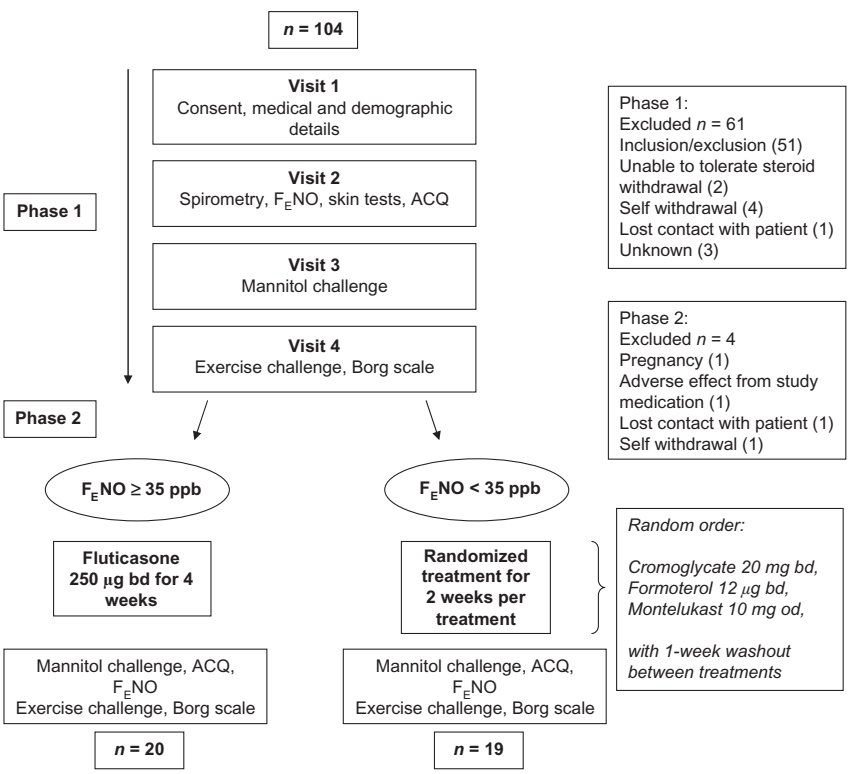

Figure 1 Consort diagram outlining the selection and treatment allocation of patients. ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide.

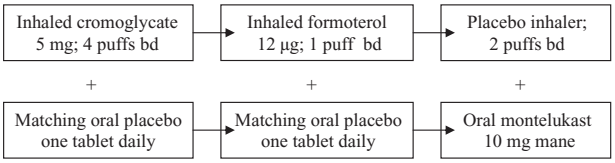

Figure 2 Scheme for treatment of patients with low fraction of exhaled nitric oxide (<35 ppb). Each treatment was given for 2 weeks with a 1-week washout separating each treatment.

and ECG performed. Patients taking ICS stopped treatment for 1 month. Short-/long-acting β-agonists were withheld for 12/72 h before testing. F_ENO measurements and spirometry were carried out in accordance with European Respiratory Society/American Thoracic Society guidelines.[30,31] Patients completed the Asthma Control Questionnaire (ACQ6).[32] Mannitol and exercise challenges were undertaken on separate days.

Progression to phase 2 depended on demonstration of a provocative dose of mannitol causing a 15% fall in FEV_1 of less than 635 mg (PD_{15} < 635 mg mannitol)[33] and/or a maximum percentage fall in post-exercise FEV_1 of 10% or more from baseline (FEV_1 $\%fall_{max} \geq 10\%$).[33]

Phase 2: trial of non-steroidal treatments (low fraction of exhaled nitric oxide group)

A randomized, placebo-blinded, cross-over trial of inhaled sodium cromoglycate, 5 mg, 4 puffs bd (Vicrom, Aventis Pharma, Auckland, New Zealand), inhaled formoterol, 12 μg, 1 puff bd (Foradil, Novartis, Auckland, New Zealand) and oral montelukast, 10 mg mane (Singulair, Merck Sharpe Dohme, Auckland, New Zealand) was conducted. Patients received a pack containing a masked inhaler and tablets (see Fig. 2). Each treatment period was for 2 weeks, separated by a 1-week washout. Patients were instructed to take one tablet daily and use the study inhaler morning and evening, 15 min before exercise, and to relieve exercise-related symptoms. Ipratropium bromide (Atrovent, Boehringer, Ingelheim, Germany) was provided as a 'reliever' during washout periods. ACQ6, F_ENO, mannitol challenge, and exercise challenge and Borg scale, were repeated on consecutive days at the end of each treatment. Patients also used their study inhaler 15 min prior to mannitol and exercise challenges.

Phase 2: trial of steroid (high fraction of exhaled nitric oxide group)

Patients received inhaled fluticasone (Flixotide metered dose inhaler, GlaxoSmithKline, Greenford, UK), 250 μg bd for 1 month. All study procedures were conducted before and after treatment as for the low F_ENO group.

Study procedures

The mannitol challenge was performed using a standardized method.[34] The exercise challenge was performed in accordance with American Thoracic Society guidelines.[35] Baseline spirometry was performed prior to an 8-min exercise protocol on a braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, the Netherlands) while breathing dry air. On completion of exercise, dyspnoea was quantified using the Borg scale.[36] Post-exercise FEV_1 was measured at 1, 3, 5, 10, 15 and 20 min. The maximum percentage fall in post-exercise FEV_1 compared with baseline was calculated, and EIB was defined as a fall of ≥10%.[33]

Statistical analysis

Low and high F_ENO groups were compared using unpaired *t*-tests, Mann–Whitney *U*-tests and chi-square tests. PD_{15} mannitol and F_ENO were logarithmically transformed and results expressed as geometric mean (95% CI). Patients were categorized as responders or non-responders using the following cut-points: ≥50% reduction in maximum fall in FEV_1 with exercise;[37] ≥1 point reduction in Borg score;[36] ≥0.5 point decrease in ACQ6[38] and ≥1 doubling dose increase in PD_{15} mannitol.[39] Paired *t*-tests were used to compare results after each treatment with baseline. Mixed-model analysis was used to compare treatments. The proportions of responders were compared using logistic regression and generalized linear mixed models. For patients with demonstrated EIB, 'complete protection' was defined as a fall in FEV_1 with exercise of less than 10% on treatment.[40]

Ethical approval was obtained from the Lower South Regional Ethics Committee (LRS/05/10/037). This study is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12605000397617).

RESULTS

A total of 104 patients were screened; 39 demonstrated AHR to mannitol and/or exercise and completed phase 2 (low F_ENO group, $n = 19$; high F_ENO group: $n = 20$) (Fig. 1). At baseline, low and high F_ENO groups had similar demographic characteristics, lung function and AHR (Table 1). AHR to mannitol and exercise was seen in 36 (92%) and 14 (36%) patients, respectively.

Response to cromoglycate, formoterol and montelukast (low fraction of exhaled nitric oxide group)—Tables 2 and 3

Compared with baseline, each treatment was associated with significant decrease in AHR to mannitol ($P < 0.05$). The magnitude of change was not significantly different between treatments ($P = 0.43$). When categorized as a 'responder' based on one doubling

Table 1 Comparison of high and low F_ENO groups at baseline

	All ($n = 39$)	Low F_ENO ($n = 19$) Geometric mean: 17.8 ppb (95% CI: 14.5–21.9)	High F_ENO ($n = 20$) Geometric mean: 66.0 ppb (95% CI: 54.9–79.3)	*P*
Male†	11 (28%)	4 (21%)	7 (35%)	0.333
Age (years)	26 (11)	28 (10)	25 (11)	0.413
Ex-smokers†	7 (18%)	5 (26%)	2 (10%)	0.184
BMI (kg/m^2)	25 (5)	26 (7)	24 (3)	0.272
Atopic†	29 (74%)	13 (68%)	16 (80%)	0.408
Taking regular ICS at baseline†	10 (26%)	6 (32%)	4 (20%)	0.408
ACQ	1.3 (0.9)	1.5 (0.9)	1.0 (0.8)	0.068
FEV_1 (L)	3.45 (0.93)	3.20 (0.85)	3.69 (0.96)	0.103
FEV_1 (% predicted)	99 (16)	95 (16)	103 (16)	0.144
FEV_1/FVC	79 (9)	78 (9)	79 (9)	0.895
FEV_1 % change post-bronchodilator	8 (9)	8 (10)	7 (9)	0.778
PD_{15} mannitol (mg)‡	131 (91–188)	119 (64–222)	143 (95–215)	0.625
PD_{15} mannitol (mg) <635 mg†	36 (92%)	16 (84%)	20 (100%)	0.064
Exercise FEV_1 $\%fall_{max}$§	7 (1–11)	8 (4–10)	5 (0–13)	0.879
Exercise FEV_1 $\%fall_{max} \geq 10\%$†	14 (36%)	6 (32%)	8 (40%)	0.584
Area under FEV_1 curve (% change·time)	55.9 (144.6)	38.6 (143.9)	72.3 (147.0)	0.474
Borg	4 (2)	4 (2)	4 (2)	0.544

Comparison of high and low F_ENO groups at baseline using unpaired *t*-tests unless otherwise stated with results expressed as mean (SD).

† Groups compared using chi-square tests and results expressed as *n* (%).

‡ Analysed after logarithmic transformation and expressed as geometric mean (95% CI).

§ Analysed using Mann–Whitney *U*-test and presented as median (IQR).

ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide; ICS, inhaled corticosteroid; IQR, interquartile range.

dose shift in PD_{15} mannitol, there were no significant differences between treatments (Table 3).

Compared with baseline, the %fall in FEV_1 with exercise was reduced with formoterol and montelukast but not with cromoglycate. However, there was a significant reduction in area under the FEV_1 curve with cromoglycate but not with formoterol or montelukast (Table 2). Overall, these changes were not significantly different between treatments, nor were there significant differences in the proportion of responders based on FEV_1 response to exercise (Table 3). Symptoms (ACQ6) decreased significantly with formoterol but not with cromoglycate or montelukast.

Response to inhaled corticosteroid in high fraction of exhaled nitric oxide group—Tables 4 and 5

With fluticasone, there was a significant reduction in AHR to mannitol, with a one doubling dose shift in 15/20 (75%) of patients. The mean fall in FEV_1 with exercise was significantly reduced whether defined by FEV_1 $\%fall_{max}$ or area under the FEV_1 curve. In the eight patients with objectively defined EIB, 7/8 (88%) had a 50% or more reduction in FEV_1 $\%fall_{max}$ and 5/8 (63%) had complete protection. Approximately half demonstrated clinically significant improvements in symptoms (ACQ6 and Borg).

DISCUSSION

In this study we undertook a phenotype-stratified approach to the management of EIW based on exhaled nitric oxide measurements before treatment allocation. Thereafter, we performed a comparative, randomized, cross-over trial of three non-steroidal treatments in patients with low F_ENO in whom ICS treatment was less likely to be effective. Overall, in this subgroup, there were no consistent between-treatment differences; the improvements with cromoglycate, formoterol and montelukast were similar. While there are a number of therapeutic options available for EIW, there are few comparative studies. Moreover, such studies focus on patients with EIB rather than EIW.[41–43] In one study, the protective effect of montelukast was superior to salmeterol.[41] In a small cross-over study, single doses of salmeterol, montelukast and zafirlukast provided similar degrees of protection.[42] In a parallel-group placebo-controlled study, budesonide plus montelukast or montelukast alone were more effective than budesonide plus formoterol or budesonide alone, although all combinations had beneficial effects.[43] The inconsistent benefits for one treatment compared with another in these studies may be because 'all comers' were included, with an uneven distribution of patients with treatment-responsive airway inflammation. Stratifying by phenotype might have improved the outcomes.

Table 2 The effects of cromoglycate, formoterol and montelukast, each given for 2 weeks, on symptoms (ACQ and Borg), airway hyperresponsiveness (to exercise and mannitol) and airway inflammation (F_ENO) in patients with low F_ENO

	Baseline	Cromoglycate	*P*	Formoterol	*P*	Montelukast	*P*	*P* (comparison of Rx[†])
ACQ	1.5 (0.9)	1.5 (0.9)	0.914	1.1 (0.7)	**0.030**	1.3 (0.9)	0.071	0.422
Borg	4 (2)	4 (2)	0.413	4 (2)	0.462	3 (1)	0.083	0.785
Exercise FEV_1 $\%fall_{max}$[‡]	8 (4–10)	4 (0–8)	0.089	2 (–1 to 5)	**0.001**	4 (–2 to 7)	**0.036**	0.543
Exercise FEV_1 $\%fall_{max}$ in those with ≥10% fall pre-Rx ($n = 6$)[‡]	12 (10–13)	5 (2–13)	0.116	4 (2–6)	**0.028**	7 (4–10)	**0.028**	0.801
Area under FEV_1 curve (% change·time)	38.6 (143.9)	–14.0 (78.2)	**0.021**	–18.3 (98.2)	0.064	–9.0 (100.3)	0.276	0.651
Area under FEV_1 curve (% change·time) in those with ≥10% fall pre-Rx ($n = 6$)	137.9 (169.8)	17.0 (67.0)	0.155	9.8 (71.1)	0.176	7.8 (46.7)	0.155	0.975
PD_{15} mannitol (mg)[§]	119 (64–222)	397 (239–660)	**<0.001**	438 (245–781)	**0.002**	216 (135–347)	**0.022**	0.432
F_ENO (ppb)[§]	17.8 (14.5–21.9)	18.0 (14.2–22.8)	0.907	23.9 (17.5–32.8)	0.082	18.5 (14.8–23.0)	0.629	0.714

Results at baseline and after cromoglycate, formoterol and montelukast, each for 2 weeks in patients with low F_ENO at baseline ($n = 19$). Paired *t*-tests were used for analysis unless otherwise stated and results presented as mean (SD).

[†] *P*-values given for comparison of cromoglycate, formoterol and montelukast using mixed-model analysis and baseline values as covariates. *P*-values for comparisons of each treatment with baseline and significant results in bold.

[‡] Analysed using Wilcoxon signed rank test and results expressed as median (IQR).

[§] Analysed after logarithmic transformation and results expressed as geometric mean (CI).

ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide; IQR, interquartile range; Rx, treatment.

Table 3 A comparison of the proportion of 'responders' in the low F_ENO group for cromoglycate, formoterol and montelukast, each given for 2 weeks, using each of the predefined criteria for clinically significant response

	Cromoglycate	Formoterol	Montelukast	*P* Cromoglycate vs formoterol	*P* Cromoglycate vs montelukast	*P* Formoterol vs montelukast
ACQ reduction ≥0.5	4/19 (21%)	8/19 (42%)	9/19 (47%)	0.134	0.073	0.709
Borg reduction ≥1	9/18 (50%)	11/19 (58%)	10/19 (53%)	0.472	0.953	0.503
Exercise FEV_1 $\%fall_{max}$ reduction ≥50%	9/18 (50%)	10/19 (53%)	9/19 (47%)	0.784	0.919	0.703
Exercise FEV_1 $\%fall_{max}$ reduction ≥50% in those with ≥10% fall pre-Rx	4/6 (67%)	5/6 (83%)	3/6 (50%)	0.447	0.487	0.216
Exercise FEV_1 $\%fall_{max}$ ≥10% to <10%	4/6 (67%)	6/6 (100%)	4/6 (67%)	NA	NA	NA
PD_{15} mannitol increase ≥1 doubling dose	12/19 (63%)	11/18 (61%)	9/19 (47%)	0.992	0.125	0.130

Proportion of patients with low F_ENO at baseline demonstrating a significant response to cromoglycate, formoterol and montelukast. Patients were categorized as responders or non-responders using the following criteria: ≥50% reduction in maximum fall in FEV_1 with exercise;[37] ≥1 point reduction in Borg dyspnoea score;[36] ≥0.5 point decrease in ACQ;[38] and ≥1 doubling dose increase in PD_{15} mannitol.[39] Analysed using logistic regression and a generalized linear mixed model. Results expressed as *n* (%).

ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide; Rx, treatment.

Table 4 The effects of fluticasone, given for 4 weeks, on symptoms (ACQ and Borg), airway hyperresponsiveness (to exercise and mannitol) and airway inflammation (F_ENO), in patients with high F_ENO

	Before	After	*P*
ACQ	1.0 (0.8)	0.7 (0.5)	0.128
PD_{15} mannitol (mg)†	143 (95–215)	428 (279–654)	**<0.001**
Exercise FEV_1 $\%fall_{max}$‡	5 (0–13)	2 (−1–3)	**0.005**
Exercise FEV_1 $\%fall_{max}$ in those with ≥10% fall pre-Rx ($n = 8$)‡	17 (12–24)	6 (2–13)	**0.017**
Area under FEV_1 curve (% change·time)	72.3 (147.0)	−10.6 (76.3)	**0.004**
Area under FEV_1 curve (% change·time) in those with ≥10% fall pre-Rx ($n = 8$)	203.0 (143.7)	8.2 (96.3)	**0.010**
Borg	4 (2)	3 (2)	0.057
F_ENO ppb†	66.0 (54.9–79.3)	20.3 (16.5–24.9)	**<0.001**

Results before and after inhaled fluticasone 500 μg daily for 4 weeks in high F_ENO group ($n = 20$). Paired *t*-tests used for comparisons unless otherwise stated. Results expressed as mean (SD) unless otherwise stated. Significant *P*-values in bold.

† Analysed after logarithmic transformation and results expressed as geometric mean (CI).

‡ Analysed using Wilcoxon signed rank test and presented as median (IQR).

ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide; IQR, interquartile range; Rx, treatment.

Table 5 The proportion of 'responders' in the high F_ENO group to fluticasone, given for 4 weeks, using each of the predefined criteria for significant response

	% responders
ACQ reduction ≥0.5	9/20 (45%)
Borg reduction ≥1	11/20 (55%)
Exercise FEV_1 $\%fall_{max}$ reduction ≥50%	11/20 (55%)
Exercise FEV_1 $\%fall_{max}$ reduction ≥50% in those with ≥10% fall pre-Rx	7/8 (88%)
Exercise FEV_1 $\%fall_{max}$ ≥10% to <10%	5/8 (63%)
PD_{15} mannitol increase ≥1 doubling dose	15/20 (75%)

Proportion of patients demonstrating a significant response to steroid as defined by predetermined criteria for improvement in ACQ, Borg, exercise FEV_1 $\%fall_{max}$ and PD_{15} mannitol. Patients were categorized as responders or non-responders using the following criteria: ≥50% reduction in maximum fall in FEV_1 with exercise;[37] ≥1 point reduction in Borg dyspnoea score;[36] ≥0.5 point decrease in ACQ;[38] and ≥1 doubling dose increase in PD_{15} mannitol.[39]

ACQ, asthma control questionnaire; F_ENO, fraction of exhaled nitric oxide; Rx, treatment.

In patients with high F_ENO, steroid therapy is likely to be beneficial[28] and we sought to confirm this in relation to EIW. In our study, fluticasone resulted in significant reductions in FEV_1 $\%fall_{max}$ and area under the FEV_1 curve in all patients with EIW, not just in the subgroup with EIB; 55% were 'responders'. Moreover, of the eight with EIB, seven (88%) had clinical protection (≥50% reduction in FEV_1 $\%fall_{max}$) and five (63%) had complete protection. These outcomes compare favourably with data from earlier studies despite the fact that these studies included only patients with EIB. Waalkens *et al.* showed that after 2-month treatment with budesonide, the prevalence of EIB was reduced by only a third and its severity reduced by approximately a half.[23] In a randomized trials, significant reductions in EIB were demonstrated with both low-dose budesonide[17] and low-dose fluticasone.[16] The findings of these studies are comparable to our own results. Finally, in a cross-over study of beclomethasone (50 and 100 μg daily) in children,[44] reductions in FEV_1 $\%fall_{max}$ occurred with both doses, although patients continued to have marked EIB despite treatment (FEV_1 $\%fall_{max}$ 28%, 21%, 21% for placebo, 50 and 100 μg, respectively, $P = 0.039$). The heterogeneity of treatment response in these studies indicates potential advantages in determining a steroid-responsive phenotype prior to treatment allocation.[45] However, the use of F_ENO in this context needs to be confirmed by a larger randomized trial in which the effects of ICS are compared in both high and low F_ENO groups.

Cromoglycate, formoterol and montelukast were all associated with a significant decrease in AHR to mannitol. To our knowledge, this is the first study in which changes in AHR to mannitol have been used to assess treatment efficacy in patients with EIW. This endpoint was selected because it is more sensitive to abnormal airway function in patients with EIW than an exercise test *per se*. Whereas 92% of our patients had a positive mannitol challenge, only 36% had a positive exercise test. Using a one doubling dose increase to define clinically significant change in PD_{15} mannitol,[39] approximately 60% benefited with each of the three treatments. In the high F_ENO group, significant decreases in AHR to mannitol occurred with ICS (overall 1.58 doubling doses), and approximately three-quarters demonstrated a one doubling dose shift. No previous studies have assessed the effect of ICS on AHR to mannitol in patients with EIW or EIB. Only two studies have measured the effect of ICS on AHR to mannitol in asthma. Brannan *et al.*[46] performed a mannitol challenge before and after 6–9 weeks of budesonide (800–2400 μg daily) in 18 asthmatic patients. A reduction in PD_{15} was seen (from 78 mg (95% CI: 51–117) to 289 mg (202–414),

$P < 0.001$, 1.89 doubling doses) and all patients had a clinically significant reduction (>0.9 doubling doses) in AHR. In the other study,[47] 17 steroid-naïve asthmatics underwent mannitol challenge at baseline and after 3- and 6-month treatment with budesonide. Mean PD_{15} was 168 mg (84–335), 512 mg (262–1002) and 606 mg (307–991) at each time point giving calculated shifts of 1.61 and 1.85 doubling doses, respectively. Thus, compared with these previous studies, the magnitude of change in AHR to mannitol in the present study is similar despite the shorter treatment duration.

We used formoterol as one of the non-steroidal treatments, and this treatment was given twice daily for 2 weeks and then acutely just prior to the exercise challenge. Thus our measurements reflect the possibility that tachyphylaxis will have occurred.[48] It is likely that without prior treatment, the benefits of acutely administered formoterol might have been greater. Furthermore, there are concerns that monotherapy with β-agonists may increase airway inflammation and responsiveness.[20] The application of our results in clinical practice requires to take these issues into consideration.

We accept that our study has some limitations. Using a single cut-point of 35 ppb for F_ENO to separate steroid-responsive from steroid-unresponsive groups was based on data obtained from a healthy population.[29] Although rational, in fact steroid responsiveness is more likely when F_ENO is even higher (>45 ppb[28]), and low F_ENO is best defined as <26 ppb.[49] Second, treatment in the low F_ENO group was placebo-blinded but not placebo-controlled. However, conclusions can still be drawn about the relative efficacy of the three treatments used in this group. Third, the study design was based on the assumption that EIW patients with low F_ENO would have poor response to ICS. This was based on previous data from our group showing that a cut-point of 35 ppb for F_ENO had a negative predictive value of 93% for change in AHR with ICS.[28] We accept that a trial of ICS in patients with low F_ENO would be required to validate this assumption more fully. Finally, the steroid arm of the study was not placebo-controlled, and as such some comparisons cannot be made. Nonetheless, our results with ICS treatment are similar to others in which a placebo was administered.

In conclusion, we have evaluated the management of EIW using a phenotype-based stratification of patients before treatment. With several treatment options available and the absence of clear guidelines, this may assist in the selection of more appropriate therapy and increase the likelihood of a positive outcome. The non-steroidal therapies tested had comparable efficacy for management of EIW in the low F_ENO population. Our results with ICS in the high F_ENO group were similar to those of other investigations in which patients with EIB have been selected.

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REFERENCES

1 Henriksen AH, Tveit KH, Holmen TL *et al.* A study of the association between exercise-induced wheeze and exercise versus methacholine-induced bronchoconstriction in adolescents. *Pediatr. Allergy Immunol.* 2002; **13**: 203–8.
2 ElHalawani SM, Ly NT, Mahon RT *et al.* Exhaled nitric oxide as a predictor of exercise-induced bronchoconstriction. *Chest* 2003; **124**(2): 639–43.
3 Rundell KW, Slee JB. Exercise and other indirect challenges to demonstrate asthma or exercise-induced bronchoconstriction in athletes. *J. Allergy Clin. Immunol.* 2008; **122**: 238–46; quiz 247–8.
4 Anderson SD, Argyros GJ, Magnussen H *et al.* Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br. J. Sports Med.* 2001; **35**: 344–7.
5 Holzer K, Anderson SD, Chan HK *et al.* Mannitol as a challenge test to identify exercise-induced bronchoconstriction in elite athletes. *Am. J. Respir. Crit. Care Med.* 2003; **167**: 534–7.
6 Anderson SD, Seale JP, Rozea P *et al.* Inhaled and oral salbutamol in exercise-induced asthma. *Am. Rev. Respir. Dis.* 1976; **114**: 493–500.
7 Woolley M, Anderson SD, Quigley BM. Duration of protective effect of terbutaline sulfate and cromolyn sodium alone and in combination on exercise-induced asthma. *Chest* 1990; **97**(1): 39–45.
8 Kemp JP, Dockhorn RJ, Busse WW *et al.* Prolonged effect of inhaled salmeterol against exercise-induced bronchospasm. *Am. J. Respir. Crit. Care Med.* 1994; **150**(6 Pt 1): 1612–15.
9 Henriksen JM, Agertoft L, Pedersen S. Protective effect and duration of action of inhaled formoterol and salbutamol on exercise-induced asthma in children. *J. Allergy Clin. Immunol.* 1992; **89**: 1176–82.
10 de Benedictis FM, Tuteri G, Bertotto A *et al.* Comparison of the protective effects of cromolyn sodium and nedocromil sodium in the treatment of exercise-induced asthma in children. *J. Allergy Clin. Immunol.* 1994; **94**: 684–8.
11 Tullett WM, Tan KM, Wall RT *et al.* Dose-response effect of sodium cromoglycate pressurised aerosol in exercise induced asthma. *Thorax* 1985; **40**: 41–4.
12 Patel KR, Wall RT. Dose-duration effect of sodium cromoglycate aerosol in exercise-induced asthma. *Eur. J. Respir. Dis.* 1986; **69**: 256–60.
13 Leff JA, Busse WW, Pearlman D *et al.* Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. *N. Engl. J. Med.* 1998; **339**: 147–52.
14 Kim JH, Lee SY, Kim HB *et al.* Prolonged effect of montelukast in asthmatic children with exercise-induced bronchoconstriction. *Pediatr. Pulmonol.* 2005; **39**: 162–6.
15 Vathenen AS, Knox AJ, Wisniewski A *et al.* Effect of inhaled budesonide on bronchial reactivity to histamine, exercise, and eucapnic dry air hyperventilation in patients with asthma. *Thorax* 1991; **46**: 811–16.
16 Hofstra WB, Neijens HJ, Duiverman EJ *et al.* Dose-responses over time to inhaled fluticasone propionate treatment of exercise- and methacholine-induced bronchoconstriction in children with asthma. *Pediatr. Pulmonol.* 2000; **29**: 415–23.
17 Jonasson G, Carlsen KH, Hultquist C. Low-dose budesonide improves exercise-induced bronchospasm in schoolchildren. *Pediatr. Allergy Immunol.* 2000; **11**: 120–5.

18 Nelson JA, Strauss L, Skowronski M *et al.* Effect of long-term salmeterol treatment on exercise-induced asthma. *N. Engl. J. Med.* 1998; **339**: 141–6.
19 Ramage L, Lipworth BJ, Ingram CG *et al.* Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir. Med.* 1994; **88**: 363–8.
20 Taylor DR. The beta-agonist saga and its clinical relevance: on and on it goes. *Am. J. Respir. Crit. Care Med.* 2009; **179**: 976–8.
21 Henriksen JM, Dahl R. Effects of inhaled budesonide alone and in combination with low-dose terbutaline in children with exercise-induced asthma. *Am. Rev. Respir. Dis.* 1983; **128**: 993–7.
22 Henriksen JM. Effect of inhalation of corticosteroids on exercise induced asthma: randomised double blind crossover study of budesonide in asthmatic children. *Br. Med. J. (Clin. Res. Ed.)* 1985; **291**(6490): 248–9.
23 Waalkens HJ, van Essen-Zandvliet EE, Gerritsen J *et al.* The effect of an inhaled corticosteroid (budesonide) on exercise-induced asthma in children. Dutch CNSLD Study Group. *Eur. Respir. J.* 1993; **6**: 652–6.
24 Berry MA, Shaw DE, Green RH *et al.* The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin. Exp. Allergy* 2005; **35**: 1175–9.
25 Yoshikawa T, Shoji S, Fujii T *et al.* Severity of exercise-induced bronchoconstriction is related to airway eosinophilic inflammation in patients with asthma. *Eur. Respir. J.* 1998; **12**(4): 879–84.
26 Duong M, Subbarao P, Adelroth E *et al.* Sputum eosinophils and the response of exercise-induced bronchoconstriction to corticosteroid in asthma. *Chest* 2008; **133**(2): 404–11.
27 Buchvald F, Hermansen MN, Nielsen KG *et al.* Exhaled nitric oxide predicts exercise-induced bronchoconstriction in asthmatic school children. *Chest* 2005; **128**(4): 1964–7.
28 Smith AD, Cowan JO, Brassett KP *et al.* Exhaled nitric oxide: a predictor of steroid response. *Am. J. Respir. Crit. Care Med.* 2005; **172**: 453–9.
29 Kharitonov SA, Gonio F, Kelly C *et al.* Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur. Respir. J.* 2003; **21**: 433–8.
30 Joint Statement of the American Thoracic Society (ATS) and the European Respiratory Society (ERS). ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am. J. Respir. Crit. Care Med.* 2005; **171**: 912–30.
31 Miller MR, Hankinson J, Brusasco V *et al.* Standardisation of spirometry. *Eur. Respir. J.* 2005; **26**: 319–38.
32 Juniper EF, O'Byrne PM, Guyatt GH *et al.* Development and validation of a questionnaire to measure asthma control. *Eur. Respir. J.* 1999; **14**(4): 902–7.
33 Anderson SD, Brannan JD. Methods for 'indirect' challenge tests including exercise, eucapnic voluntary hyperpnea, and hypertonic aerosols. *Clin. Rev. Allergy Immunol.* 2003; **24**(1): 27–54.
34 Anderson SD, Brannan J, Spring J *et al.* A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am. J. Respir. Crit. Care Med.* 1997; **156**(3 Pt 1): 758–65.
35 Crapo RO, Casaburi R, Coates AL *et al.* Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am. J. Respir. Crit. Care Med.* 2000; **161**(1): 309–29.
36 Wilson RC, Jones PW. Long-term reproducibility of Borg scale estimates of breathlessness during exercise. *Clin. Sci. (Lond.)* 1991; **80**: 309–12.
37 Dahlen B, O'Byrne PM, Watson RM *et al.* The reproducibility and sample size requirements of exercise-induced bronchoconstriction measurements. *Eur. Respir. J.* 2001; **17**: 581–8.
38 Juniper EF, Svensson K, Mork AC *et al.* Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir. Med.* 2005; **99**: 553–8.
39 Brannan JD, Anderson SD, Gomes K *et al.* Fexofenadine decreases sensitivity to and montelukast improves recovery from inhaled mannitol. *Am. J. Respir. Crit. Care Med.* 2001; **163**(6): 1420–5.
40 Anderson SD, Rodwell LT, Du Toit J *et al.* Duration of protection by inhaled salmeterol in exercise-induced asthma. *Chest* 1991; **100**(5): 1254–60.
41 Villaran C, O'Neill SJ, Helbling A *et al.* Montelukast versus salmeterol in patients with asthma and exercise-induced bronchoconstriction. Montelukast/Salmeterol Exercise Study Group. *J. Allergy Clin. Immunol.* 1999; **104**(3 Pt 1): 547–53.
42 Coreno A, Skowronski M, Kotaru C *et al.* Comparative effects of long-acting beta2-agonists, leukotriene receptor antagonists, and a 5-lipoxygenase inhibitor on exercise-induced asthma. *J. Allergy Clin. Immunol.* 2000; **106**: 500–6.
43 Stelmach I, Grzelewski T, Majak P *et al.* Effect of different antiasthmatic treatments on exercise-induced bronchoconstriction in children with asthma. *J. Allergy Clin. Immunol.* 2008; **121**: 383–9.
44 Petersen R, Agertoft L, Pedersen S. Treatment of exercise-induced asthma with beclomethasone dipropionate in children with asthma. *Eur. Respir. J.* 2004; **24**: 932–7.
45 Szefler SJ, Martin RJ, King TS *et al.* Significant variability in response to inhaled corticosteroids for persistent asthma. *J. Allergy Clin. Immunol.* 2002; **109**: 410–18.
46 Brannan JD, Koskela H, Anderson SD *et al.* Budesonide reduces sensitivity and reactivity to inhaled mannitol in asthmatic subjects. *Respirology* 2002; **7**(1): 37–44.
47 Koskela HO, Hyvarinen L, Brannan JD *et al.* Sensitivity and validity of three bronchial provocation tests to demonstrate the effect of inhaled corticosteroids in asthma. *Chest* 2003; **124**(4): 1341–9.
48 Hancox RJ, Subbarao P, Kamada D *et al.* Beta2-agonist tolerance and exercise-induced bronchospasm. *Am. J. Respir. Crit. Care Med.* 2002; **165**: 1068–70.
49 Shaw DE, Berry MA, Thomas M *et al.* The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am. J. Respir. Crit. Care Med.* 2007; **176**: 231–7.