

Exhaled nitric oxide levels in asthma: Personal best versus reference values

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Background: Factors affecting the fraction of nitric oxide in exhaled air (FE_{NO}) are multiple. Interpreting values when assessing airways disease may be problematic. Clinically optimum levels have not been defined.

Objectives: We aimed to establish the relationship between predicted values for FE_{NO} obtained from equations by Olin et al, Travers et al, and Dressel et al, and normalized levels after oral prednisone. We also compared postprednisone FE_{NO} levels with those obtained during optimized treatment with inhaled fluticasone.

Methods: Data were obtained before and after a trial of oral prednisone (30mg/d for 14 days), and also from a previously published study in which patients had their dose of inhaled corticosteroid adjusted using either FE_{NO} or symptoms/lung function to optimize treatment.

Results: Seventy-three patients completed the study. The geometric mean FE_{NO} after prednisone (17.7 parts per billion [ppb]; 95% CI, 15.5-20.2) was significantly lower than mean FE_{NO} at the optimized fluticasone dose (20.2 ppb; 95% CI, 17.1-23.8; $P=.04$) and at loss of control (27.6 ppb; 95% CI, 22.8-33.4; $P<.001$). FE_{NO} levels after prednisone did not differ significantly from the predicted values of Olin et al (16.8 ppb, 95% CI, 16.0-17.5; $P=.44$), but were significantly lower than values of Travers et al (predicted, 21.5 ppb; 95% CI, 20.9-22.2; $P=.005$) and Dressel et al (predicted, 27.8 ppb; 95% CI, 26.7-28.9; $P<.001$).

Conclusions: Optimum FE_{NO} levels are best established by using oral rather than inhaled steroid treatment, and these approximate to predicted values from the reference equation by Olin et al. However, at optimized doses of inhaled corticosteroid, although FE_{NO} levels were higher than predicted, asthma was well controlled. Targeting FE_{NO} on reference values is not justified. (J Allergy Clin Immunol 2009;■■■:■■■-■■■.)

Key words: Asthma, exhaled nitric oxide, inhaled corticosteroid, prednisone, reference values

Abbreviations used

FE_{NO}: Fraction of nitric oxide in exhaled air
ICS: Inhaled corticosteroid
LOC: Loss of control
ppb: Parts per billion

Exhaled nitric oxide is a surrogate marker for eosinophilic airway inflammation.¹ Measurements are used to interpret the etiology of nonspecific respiratory symptoms, identify potential responders to inhaled corticosteroid (ICS) therapy,² and monitor underlying disease activity in asthma.^{3,4} However, it remains unclear how best to interpret individual FE_{NO} measurements, particularly in the ongoing management of asthma. This is because, unlike induced sputum eosinophils, which are absent unless airway inflammation is present, a number of nonpathological factors may influence FE_{NO}, occasionally giving rise to increased levels even in healthy individuals.⁵

To date, clinically significant cut-points for FE_{NO} have been based on the relationship between FE_{NO} and induced sputum eosinophil counts,⁶ as well as FE_{NO} levels which are prognostically important in relation to ICS therapy.^{7,8} In general, low FE_{NO} values (<25 parts per billion [ppb]) are associated with minimal airway eosinophilia. In patients with diagnosed asthma, this generally implies good asthma control or, if the patient is symptomatic, the need to consider explanations for their symptoms other than active eosinophilic airway inflammation. Conversely, high values (>50 ppb) indicate active eosinophilic airway inflammation and the likelihood of deterioration in asthma control if the dose of ICS is reduced, even if the patient is asymptomatic.³

However, this broad approach leaves open some unresolved questions. These have become more important in the light of recent publications describing reference ranges for FE_{NO} in healthy individuals.⁹⁻¹⁴ First, should reference ranges derived from healthy individuals that correct for underlying factors such as age, sex, height, atopy, and smoking history be applied when interpreting FE_{NO} levels in patients with asthma with current respiratory symptoms? Second, should high levels prompt an increase in ICS dose? Some patients with asthma may have FE_{NO} levels that are higher than normal despite good asthma control. Pijnenburg et al¹⁵ have previously argued that normalizing FE_{NO} is an unrealistic objective using anti-inflammatory treatment. Third, is it possible that individualized personal best values might be more clinically useful than population-derived reference values? Values deemed to be clinically normal and abnormal should perhaps be based on FE_{NO} levels obtained when an individual patient's asthma is respectively well controlled and poorly controlled.

The aim of the current study was to identify the relationship between reference values for FE_{NO} and personal best levels obtained after a course of oral prednisone in patients with mild

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to moderate persistent asthma. Second, we compared the personal best FE_{NO} levels after prednisone with those obtained at loss of control and during optimized treatment with inhaled steroid. These comparisons were designed to clarify whether anti-inflammatory treatment ought to be guided by reference values or individual clinically-based cut-points for FE_{NO}.

METHODS

Patients with chronic asthma receiving ICS treatment were recruited. The study design has been reported previously.¹⁶ After a run-in, at visit 2, patients commenced a 14-day open-label course of oral prednisone 30 mg/d, designed to achieve personal best FE_{NO}. Relevant postprednisone data were recorded at visit 3. This aspect of the study has not been reported previously.

At visit 3, patients commenced inhaled fluticasone 750 µg/d (or 500 µg/d if prestudy ICS requirements were <200 µg/d fluticasone or equivalent (Flixotide; GlaxoSmithKline, Greenford, United Kingdom). After 4 weeks, subjects were randomly allocated to 1 of 2 groups, each having an algorithm for fluticasone dose titration. One algorithm was based on changes in symptoms, bronchodilator use, diurnal peak flows, and spirometry (conventional group).¹⁷ In the other, ICS dose adjustments were based on FE_{NO} measurements (FE_{NO} group), with a cut-point of 15 ppb at a flow rate of 250 mL/s (equivalent to 43 ppb at a flow rate of 50 mL/s). The fluticasone dose was stepped down or up (to a maximum of 1000 µg/d). Six dose steps were available: 1000, 750, 500, 250, 100, and 0 µg/d (matching placebo). Down titration was repeated 1 step at a time at 4-weekly intervals until asthma became uncontrolled in relation to either high FE_{NO} or *a priori* clinical criteria (loss of control [LOC]). At LOC, the dose of fluticasone was increased, 1 step at a time at 4-weekly intervals, until asthma was again controlled. At this point, the optimized ICS dose was deemed to have been established.

Measurements

Spirometry was measured according to American Thoracic Society criteria.¹⁸ Exhaled nitric oxide measurements were measured in accordance with American Thoracic Society recommendations by using a NIOX analyzer (Aerocrine AB, Solna, Sweden).¹⁹ All FE_{NO} results are reported as for an exhalation flow rate of 50 mL/s. During the course of the study, to comply with published guidelines, the flow rate used for FE_{NO} measurements was changed from 250 mL/s to 50 mL/s, but we continued to measure FE_{NO} at both flow rates, and data at both flow rates were obtained from 94 patients. The correlation between the 2 was highly significant ($r=0.97$; $P<.0001$) and a conversion factor was then applied to all data:

$$\text{FE}_{\text{NO}} @ 50 \text{ mL/s} = 2.866 \times \text{FE}_{\text{NO}} @ 250 \text{ mL/s}$$

Predicted values for FE_{NO} measurements

Comparisons were made between FE_{NO} levels obtained both after oral prednisone and at optimized doses of ICS with predicted values derived from recently published reference equations. The first, reported by Olin et al,¹¹ included corrections for atopy, age, and height:

$$\text{Log}_e \text{FE}_{\text{NO}} = -0.00082 + 0.21 (\text{if atopy} = \text{yes}) + 0.013 \times \text{height (cm)} + 0.01 \times \text{age (y)}$$

The second, reported by Travers et al,¹² does not provide reference equations in the published article. The authors were contacted, and relevant information was provided (Weatherall M, personal communication, October 2008):

$$\text{Log FE}_{\text{NO}} = 2.818 + 0.1704 (\text{if atopy} = \text{yes}) + 0.235 (\text{if sex} = \text{male}) - 0.15 (\text{if exsmoker}) - 0.224 (\text{if current smoker})$$

The third, reported by Dressel et al,¹⁴ included corrections for respiratory allergy, smoking status, current infection, sex, and height:

TABLE I. Demographic and clinical data at baseline for the 73 patients who completed the course of prednisone

Age (y)	44 (range,13-73)
Sex (male:female)	32:41
Duration of asthma (y)	25.4 (range, 1-65)
Baseline FE _{NO} (ppb), geometric mean	20.5 (17.8-23.6)
ICS dose at study entry, BDP equivalent (µg/d)	900 (780-1020)
Optimized ICS dose after titration, BDP equivalent (µg/d)	958 (764-1151)
FEV ₁ (% predicted) at baseline	82.8 (77.6-88.0)
FEV ₁ (% predicted) at LOC	78.6 (73.1-84.0)
FEV ₁ (% predicted) after prednisone	84.0 (78.9-89.2)

Data are presented as means and 95% CIs unless otherwise stated.

BDP, Beclomethasone.

$$\begin{aligned} \text{FE}_{\text{NO}} = & 17.49 \times 1.49 (\text{if atopy} = \text{yes}) \times 0.627 (\text{if smoking} = \text{yes}) \times \\ & 1.235 (\text{if current infection} = \text{yes}) \times 1.174 (\text{if sex} = \text{male}) \times \\ & 1.113^{(\text{height in cm} - 170)/10} \end{aligned}$$

Analyses

The principal analyses were performed by using geometrically transformed values for FE_{NO}. Student *t* tests and Mann-Whitney *U* tests were used to compare measured and predicted values. Mann-Whitney *U* tests were used for between-group comparisons.

Ethics

The study was approved by the Otago Ethics Committee, and participants gave written informed consent.

A fuller version of this article's Methods section is provided in the Online Repository at www.jacionline.org.

RESULTS

Of the 94 patients who completed the original trial, 73 received a course of oral prednisone and completed the current study protocol. The remaining 21 subjects did not give consent for the prednisone trial and are excluded from further analysis. The demographic and relevant clinical data are provided in Table I. Thirty-eight patients were treated according to the conventional algorithm, and 35 were treated according to the FE_{NO} algorithm. There were no significant differences in the clinical status (symptom scores, spirometric values, and so forth) at LOC or when optimum ICS dosing had been established between the 2 groups (Table II).

The principal study results are shown in Tables III and IV. These include the mean FE_{NO} levels derived from the prediction equations by Olin et al,¹¹ Travers et al,¹² and Dressel et al,¹⁴ together with the FE_{NO} levels measured after the 2-week course of oral prednisone (theoretically lowest), at loss of control (theoretically highest), and with optimized doses of inhaled fluticasone. Separate results for male and female subjects are also reported, given that there may be significant differences in FE_{NO} in relation to sex.²⁰ Further supplementary data are provided in this article's Table E1, E2, and E3 in the Online Repository at www.jacionline.org.

The geometric mean FE_{NO} level after the course of prednisone (17.7 ppb) was significantly lower than that obtained at loss of control (27.6 ppb; $P<.001$). It was also significantly lower than the FE_{NO} level at the optimum dose of fluticasone (20.2 ppb; $P=.04$). The mean FE_{NO} levels after prednisone were significantly lower than the predicted values derived from the

TABLE II. Data obtained in the period immediately before or at LOC after progressive reduction in ICS dose and at optimization of ICS dose according to the 2 treatment strategy algorithms

Parameter	At LOC after inhaled steroid withdrawal			After inhaled steroid dose optimization		
	Conventional group (n = 38)	FE _{NO} group (n = 35)	P value for between-group comparison	Conventional group (n = 38)	FE _{NO} group (n = 35)	P value for between-group comparison
Symptom score (mean of 7 days before LOC)	1.2 (0.9-1.5)	1.0 (0.7-1.4)	.39	0.6 (0.3-0.9)	0.5 (0.2-0.7)	.39
Night waking (nights/wk, previous 7 d)	0.9 (0.4-1.4)	1.0 (0.4-1.6)	.81	0.0 (0-0.1)	0.0 (0-0.0)	.09
Bronchodilator use (occasions/d, previous 7 d)	2.6 (1.2-3.9)	2.0 (0.7-3.3)	.54	0.4 (0.2-0.7)	0.3 (0.1-0.6)	.56
Morning peak flow (mean of previous 7 d)	392 (356-428)	387 (354-420)	.85	404 (368-439)	401 (370-433)	.93
FEV ₁ % predicted	75.4 (68.0-82.8)	82.0 (74.0-90.0)	.24	79.4 (72.4-86.5)	85.5 (75.6-92.5)	.23

The first group (conventional group) had their ICS dose adjusted in relation to reported symptoms and lung function. The second group (FE_{NO} group) had their ICS dose adjusted in relation to FE_{NO} levels where *a priori*, a level of less than 43 ppb was deemed to indicate controlled airway inflammation. Data are given as means and 95% CIs.

TABLE III. Geometric mean FE_{NO} values predicted by each of the reference equations, and at each time point during the study in 73 subjects, stratified by sex

Parameter	FE _{NO} (ppb)						P
	All subjects (n = 73)		Male subjects (n = 32)		Female subjects (n = 41)		
	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	
Predicted value based on Olin et al ¹¹	16.8	16.0-17.5	19.0	18.0-20.0	15.2	14.5-16.0	<.001
Predicted value based on Travers et al ¹²	21.5*	20.9-22.2	24.6	24.1-25.1	19.4	19.0-19.7	<.001
Predicted value based on Dressel et al ¹⁴	27.8*	26.7-28.9	32.8	32.1-33.6	24.4	23.8-25.0	<.001
After course of oral prednisone	17.7	15.5-20.2	20.1	16.8-24.1	16.0	13.3-19.4	.12
At optimized dose of fluticasone	20.2	17.1-23.8	20.5	16.5-25.5	20.0	15.7-25.4	.96
At LOC	27.6	22.8-33.4	29.5	22.3-38.9	26.2	20.1-34.2	.71

Subjects received a trial of prednisone, and then all inhaled and oral steroid treatment was withdrawn until LOC. Thereafter the inhaled fluticasone dose was titrated until asthma was well controlled.¹⁶ Final column (P) represents comparison between male and female subjects. See text for other important comparisons.

*P <.01 for comparisons with FE_{NO} after oral prednisone.

equations from Travers et al¹² (predicted = 21.5 ppb; *P* = .005) and those based on the equations from Dressel et al¹⁴ (predicted = 27.8 ppb; *P* < .001) but were not significantly different from the values obtained by using equations from Olin et al.¹¹ (16.8 ppb; *P* = .44). There was no significant correlation between the predicted values obtained for individual patients and the actual FE_{NO} levels obtained after prednisone using any of the reference equations (Olin et al,¹¹ *r* = −0.01, *P* = .94; Travers et al,¹² *r* = 0.18, *P* = .12; Dressel et al,¹⁴ *r* = 0.15, *P* = .19).

DISCUSSION

Although FE_{NO} measurements are increasingly being used in clinical practice to assess airway inflammation, there are significant areas of uncertainty regarding how results ought to be interpreted, especially in the treatment of patients with chronic asthma over time. The results of the current study shed new light on the relationships between currently available reference values for FE_{NO}, values obtained when airway inflammation is optimally treated (using prednisone), and values obtained when asthma is poorly controlled.

In our study, FE_{NO} values were significantly lower after a course of oral prednisone (17.7 ppb) than with inhaled fluticasone (20.2 ppb). Although the mean difference between these 2 values, 2.5 ppb, may not seem to be clinically relevant, it ranged from

−39.5 to +62.6 ppb. In 30 of 73 cases, the difference in FE_{NO} with ICS compared with postprednisone values was greater than +25%, which is the coefficient of variation for repeated FE_{NO} measurements in patients with asthma.⁹ Thus, in a significant proportion of patients, FE_{NO} remained high despite apparently optimized doses of ICS, when in fact greater control of airway inflammation, and hence reduction in FE_{NO}, was at least theoretically possible using oral prednisone. There are a number of possible reasons for this. First, some patients may have been noncompliant with inhaled fluticasone but were more compliant with oral prednisone, although in the context of this randomized trial in which patients were carefully monitored, this is unlikely. Second, it is possible that although patients may have been taking ICS therapy as prescribed, there was suboptimal drug deposition in the affected airways because of poor inhaler technique. These issues were previously addressed by Pijnenburg et al¹⁵ in a controlled study in which detailed attention was given to inhaler technique and compliance among 40 children with asthma. High FE_{NO} levels persisted in the majority of children: only 6 of 40 achieved normal values (deemed to be less than 20 ppb) with treatment. The authors concluded that their corrective interventions were ineffective and that achieving normal values is not possible in patients with asthma. Third, persistently high FE_{NO} levels during ICS treatment may also reflect alveolar rather than airway nitric oxide production, in which case only systemic rather than topical anti-

TABLE IV. Factor difference in log-FE_{NO} measured at each of the important time points in relation to the predicted values obtained from each of 3 reference equations

	After course of oral prednisone			At optimized dose of fluticasone			At LOC		
	All	Male subjects	Female subjects	All	Male subjects	Female subjects	All	Male subjects	Female subjects
Comparison with log-predicted values based on Olin et al ¹¹	1.02 (0.97-1.07)	1.02 (0.95-1.09)	1.02 (0.87-1.00)	1.07* (1.01-1.13)	1.03 (0.95-1.10)	1.10* (1.01-1.20)	1.18* (1.11-1.25)	1.15* (1.05-1.25)	1.20* (1.10-1.30)
Comparison with log-predicted values based on Travers et al ¹²	0.94* (0.89-0.98)	0.94* (0.88-0.99)	0.94 (0.87-1.00)	0.98 (0.93-1.04)	0.94 (0.87-1.01)	1.01 (0.93-1.09)	1.08* (1.02-1.14)	1.06 (0.97-1.14)	1.10* (1.01-1.19)
Comparison with log-predicted value based on Dressel et al ¹⁴	0.87* (0.82-0.91)	0.86* (0.81-0.91)	0.87* (0.81-0.93)	0.91* (0.85-0.96)	0.86* (0.80-0.92)	0.94 (0.86-1.02)	1.00 (0.94-1.06)	0.97 (0.89-1.05)	1.02 (0.94-1.11)

Values in parentheses are 95% CIs.

**P* < .05 for the difference between predicted values and actual values.

inflammatory therapy will be effective. This possibility has been investigated by Gelb et al,²¹ who reported that in 10 patients already receiving ICS treatment, a course of oral prednisone resulted in a significant fall in alveolar nitric oxide concentrations.

The FE_{NO} values obtained after prednisone were not significantly different from the predicted values obtained using the equation from Olin et al,¹¹ and were statistically but not clinically significantly different from the values derived from Travers et al.¹² This suggests that at least in theory, personal best FE_{NO} levels in patients with asthma who have high levels when they are symptomatic do indeed coincide with this particular set of predicted values. This finding does not contradict the conclusions offered by Pijnenburg et al¹⁵: in that study, the criterion for success using ICS was to reduce FE_{NO} levels below a specific fixed cut-point (20 ppb). This may be unrealistic. It is possible that normal values based on reference equations are higher in some individuals than an *a priori* target cut-point for FE_{NO} that is being used to guide treatment. Comparisons between FE_{NO} levels obtained with prednisone and those derived from the equation by Dressel et al¹⁴ were often widely discrepant, suggesting that the equation by Dressel et al¹⁴ are unhelpful in this context. Not only was there a statistically significant difference between the predicted and the observed postprednisone values, but also the magnitude of this difference was often clinically significant (>25%). The reasons for this are not clear, but 1 possibility is that the population studied by Dressel et al¹⁴ included individuals with airway inflammation who were steroid-naïve and not in fact normal.

It is debatable whether aiming for optimum FE_{NO} levels is clinically justified. There is evidence that airway inflammation is still present in patients with a history of asthma who are currently in remission,²² and that this can be further reduced by more intensive inhaled anti-inflammatory treatment.²³ However, is this desirable? In our study, the optimized titrated dose of fluticasone was sufficient to provide for well controlled asthma. This clinical status was associated with FE_{NO} levels that were higher than those obtained postprednisone, and higher than the normal values predicted by the equation of Olin et al¹¹ (by a logarithmically converted factor of 1.07 (95% CI, 1.01-1.13)). These comparisons suggest that overall, achieving optimum FE_{NO} levels is not an important objective,

although from time to time in individual cases, it may be helpful to know where current FE_{NO} levels lie in relation to both personal best and population-based normal values.

Our study provides further information regarding the magnitude of changes in FE_{NO} that are clinically relevant. Recently Michils et al⁴ reported that a change from well controlled to poorly controlled asthma is associated with a change in FE_{NO} of greater than 30%. In the current study, the mean change in FE_{NO} between well controlled (optimum doses of ICS) and poorly controlled asthma (LOC) was 12.1 ppb (95% CI, 6.1-18.2), equivalent to 67% change (95% CI, 39.9-94.1). The mean absolute change (ppb) appears relatively low when compared with data from other studies. For example, Pijnenburg et al⁷ reported that after withdrawal of ICS, the mean FE_{NO} increased by 26 ppb in children whose asthma relapsed. However, these between-study differences are probably explained differences in the clinical criteria used to determine LOC.

A weakness of our study is that factors other than asthma that may have caused symptoms, such as rhinosinusitis or gastroesophageal reflux, were not specifically controlled for. This means that in some patients, asthma control may have been determined inaccurately. This might potentially have influenced the relationship between FE_{NO} and clinical status and hence the interpretation of our results. However, as far as possible, clinical confounders were excluded at screening, and we do not believe that our results were likely to have been affected significantly.

In conclusion, we have shown that in patients with persistent asthma whose phenotype is characterized by elevated FE_{NO} levels when symptomatic, personal best levels were lower using oral rather than inhaled steroid treatment, and that these approximated to predicted values derived from recently published reference equations by Olin et al¹¹ and to a lesser extent Travers et al.¹² The predicted values based on Dressel et al¹⁴ were less reliable. However, given that the majority of patients had well controlled asthma using ICS, with higher than predicted FE_{NO} levels, aiming for a target FE_{NO} based on reference equations does not appear to be justified in clinical practice. Overall, our data indicate that when measuring FE_{NO} in relation to asthma control and its treatment, target FE_{NO} levels based on group mean data or reference

equations have limited value. The absolute values and/or the magnitude of changes in FE_{NO} in relation to personal best obtained in individual patients when asthma is well controlled are more likely to be informative.

Clinical implications: When monitoring patients with asthma, interpreting FE_{NO} levels is best done using the patient's personal best value as a reference point, rather than predicted values based on reference equations. Targeting treatment against predicted FE_{NO} values is not justified.

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METHODS

The inclusion and exclusion criteria, design, and methods for the study have been reported in detail previously.^{E1} In brief, 110 patients age 12 to 75 years with chronic persistent asthma receiving regular treatment with ICS for 6 months or longer were recruited. Long-acting β -agonists were discontinued at visit 1. After a 2-week run-in, at visit 2, patients entered a 2-week open-label period of treatment with oral prednisone 30 mg/d without altering their pre-study ICS dose. This was designed to achieve best possible FE_{NO} and spirometric measurements. These were recorded at visit 3. This aspect of the study has not been previously reported.

At visit 3, all ICS treatment was substituted by inhaled fluticasone (Flixotide; GlaxoSmithKline, Greenford, United Kingdom) administered twice daily by using a combination of 2 identical blind metered-dose inhalers via a large volume spacer (Volumatic; GlaxoSmithKline). Subjects were commenced at 750 μ g/d (or 500 μ g/d if prestudy inhaled steroid requirements were less than 200 μ g/d fluticasone or equivalent). Subjects returned after 4 weeks and were then randomly allocated to 1 of 2 groups, each of which had an algorithm for fluticasone dose titration. Six treatment steps were available: 1000, 750, 500, 250, 100, and 0 μ g/d (matching placebo). Subjects were blind to their group allocation and to the prescribed dose of fluticasone. The algorithm for fluticasone dose adjustment in 1 group was based on Global Initiative for Asthma criteria for asthma control.^{E2} This included changes in symptoms, bronchodilator use, diurnal peak flows, and also spirometry (conventional group). In the second group, ICS dose adjustments were based solely on FE_{NO} measurements (FE_{NO} group), with a cut-point of 15 ppb at a flow rate of 250 mL/s (equivalent to 43 ppb at a flow rate of 50 mL/s) used to determine uncontrolled airway inflammation. The dose of inhaled fluticasone was stepped down or up (to a maximum of 1000 μ g/d) depending on whether asthma was deemed to be controlled or uncontrolled. Down-titration was repeated 1 step at a time at 4-weekly intervals until asthma became uncontrolled according to *a priori* criteria (LOC), at which point the dose of fluticasone was increased, again 1 step at a time at 4-weekly intervals, until asthma was again controlled. At the end of this titration procedure, the optimized ICS dose for that individual was deemed to have been established. Subjects remained on the optimized dose and were reviewed at 2-monthly intervals for 12 months. Details regarding the last phase of the study are not relevant to the current report and are not described further.^{E1}

Measurements

Spirometry was measured according to American Thoracic Society criteria.^{E3} FE_{NO} measurements were obtained in accordance with American Thoracic Society recommendations.^{E4} Initially, subjects had FE_{NO} levels measured at a flow rate of 250 mL/s. After the introduction of guidelines recommending a flow rate of 50 mL/s, all FE_{NO} measurements were made at both 50 and 250 mL/s, and a correction factor was calculated and applied. All FE_{NO} results are reported as for an exhalation flow rate of 50 mL/s. During the course of the study, to comply with published guidelines, the flow rate used for FE_{NO} measurements was changed from 250 mL/s to 50 mL/s, but we continued to measure FE_{NO} at both flow rates, and data at both flow rates were obtained from 94 patients. The correlation between the 2 was highly significant ($r = 0.97$; $P < .0001$), and a conversion factor was then applied to all data:

$$\text{FE}_{\text{NO}} @ 50 \text{ mL/s} = 2.866 \times \text{FE}_{\text{NO}} @ 250 \text{ mL/s}$$

Predicted values for FE_{NO} measurements

Comparisons were made between FE_{NO} levels obtained both after oral prednisone and at optimized doses of ICS with predicted values derived from recently published reference equations. The first, reported by Olin et al,^{E5} included corrections for atopy, age, and height:

$$\text{Log}_n \text{FE}_{\text{NO}} = -0.00082 + 0.21 (\text{if atopy} = \text{yes}) + 0.013 \times \text{height (cm)} + 0.01 \times \text{age (y)}$$

The second, reported by Travers et al,^{E6} does not provide reference equations in the published article. The authors were contacted and relevant information was provided (Weatherall M, personal communication, October 2008):

$$\text{Log FE}_{\text{NO}} = 2.818 + 0.1704 (\text{if atopy} = \text{yes}) + 0.235 (\text{if sex} = \text{male}) - 0.15 (\text{if exsmoker}) - 0.224 (\text{if current smoker})$$

The third, reported by Dressel et al,^{E7} included corrections for respiratory allergy, smoking status, current infection, sex, and height:

$$\begin{aligned} \text{FE}_{\text{NO}} = & 17.49 \times 1.49 (\text{if atopy} = \text{yes}) \times 0.627 (\text{if smoking} = \text{yes}) \times \\ & 1.235 (\text{if current infection} = \text{yes}) \times 1.174 (\text{if sex} = \text{male}) \times \\ & 1.113^{(\text{height in cm} - 170)/10} \end{aligned}$$

Analyses

The principal analyses were performed by using geometrically transformed values for FE_{NO}. Student *t* tests and Mann-Whitney *U* tests were used to compare measured and predicted values. Mann-Whitney *U* tests were used for between-group comparisons.

Ethics

The study was approved by the Otago Ethics Committee, and participants gave written informed consent.

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TABLE E1. FE_{NO} measurements for the 73 subjects, stratified by sex

Parameter	FE _{NO}						P value
	All subjects (n = 73)		Male subjects (n = 32)		Female subjects (n = 41)		
	Geometric mean	95% CI	Geometric mean	95% CI	Geometric mean	95% CI	
Predicted value based on Olin et al ^{E5}	16.8	16.0, 17.5	19.0	18.0, 20.0	15.2	14.5, 16.0	<.001
Predicted value based on Travers et al ^{E6}	21.5	20.9, 22.2	24.6	24.1, 25.1	19.4	19.0, 19.7	<.001
Predicted value based on Dressel et al ^{E7}	27.8	26.7, 28.9	32.8	32.1, 33.6	24.4	23.8, 25.0	<.001
After course of oral prednisone	17.7	15.5, 20.2	20.1	16.8, 24.1	16.0	13.3, 19.4	.12
Difference from Olin at al*	3.9	0.8, 7.0	3.9	-1.2, 9.1	3.9	0.0, 7.8	.89
Difference from Travers et al*	-0.7	-3.7, 2.3	-1.5	-6.3, 3.4	-0.1	-3.9, 3.7	.42
Difference from Dressel et al*	-7.2	-10.2, -4.1	-9.7	-14.5, -5.0	5.2	-9.1, -1.3	.05
At LOC	27.6	22.8, 33.4	29.5	22.3, 38.9	26.2	20.1, 34.2	.71
Difference from Olin at al*	20.8	19.9, 36.7	21.3	9.0, 33.7	20.4	12.5, 28.4	.66
Difference from Travers et al*	16.2	9.4, 23.1	15.9	3.8, 28.0	16.5	8.5, 24.4	.44
Difference from Dressel et al*	9.8	2.9, 16.7	7.7	-4.4, 19.7	11.4	3.4, 19.4	.17
At optimized dose of fluticasone	20.2	17.1, 23.8	20.5	16.5, 25.5	20.0	15.7, 25.4	.96
Difference from Olin at al*	8.7	4.3, 13.1	5.7	-0.3, 11.7	11.1	4.8, 17.3	.35
Difference from Travers et al*	4.1	-0.3, 8.5	0.3	-5.9, 6.4	7.1	0.9, 13.2	.10
Difference from Dressel et al*	-2.4	-6.9, 2.1	-8.0	-13.1, -2.1	2.0	-4.2, 8.3	.01

Subjects received prednisone trial, and then all inhaled and oral steroid treatment was withdrawn until LOC. Thereafter, the inhaled fluticasone dose was titrated until asthma was well controlled.¹⁶ Column with *P* values is for the comparison between male and female subjects.

*The differences between measured and reference values are reported as arithmetic means.

TABLE E2. FE_{NO} measurements for the 73 subjects, stratified by sex

Parameter	FE _{NO}						P value
	All subjects (n = 73)		Male subjects (n = 32)		Female subjects (n = 41)		
	Arithmetic mean	95% CI	Arithmetic mean	95% CI	Arithmetic mean	95% CI	
Predicted value based on Olin et al ^{E5}	17.1	16.3, 17.8	19.2	18.2, 20.2	15.4	14.7, 16.2	<.001
Predicted value based on Travers et al ^{E6}	21.7	21.0, 22.3	24.6	24.2, 25.1	19.4	19.1, 19.7	<.001
Predicted value based on Dressel et al ^{E7}	28.1	27.1, 29.2	32.9	32.2, 33.6	24.5	23.9, 25.1	<.001
After course of oral prednisone	21.0	17.9, 24.0	23.1	18.3, 28.0	19.3	15.5, 23.1	.12
Difference from Olin at al	3.9	0.8, 7.0	3.9	−1.2, 9.1	3.9	0.0, 7.8	.89
Difference from Travers et al	−0.7	−3.7, 2.3	−1.5	−6.3, 3.4	−0.1	−3.9, 3.7	.42
Difference from Dressel et al	−7.2	−10.2, −4.1	−9.7	−14.5, −5.0	5.2	−9.1, −1.3	.05
At LOC	37.9	31.0, 44.8	40.6	28.4, 52.7	35.8	27.9, 43.8	.71
Difference from Olin at al	20.8	19.9, 36.7	21.3	9.0, 33.7	20.4	12.5, 28.4	.66
Difference from Travers et al	16.2	9.4, 23.1	15.9	3.8, 28.0	16.5	8.5, 24.4	.44
Difference from Dressel et al	9.8	2.9, 16.7	7.7	−4.4, 19.7	11.4	3.4, 19.4	.17
At optimized dose of fluticasone	25.8	21.5, 30.1	24.9	18.8, 30.9	26.5	20.4, 32.6	.96
Difference from Olin at al	8.7	4.3, 13.1	5.7	−0.3, 11.7	11.1	4.8, 17.3	.35
Difference from Travers et al	4.1	−0.3, 8.5	0.3	−5.9, 6.4	7.1	0.9, 13.2	.10
Difference from Dressel et al	−2.4	−6.9, 2.1	−8.0	−13.1, −2.1	2.0	−4.2, 8.3	.01

Subjects received prednisone trial, and then all inhaled and oral steroid treatment was withdrawn until LOC. Thereafter, the inhaled fluticasone dose was titrated until asthma was well controlled.¹⁶ Column with *P* values is for the comparison between male and female subjects.

TABLE E3. FE_{NO} measurements for the 73 subjects, stratified by sex

Parameter	FE _{NO}						P value
	All subjects (n = 73)		Male subjects (n = 32)		Female subjects (n = 41)		
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	
Predicted value from Olin et al ^{E5}	16.5	14.6, 20.1	20.2	17.7, 21.3	15.1	14.0, 16.9	<.001
Predicted value from Travers et al ^{E6}	19.9	19.9, 25.1	25.1	25.1, 25.1	19.9	19.9, 19.9	<.001
Predicted value from Dressel et al ^{E7}	26.4	24.0, 32.8	33.3	31.6, 34.5	24.3	23.5, 25.9	<.001
After course of oral prednisone	16.1	11.9, 28.9	19.3	12.8, 29.6	14.5	11.0, 25.0	.12
Difference from Olin at al*	−1.1	−5.3, 11.1	−1.2	−5.5, 10.1	−1.1	−5.4, 12.3	.89
Difference from Travers et al*	−5.3	−10.4, 4.4	−5.8	−11.1, 4.5	−5.3	−8.4, 5.1	.42
Difference from Dressel et al*	−11.5	−17.0, −1.7	−13.3	−18.9, −3.4	−10.2	−13.8, 2.6	.05
At LOC	29.0	14.4, 53.8	23.9	15.2, 65.3	34.1	12.8, 51.8	.71
Difference from Olin at al*	10.9	−3.0, 38.0	7.6	−3.2, 44.0	18.4	−1.9, 37.9	.66
Difference from Travers et al*	5.5	−7.1, 33.0	−1.2	−9.0, 40.1	14.2	−6.3, 32.0	.44
Difference from Dressel et al*	−1.3	−14.6, 28.2	−7.6	−17.9, 29.3	8.4	−10.5, 28.2	.17
At optimized dose of fluticasone	22.7	10.8, 35.3	22.1	13.9, 28.9	22.7	10.1, 36.7	.96
Difference from Olin at al*	2.7	−4.6, 19.9	1.2	−4.5, 8.4	5.9	−4.8, 20.4	.35
Difference from Travers et al*	−1.5	−9.2, 11.4	−3.0	−9.3, 3.8	2.9	−9.6, 16.9	.10
Difference from Dressel et al*	−9.7	−16.3, 7.4	−12.3	−18.8, −5.4	−3.7	−15.1, 13.6	.01

Subjects received prednisone trial, and then all inhaled and oral steroid treatment was withdrawn until LOC. Thereafter, the inhaled fluticasone dose was titrated until asthma was well controlled.¹⁶ Column with *P* values is for the comparison between male and female subjects.

*All differences between measured and reference values are reported as medians.