

RESEARCH ARTICLE

Effect of Inhaled β_2 -Agonist on Exhaled Nitric Oxide in Chronic Obstructive Pulmonary Disease

Mostafa Amer¹, Jan Cowan¹, Andrew Gray², Ben Brockway¹, Jack Dummer^{1*}

1 Otago Respiratory Research Unit, Department of Medicine, University of Otago, Dunedin, New Zealand,

2 Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand

* jack.dummer@otago.ac.nz

Abstract

The fractional exhaled nitric oxide measured at an expiratory flow of 50mL/s (FE_{NO50}) is a marker of airway inflammation, and high levels are associated with greater response to steroid treatment. In asthma, FE_{NO50} increases with bronchodilation and decreases with bronchoconstriction, the latter potentially causing an underestimate of the degree of airway inflammation when asthma worsens. It is unknown whether the same effect occurs in chronic obstructive lung disease (COPD). Likewise, it is not known whether changes in airway calibre in COPD patients alter flow-independent parameters describing pulmonary nitric oxide exchange, such as the maximal flux of nitric oxide (NO) from the proximal airway compartment ($J'aw_{NO}$) and the distal airway/alveolar concentration of NO (CA_{NO}). We recruited 24 patients with COPD and performed FE_{NO} analysis at multiple expiratory flows before and after treatment with inhaled β_2 -agonist bronchodilator therapy. For the 21 patients analysed, FE_{NO50} rose from 17.1 (1.4) ppb (geometric mean (geometric SD)) at baseline, to 19.3 (1.3) ppb after bronchodilator therapy, an increase of 2.2 ppb (95% CI, 0.7–3.6; $P = 0.005$). There were non-significant changes in flow-independent NO parameters. The change in FE_{NO50} correlated positively with the change in $J'aw_{NO}$ ($r_s = 0.67$, $P < 0.001$; $r_s = 0.62$, $P = 0.002$ before and after correction for axial back-diffusion respectively) following bronchodilation. Inhaled bronchodilator therapy can increase exhaled nitric oxide measurements in COPD. The standardisation of inhaled bronchodilator therapy before FE_{NO} analysis in COPD patients should therefore be considered in both research and clinical settings.

Introduction

The fraction of exhaled nitric oxide (FE_{NO50}) is a non-invasive biomarker of inflammation associated with T-helper type 2 cells and eosinophils in the airways, which typically occurs in asthma and responds to inhaled corticosteroid (ICS) [1–3]. FE_{NO50} is therefore useful for predicting whether or not a patient with airways disease will respond to ICS, with higher levels being associated with greater responsiveness [4].



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Nonetheless, as a clinical tool, FE_{NO}50 has some limitations, and one of these is that it is influenced by airway calibre. Previous studies in asthma patients have shown that administration of inhaled salbutamol causes an increase in FE_{NO}50 of approximately 10% [5]. More recent studies have shown that acute bronchoconstriction is associated with a drop in FE_{NO}50, a reduction in FEV₁ of around 30% being associated with a similar reduction in FE_{NO}50 [6, 7]. This is problematic because, at a time of deteriorating asthma control, bronchoconstriction may result in a falsely reassuring FE_{NO}50 implying minimal airway inflammation when, in fact, the inflammatory state has worsened.

Many patients with COPD exhibit bronchodilator reversibility [8] but little is known about the effect of changes in airway calibre on FE_{NO}50 in COPD. There is some evidence to suggest that a greater degree of reversibility of airway obstruction is associated with an elevated FE_{NO}50 and eosinophilic airway inflammation [9, 10]. COPD patients with this phenotype of high FE_{NO}50 and airway eosinophilia are also more likely to respond to corticosteroid [11, 12]. At present, the effect of change in airway calibre on FE_{NO}50 in COPD patients is uncertain. This is important to investigate because it may be more difficult to detect corticosteroid-responsive COPD patients if FE_{NO}50 measurements are performed when their airways are constricted, and FE_{NO}50 is lower than it otherwise might be.

COPD is typically associated with inflammation of the distal airways, so measures of nitric oxide concentration or production in the distal airways may be of value in COPD [13]. Using a two-compartment model of pulmonary nitric oxide (NO) exchange, flow-independent NO parameters can be derived from the measurement of exhaled nitric oxide concentration at multiple expiratory flows: the maximal flux of nitric oxide (NO) from the proximal airway compartment (J'_{awNO}) and the distal airway/alveolar concentration of NO (CA_{NO}) [14]. More recently, a simplified method has been proposed, using only two expiratory flows, to determine surrogate markers of J'_{awNO} and CA_{NO} : the area under the curve of the NO concentration versus time plot (AUC-NO) at the expiratory flow of 200 mL/s (AUC_{200}) represents the CA_{NO} , and the difference in AUC-NO between the 50 and 200 mL/s exhalations (ΔAUC_{50-200}) represents the J'_{awNO} [15]. The effects of bronchodilation on these flow-independent NO exchange parameters, in patients with COPD, is unknown.

We hypothesised that, in COPD patients, FE_{NO}50 would increase after administration of inhaled β_2 -agonist. The primary aim of the study was to determine any change in FE_{NO}50 following the administration of bronchodilator. The secondary aim was to determine any change in flow-independent NO parameters following the administration of bronchodilator.

Methods

Participants

Twenty-four patients with COPD were recruited and attended a single visit between December 2014 and January 2015 inclusive. Patients were aged 45 years or older, had a smoking history of more than 10 pack years, a post-bronchodilator FEV₁/FVC of less than 70% and FEV₁ < 80% predicted. Patients had stable COPD with no exacerbations or use of antibiotics in the two weeks preceding study participation. For each patient, a history of current and past respiratory symptoms, smoking history and medications were obtained, and beclomethasone dipropionate (BDP) equivalents were calculated as described previously [16]. A modified Medical Research Council (mMRC) dyspnoea score [17] and COPD assessment test (CAT) [18] were also completed. Patients with diagnosed lung cancer, bronchiectasis, or other significant co-morbidity were excluded from the study, as were patients unable to perform the 50mL/s exhaled nitric oxide (FE_{NO}50) manoeuvres in accordance with American Thoracic Society (ATS) guidelines [19]. A study protocol is provided for further information (S1 Protocol). This study was

registered at the Australian New Zealand Clinical Trials Registry: ACTRN12616000140459, it was approved by the Northern B Health and Disability Ethics Committee (reference 14/NTB/164), and all patients gave written, informed consent. Māori consultation was undertaken with Ngāi Tahu.

Procedures

Participants performed the following sequence of tests to allow for the known effects of spirometry on exhaled nitric oxide measurements: (1) FE_{NO}50 analysis; (2) FE_{NO} analysis at multiple expiratory flows; and (3) spirometry. Forty-five minutes after spirometry, 400mcg salbutamol was administered via spacer, and, 15 minutes later, steps 1–3 were repeated. This sequence of tests allowed one hour to pass between baseline spirometry and post-bronchodilator FE_{NO} analysis, so the former did not affect the latter [5, 20, 21]. Fifteen minutes elapsed between administration of salbutamol and the second set of tests to allow bronchodilation to occur fully [22, 23]. All subjects were required to withhold tiotropium for 24 hours and all other inhalers for 12 hours prior to attendance, and current smokers were required to abstain from smoking within one hour of the study visit as currently recommended [19].

A chemiluminescence nitric oxide analyser (NOA 280i; Sievers, Boulder, CO) was used to measure FE_{NO}50 as per ATS guidelines [19], and FE_{NO} was measured at 100, 150, 200, and 250 mL/s as described previously [14, 15]. Briefly, patients performed two exhalations at each expiratory flow, by inhaling NO-free air and exhaling against resistance to increase mouth pressure to 10cmH₂O, thereby closing the soft palate and isolating the nasopharynx [24]. Pressure and NO concentration were recorded simultaneously for each exhalation manoeuvre, and subjects were encouraged to maintain the required pressure and flow through a visual biofeedback system. The nitric oxide analyser was calibrated weekly with known NO concentration (50 parts per million) and zero NO gases, as per the manufacturer's guidelines. To examine consistency in the measurement of FE_{NO} between subjects, a random sample of 40 NO plateaus at different expiratory flows were subsequently re-read by a trained investigator blinded to the subject and test phase (pre- or post-bronchodilator).

CA_{NO} and J_{awNO} were calculated using the slope-intercept method with and without adjustment for axial back-diffusion [14]. AUC₂₀₀ and Δ AUC₅₀₋₂₀₀ were also calculated according to the recently published method [15].

Spirometry (FEV₁) was performed using a flow-volume device (VMax 1022; SensorMedics; Yorba Linda, CA) according to current standards [22], and used to determine reversibility [21] and the degree of airflow limitation [25]. The spirometer was calibrated daily with a 3L calibration syringe prior to subject testing.

Statistical analysis

Summary statistics are provided for all patient characteristics of interest and all clinical measures. Where continuous variables are log-normally distributed, geometric means and geometric standard deviations are provided. The changes in pre- and post-bronchodilator measurements were reviewed to ascertain their distributions, and, given no indication that changes were non-normally distributed, compared with paired t-tests using untransformed data. The associations between the change in FE_{NO}50 level and change in FEV₁ and between other pairs of continuous variables were assessed by Spearman's rank correlation as it was not felt that linearity could be assumed a priori. Two-sided p values <0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism version 6.05 for Windows (GraphPad Software, La Jolla California USA).

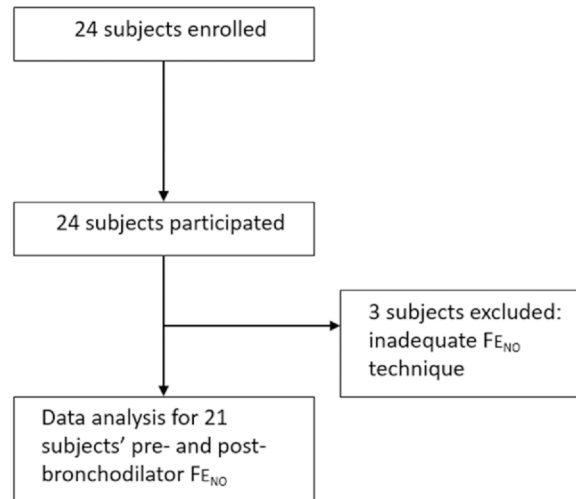


Fig 1. Study profile.

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Results

Twenty-four participants were recruited to the study, of whom three were excluded from the analysis because their FE_{NO50} exhalations did not meet ATS criteria for acceptable manoeuvres (Fig 1). The characteristics of the remaining 21 subjects are shown below (Table 1), and a minimal dataset is available as supporting information (S1 Table).

FE_{NO50} rose from 17.1 (1.4) ppb (geometric mean (geometric SD)) at baseline, to 19.3 (1.3) ppb after bronchodilator therapy, an increase of 2.2 ppb (95% CI, 0.7–3.6; $P = 0.005$) (Fig 2). There were non-significant changes in the flow-independent NO parameters (Table 2).

Both with and without adjustment for axial back-diffusion, the change in FE_{NO50} correlated positively with the change in J'_{awNO} ($r_s = 0.62$, $P = 0.002$; $r_s = 0.67$, $P < 0.001$ respectively) and negatively with the change in CA_{NO} ($r_s = 0.52$, $P = 0.02$; $r_s = 0.36$, $P = 0.11$) following administration of inhaled β_2 -agonist (Fig 3). We did not observe a similar relationship between the change in FE_{NO50} and change in FEV_1 ($r_s = 0.19$, $P = 0.42$).

The ΔAUC_{50-200} correlated with the J'_{awNO} calculated using the slope-intercept method ($r_s = 0.74$, $P < 0.001$; $r_s = 0.72$, $P < 0.001$ pre- and post-bronchodilator respectively) [26]. The ΔAUC_{50-200} also correlated with the J'_{awNO} when calculated using the Condorelli adjustment ($r_s = 0.82$, $P < 0.001$; $r_s = 0.79$, $P < 0.001$ pre- and post-bronchodilator respectively) [14]. There was a correlation between the AUC_{200} and the CA_{NO} calculated using the slope-intercept method [26] ($r_s = 0.59$, $P = 0.005$; $r_s = 0.58$, $P = 0.006$ pre- and post-bronchodilator respectively). No correlation was observed between the AUC_{200} and the CA_{NO} calculated using the Condorelli adjustment ($r_s = 0.23$, $P = 0.32$; $r_s = 0.26$, $P = 0.26$ pre- and post-bronchodilator respectively) [14].

Discussion

FE_{NO50} increased by 2.2 ppb, or 13% of baseline levels, in COPD patients after administration of inhaled β_2 -agonist ($P = 0.005$). This finding is consistent with a number of previous studies of asthma patients. Silkoff *et al* previously showed that, after administration of inhaled β_2 -agonist, FE_{NO50} increased by approximately 10% in patients with asthma [5]. More recent studies of asthmatic patients showed that an acute reduction in airway calibre led to a parallel drop in FE_{NO50} [6, 7]. Other studies, however, have shown no change in FE_{NO} with changes in airway

Table 1. Subject characteristics.

n (female/male)	21 (10/11)
Age, years	68 ± 10
NZ European, n (%)	20 (95)
Māori, n (%)	1 (5)
Body mass index, kg/m ²	25.4 ± 4.3
Current smoker, n (%)	5 (24)
Ex-smoker, n (%)	16 (76)
Smoking pack-years	33 ± 14
Inhaled steroid, n (%)	17 (81)
BDP equivalent	1062 ± 847
mMRC score	1.8 ± 1.0
CAT score	19 ± 6
Post-bronchodilator FEV ₁ /FVC ratio (%)	41.8 ± 11.2
Post-bronchodilator FEV ₁ (L)	1.15 ± 0.33
Post-bronchodilator FEV ₁ (% predicted)	43.3 ± 12.5
Reversible airflow obstruction, [‡] n (%)	5 (24)
GOLD group A, ^ψ n (%)	3 (14)
GOLD group B, ^ψ n (%)	1 (5)
GOLD group C, ^ψ n (%)	6 (29)
GOLD group D, ^ψ n (%)	11 (52)

Data are presented as mean ± SD unless stated otherwise.

[‡] Based on ≥ 12% and 200mL increase in FEV₁ post-bronchodilator compared to baseline [21].

^ψ Global initiative for chronic Obstructive Lung Disease (GOLD) classification [25].

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calibre in asthma and COPD [27, 28]. Any increase in FE_{NO}50 after inhaled β_2 -agonist is most likely explained by the changes in airway wall dynamics that accompany the changes in airway calibre and influence NO diffusion. Since J'_{awNO} is proportional to airway NO diffusion capacity (D_{awNO}), an increase in airway surface area and reduction in wall thickness through bronchodilation would be expected to increase D_{awNO} , and in turn, J'_{awNO} and FE_{NO}50 [6].

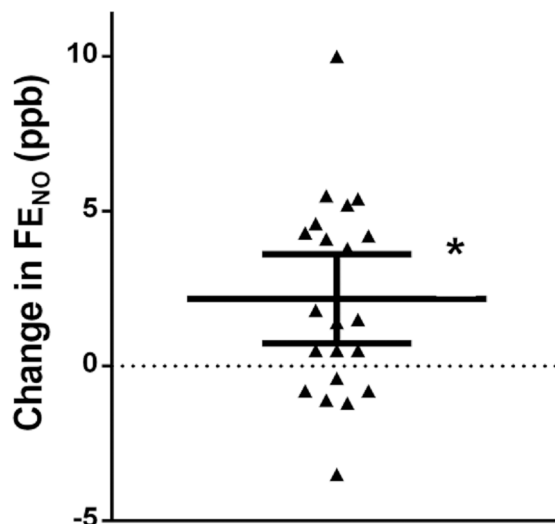


Fig 2. Plot showing the individual values and the mean with 95% confidence interval post-bronchodilator changes in FE_{NO}50, ppb. (*P = 0.005 for the change in FE_{NO}50).

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Table 2. Pre- and post-bronchodilator spirometry, FE_{NO}50 and flow-independent pulmonary NO parameters in 21 patients with COPD.

	Pre-bronchodilator	Post-bronchodilator	P-value [‡]
FEV ₁ (L)	1.00 ± 0.26	1.15 ± 0.33	< 0.001
FVC (L)	2.50 ± 0.64	2.81 ± 0.76	< 0.001
FE _{NO} 50 (ppb) [§]	17.1 ± 1.4	19.3 ± 1.3	0.005
J'aw _{NO} (pL/s) [¶]	872 ± 866	924 ± 760	0.54
C _{ANO} (ppb) [¶]	3.2 ± 2.0	2.9 ± 2.2	0.44
J'aw _{NO} (pL/s) [¶]	1316 ± 1111	1489 ± 1295	0.09
C _{ANO} (ppb) [¶]	2.2 ± 1.6	1.7 ± 1.4	0.34
Δ AUC ₅₀₋₂₀₀ (ppb/s)	13.0 ± 8.7	15.6 ± 11.8	0.11
AUC ₂₀₀ (ppb/s)	7.6 ± 5.7	7.7 ± 6.2	0.85

Data are presented as mean ± SD unless stated otherwise.

[‡]Pre- vs post-bronchodilator

[§] geometric mean ± geometric SD.

[¶]Calculated using the slope-intercept method of Tsoukias and George [26].

[¶]Adjusted for axial back-diffusion using the Condorelli equation [14].

AUC: area under the curve of the NO concentration vs time plot—between the 50 and 200 mL/s exhalations (Δ AUC₅₀₋₂₀₀), and at the 200 mL/s exhalation (AUC₂₀₀) [15].

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While the above mechanism is plausible, it should be noted that, in COPD, the distribution of nitric oxide production in the airways is incompletely understood, and may be quite different from that observed in asthma [29]. The increase in FE_{NO}50 in response to inhaled β_2 -agonist may therefore be a result of different mechanisms in asthma and COPD. Proposed alternative mechanisms include the increased recruitment of airways, indicated by the increase in FVC, which may lead to increased release of “trapped” nitric oxide from recently constricted and hypoventilated airways (4, 5). There is also some *in vitro* evidence that salbutamol directly upregulates inducible nitric oxide synthase in bronchial epithelial cells and, if this occurs *in vivo*, it could result in increased airways production of nitric oxide and a rise in FE_{NO}50 [30].

We were unable to show a relationship between change in FEV₁ and change in FE_{NO}50. This was in contrast to previous work demonstrating a positive correlation between changes in these parameters after bronchoconstriction in asthma [31]. Our study participants had a mean post-bronchodilator FEV₁ of only 1.15 litres, so absolute changes in FEV₁ before and after salbutamol were small and close to the accuracy limits of spirometry. This may have made it more difficult to determine any relationship between change in FEV₁ and change in FE_{NO}50.

C_{ANO} is commonly partitioned from J'aw_{NO} by a two-compartment model, requiring the subject to exhale at three different flows [14, 26]. In contrast, the AUC-NO has recently been proposed as a simpler procedure for the patient in order to obtain surrogates of J'aw_{NO} and

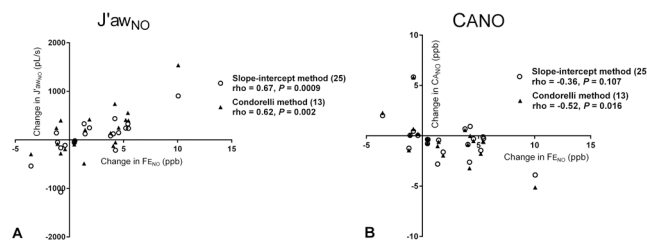


Fig 3. Scatter plots showing the correlation between the individual post-bronchodilator changes in FE_{NO}50, and J'aw_{NO}, pL/s (A), and C_{ANO}, ppb (B), relative to baseline.

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CA_{NO} by using only two expiratory flows [15]. We found a strong correlation between the ΔAUC_{50-200} and $J'_{aw_{NO}}$ calculated with and without the Condorelli adjustment. However, the correlation between the AUC_{200} and the CA_{NO} was weaker and was not observed when the Condorelli adjustment was applied. It has recently been noted, on modelling, that the conducting airways can make a significant contribution to the AUC_{200} and, because of this, it cannot be used to reliably estimate CA_{NO} [32]. Our experimental results are consistent with this.

We have found that, in patients with COPD, administration of a bronchodilator can significantly change FE_{NO50} , an increase in FEV_1 of over one-eighth of baseline resulting in a similar increase in FE_{NO50} . Thus, FE_{NO50} may be underestimated in a patient if measurement is performed in the presence of bronchoconstriction. The absolute change in FE_{NO50} was too small, for the most part, to mask an individual's change in inflammatory status using population-derived cut-points for the presence or absence of steroid-responsive eosinophilic airway inflammation at >50 ppb or <25 ppb respectively.

Despite having FE_{NO50} levels that are similar to those reported in subjects without lung disease [29], as many as two thirds of COPD patients have evidence of eosinophilic airway inflammation [33]. When assessing the effect of an intervention in such a group, a change of at least 20% has been recommended as indicating a significant rise or fall in FE_{NO50} [34]. In this context, it would be important to consider FE_{NO50} measurement in the presence of a standardised amount of inhaled bronchodilator treatment in order to avoid variability in the measurement of up to the 13% observed in this study.

Our findings support the recent suggestion, by Haccuria *et al.*, that change in airway calibre should be listed amongst the patient factors that influence measurement of FE_{NO50} in future ATS guidelines [7]. The same authors also suggest that, in asthma, a biomarker of airway inflammation incorporating both FE_{NO50} and FEV_1 may have potential in guiding ICS treatment where, as yet, the use of FE_{NO50} alone has been disappointing [35]. A similar case could be made for such a biomarker in COPD.

In conclusion, our study shows that administration of inhaled β -agonist increases FE_{NO50} in COPD patients. Therefore, when performing FE_{NO50} analysis in both research and clinical settings, in patients with COPD, the standardisation of pre-test bronchodilator therapy should be considered.

Supporting Information

S1 Protocol. Study protocol.

(PDF)

S1 Table. Minimal data set.

(PDF)

Author Contributions

Conceived and designed the experiments: JD. Performed the experiments: MA JC BB. Analyzed the data: MA AG JD. Contributed reagents/materials/analysis tools: JC BB JD. Wrote the paper: MA JC AG BB JD.

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