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## **Non-invasive sampling methods of inflammatory biomarkers in asthma and allergic rhinitis**

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## CHAPTER 4

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# Reversal of the late asthmatic response increases exhaled nitric oxide

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# Introduction

Exhaled nitric oxide (eNO) is indicative of the severity of the airway inflammation in asthma (1,2). Consequently, this non-invasive, patient-friendly methodology has recently been introduced into clinical practice as a diagnostic and monitoring tool especially for children (3-5). However, despite standardization of the eNO protocol (6), there are still some issues that need to be addressed. For example, whether there does or does not exist a relationship between the airway calibre and the eNO levels. A clear-cut relationship of eNO and the airway calibre may have implications for the measurements (7-10).

Late asthmatic airway response (LAR) to inhaled allergen, defined as a fall in forced expiratory volume in 1 second (FEV<sub>1</sub>) of at least 15% from pre-allergen baseline (11), have been shown to be associated with airway inflammation, including increased levels of eNO (12). We hypothesized that eNO levels during LAR are related to the degree of airway narrowing. Therefore, we studied the effect of vigorous bronchodilation with inhaled salbutamol during LAR on eNO levels.

## Subjects and methods

### SUBJECTS

Data from 12 asthmatics (6M/6F, 21-40 y) PC<sub>20</sub>FEV<sub>1</sub> methacholine <8 mg/mL with mild to moderate persistent asthma (FEV<sub>1</sub> 73.6-121.4 % predicted) and dual responses to inhaled house-dust mite (HDM) extract participating in an intervention study were used. All subjects had a history of persistent asthma for at least 1 year (according to criteria by GINA 2002), without any other clinically relevant disorders. None of the subjects had smoked tobacco during the past year. None was using concomitant anti-asthma or anti-allergy medication for at least 6 weeks prior to and during the study, except for inhaled short-acting β<sub>2</sub>-agonists *prn*. There was no history of viral infections of the lower airways for at least 4 weeks. The study was approved by the Ethics Committee of the Leiden University Medical Center and all participants gave written consent.

### STUDY DESIGN

The allergen-induced airway response during the LAR was measured at 1-hourly intervals until 9-h post-allergen. To reverse the LAR, 600 µg salbutamol was administered through an aerochamber (Volumatic, GlaxoSmithKline, Zeist, The Netherlands), and the FEV<sub>1</sub> was repeated 15 min

later. Exhaled NO was measured before and approximately 30 minutes post-salbutamol. For ethical reasons, no placebo-arm was included in this study.

## Methods

### ALLERGEN BRONCHOPROVOCATION TEST

The allergen bronchoprovocation test was performed using the standardized 2-minute tidal breathing method according to Cockcroft (11). Purified aqueous allergen extract of *Dermatophagoides Pterinysinus* (SQ503, ALK-BPT, ALK-Abelló, Nieuwegein, The Netherlands), with 0.5% phenol as a preservative was used for the bronchoprovocation tests (BPT). Preparation of the HDM extract dilutions was performed according to a previously validated protocol (13). The allergen aerosols were generated by a DeVilbiss 646 nebulizer (output 0.13 mL/min) connected to an in- and expiratory valve box with an expiratory aerosol filter (Pall Ultipor BB50T, Medica BV, Den Bosch, The Netherlands). Subjects first inhaled the allergen diluent, and provided the subsequent fall in FEV<sub>1</sub> remained <10% of baseline, they subsequently inhaled a total of 3 doubling concentrations of HDM extract at 12 min intervals that previously caused a LAR. The airway response during the LAR was measured at 1-hourly intervals until 9 h post-allergen or earlier if subjects experienced unbearable discomfort or the FEV<sub>1</sub> fell below 1.4 L.

### AIRWAY RESPONSE

The airway response to the inhaled aerosols was measured by FEV<sub>1</sub> according to standardized lung function techniques and recorded by a spirometer connected to a PC (Vmax Spectra, Sensor Medics, Bilthoven, The Netherlands) (14). At each specified timepoint, the FEV<sub>1</sub> was measured in duplicate, and the highest, technically satisfactory FEV<sub>1</sub> was implicated in the analysis. The airway response was quantified as percentage change from pre-allergen baseline FEV<sub>1</sub>.

### EXHALED NO MEASUREMENTS

Exhaled NO levels were measured in triplicate (within 10%) by a chemoluminescence analyzer (Ecomedics CLD88sp, Duernten, Switzerland) at the specified timepoints. The mean ppb-value was implicated in the analysis; the response was quantified as percentage change from pre- to post-salbutamol value.

### ANALYSIS

FEV<sub>1</sub> and eNO responses were correlated using a Spearman Rank Order Correlation Coefficient.

## Results

All subjects had a LAR. As compared to pre-allergen baseline, the mean fall in FEV<sub>1</sub> at the time of reversal was 33.3% (range 15.1-57.7%). Salbutamol increased FEV<sub>1</sub> on average by 43% (SD:16%) as compared to pre-salbutamol FEV<sub>1</sub>. At the end of the allergen challenge, the mean eNO pre-salbutamol was 60.2 ppb (30.6-108.1 ppb). Salbutamol increased eNO on average by 30% (range: 39.06–140.6 ppb; SD:17%). The Spearman Rank Order Correlation between the % change in FEV<sub>1</sub> and the % change in eNO (pre- versus post-salbutamol) was 0.51 ( $p < 0.02$ ) (Figure).

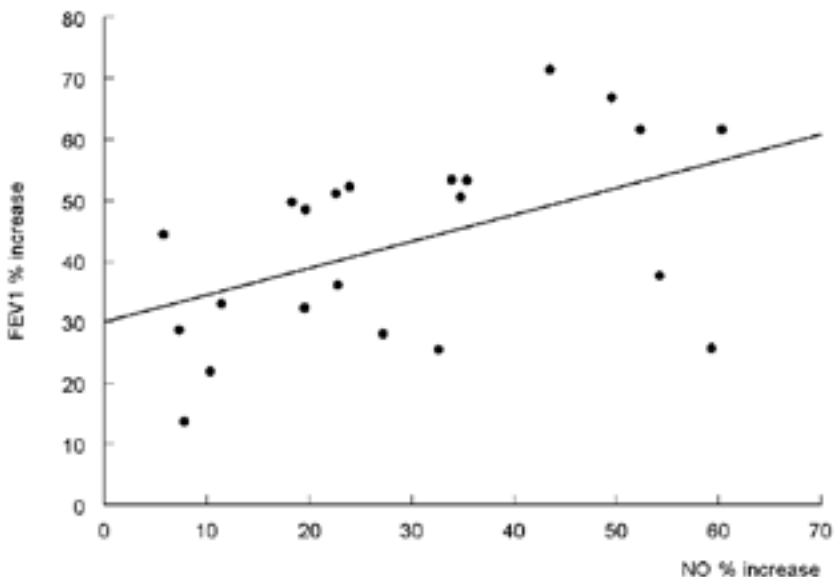


FIGURE 1 There was a significant correlation between the % change in FEV<sub>1</sub> and the % change in eNO (pre- versus post-salbutamol); the spearman rank order correlation was 0.51 ( $P < 0.02$ ).

## Discussion

We found a raised eNO level following reversal of airways obstruction with inhaled salbutamol during the LAR at 9 hours post-allergen. Our data underscore and extend earlier findings.

First, late asthmatic responses (LAR) are associated with increased airway inflammation and accordingly, Kharitonov *et al* found increased eNO levels 10 hours post-allergen in asthmatic subjects, corresponding with the magnitude

of the LAR at 9 h post-allergen (12). Second, eNO levels have been found to relate to the airway diameter. Various studies in asthma showed decreases in eNO levels following bronchoconstrictor stimuli including methacholine, histamine, hypertonic saline, adenosine monophosphate (AMP) and exercise-induced bronchoconstriction (7,9,10). Alternatively, following inhalation of salbutamol, Silkoff *et al* found increased levels of eNO corresponding with increases in FEV<sub>1</sub> in asthmatic subjects in absence of allergen challenge (8). The present study combines all abovementioned observations: in agreement with Kharitonov's data (12), we found similar pre-bronchodilator eNO values during the LAR at 9 h post-allergen. And, according to the observations of Silkoff *et al* in the absence of allergen, there was a 30% raise in eNO levels following bronchodilation (8).

Our data imply that as a result of allergen-induced bronchoconstriction, the eNO level during the LAR is usually underestimated. Although eNO has been shown to reflect the degree of airway inflammation in asthma (15,16), Ricciardolo *et al* have demonstrated that the allergen-induced eNO may also act as an endogenous bronchoprotective mechanism (17). Therefore, using a bronchodilator following allergen challenge may not only relieve the bronchoconstriction, but may also support this endogenous bronchoprotective mechanism. Another implication of our study may be that for a correct non-invasive assessment of airway inflammation in asthma, eNO should be measured after (appropriate) bronchodilatation. To enable comparison with other studies or measurements in individuals, we suggest that both the bronchodilator dose and timepoint of post-bronchodilator eNO measurements should be standardized.

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