PERIPHERAL NITRIC OXIDE IS INCREASED IN RHINITIC PATIENTS WITH ASTHMA COMPARED TO BRONCHIAL HYPERRESPONSIVENESS

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Summary

Allergic rhinitis is a predisposing factor for developing clinical asthma. Moreover, allergic rhinitis is often associated with bronchial hyperresponsiveness (BHR). We hypothesise that patients with asthma have more small airway involvement than those with allergic rhinitis and BHR alone. The aim of this study was to assess peripheral and proximal NO concentration in rhinitic subjects, and to correlate the peripheral NO concentration to the peripheral obstruction in response to methacholine.

Patients with allergic rhinitis with or without BHR, or clinical asthma were investigated in and out of the allergy season. Healthy subjects served as controls. Fractional exhaled NO was performed, and peripheral NO concentration and proximal flux of NO was calculated. Methacholine test was performed including Impulse Oscillometry.

Rhinitic patients with asthma demonstrate an increase in both proximal and peripheral NO compared to those with rhinitis alone or those with BHR. There is a trend of increased peripheral NO from patients with rhinitis only, rhinitis and BHR, to rhinitis with asthma. The increase in peripheral NO correlated with an increased peripheral obstruction in response to methacholine. Patients with seasonal allergic rhinitis demonstrated a decrease in both proximal and peripheral NO in the off-season.

The results support our hypothesis that rhinitic patients with asthma have more peripheral lung inflammation and small airway involvement compared to rhinitic patients with BHR alone.

Introduction

Asthma and rhinitis commonly occur together and many of those with rhinitis also have bronchial hyperresponsiveness (BHR) to methacholine. Among the atopic asthmatics more than 90 % have allergic rhinitis, and having allergic rhinitis has shown to be a predisposing factor for later developing asthma (1). The transition from rhinitis only to the development of clinical asthma is probably a gradual one, with bronchial hyperresponsiveness representing an intermediate step, associated with increasing lower airway inflammation and an increased risk of future asthma development (2;3). We have previously reported that patients with allergic rhinitis and bronchial hyperresponsiveness differ from patients with clinical asthma as the asthmatics better perceived induced bronchoconstriction. From there, we hypothesised that the main difference between patients with rhinitis and asthma compared to those with rhinitis and bronchial hyperresponsiveness was due mainly to differences in geographical distribution of lower airway inflammation, i.e. that the asthmatics had more evidence of small airway involvement (4).

Nitric oxide (NO) can be measured in exhaled air, and high levels of NO have been shown in patients with active asthma (5;6). Thus, high levels of NO may reflect ongoing inflammation in the airways of the patients, and can therefore be regarded as a non-invasive potential clinical tool to monitor asthma (7). Smith et al. (8) have recently used NO measurements as a single successful tool to guide treatment adjustment. In recent years it has been possible to measure NO at different exhalation flows, and approximate the NO concentration in the peripheral region as well as the conducting airways (9).

Involvement of peripheral airways can also be estimated by various respiratory physiological methods. Impulse Oscillometry (IOS) is based on the principle of oscillating pulses with different frequencies introduced to the airways during tidal breathing. Patterns of reflecting waves can be interpreted as airway resistance, where high frequency waves (≥20 Hz) are

believed to reflect resistance in central airways, while lower frequencies (5 Hz) measures total resistance. The difference between these two parameters (ΔR5-R20) subsequently reflects peripheral properties of the respiratory tract (10).

In a previous study we showed that patients with seasonal allergic rhinitis in combination with asthma have significantly higher levels of exhaled NO compared to patients with rhinitis alone or patients with rhinitis and BHR to methacholine, both during season and off season (11). The aim of this study was to further investigate these patient groups by using measurements of NO at different flows to estimate the peripheral (previously described as alveolar) NO concentration as well as proximal (previously described as bronchial) NO flux. It has previously been shown that there is an increase in peripheral NO concentration in asthmatic patients (12-15), and we hypothesize that involvement of the peripheral airways differs between patients with rhinitis and concomitant asthma and patients with rhinitis with (or without) BHR. In addition we aimed to correlate the peripheral NO concentration to the peripheral obstruction in response to methacholine, to strengthen our hypothesis regarding peripheral airway involvement in asthma.

Materials and Methods

Patients

Fifty-one adult patients (30 females) with allergic rhinitis were investigated. Twenty-six of those had concomitant clinically diagnosed mild asthma, according to Global Initiative for Asthma (GINA) standards (16). The age ranged from 18-58 (median 27), and they had a positive skin prick test with sensitization to birch, timothy and/or mugwort (ALK Abello, Copenhagen, Denmark)(11). Thirty-six patients had seasonal symptomatic allergic rhinitis and were investigated both once during pollen season (having symptoms from nose and eyes) as well as off season (at least one month after last reported symptom). Only those with pure

seasonal allergy were investigated, i.e. those with confirmed sensitization to perennial allergens (cat, dog, horse, house dust mite or moulds) were excluded. Three of the asthmatic patients inhaled corticosteroids daily (200-400 μg budesonide).

A control group of 12 healthy subjects, age ranging from 19-56 (median 43), who did not report a history of rhinitis or asthma, was investigated. These subjects had negative skin prick tests and were not hyperresponsive to methacholine.

All subjects were non-smokers without upper respiratory tract infection within three weeks prior to the investigation. Caffeine was not allowed four hours, and strenuous physical exercise two hours, prior to the investigation.

All subjects gave written informed consent, and the ethical committee in Lund approved the study (LU412-03).

NO measurements and analysis

NO measurements were performed in accordance with international American Thoracic Society recommendations (17), using a NIOX, nitric oxide gas analyser (Aerocrine, AB, Stockholm, Sweden). Patients were comfortably seated, inhaled NO depleted ambient air, and exhaled at different flow rates (10, 50, 100 and 400 ml/s) 2-4 times depending on divergence.

Peripheral NO concentration (or alveolar concentration, $C_{A_{NO}}$) and proximal maximal NO flux ($J'_{aw_{NO}}$) was approximated by plotting NO-output (product of concentration and flow) against exhalation flow (at flow 100 and 400 ml/s)(18). The slope and intercept of this line approximate $C_{A_{NO}}$ and $J'_{aw_{NO}}$, respectively (9;19). Calculations using the flow 50 ml/s were also performed, but were not used as an increase in slope and a decrease in the intercept was observed confirming previous reports that linearity between NO-output and flow is valid only for approximately > 50 ml/s (20).

All NO measurements were done prior to bronchial challenge test.

Airway resistance and lung reactance using methacholine inhalation challenge test

Measurement of airway resistance and lung reactance was performed during a methacholine challenge test as previously described (11). A MasterScope spirometer, software version 4.5 (Erich Jaeger GmbH, Wurzburg, Germany) was used for flow-volume spirometry. The test was carried out with tidal volume triggered equipment (Aerosol Provocation System, APS, Erich Jaeger GmbH, Wurzburg, Germany). The APS delivered a cumulative dose of 2000 μg methacholine in five increments, following an initial dose of 0.9 % NaCl. The challenge was discontinued if the FEV_1 declined more than 20 % during the protocol, and the $PD_{20}FEV_1$ was determined When FEV_1 fell below 80 % of the baseline value or when the total amount of 2000 μg methacholine was delivered, 400 μg of salbutamol were given to the subject. After ten minutes a new flow-volume spirometry was carried out, to ensure that the subjects were recuperating properly.

Impulse Oscillometry (IOS) was performed (using a Jaeger MasterScreen Impulse Oscillometry System, Erich Jaeger GmbH), 90 s after each challenge step, prior to the spirometry to avoid the influence of deep inspiration on the IOS parameters. FEV_1 was performed 120-150 s after each challenge step. The subjects used nose clips and pressed their hand palms against the cheeks to decrease the upper airways shunt. For about 30 seconds, oscillometric pressure impulses were superimposed to the tidal breathing of the subject, having a pulse sequence of 5 per second and a frequency spectrum between 5-35 Hz. The IOS parameters were plotted against the methacholine dose at each challenge step and a slope value was calculated (in press (21).

Statistical analysis

Data is shown as median (range). As normal distribution could not be assumed, nonparametric tests were used. Kruskal-Wallis test for unpaired samples was used for comparison among the groups, and Mann-Whitney's U-test for unpaired samples was used for comparison between separate groups. Wilcoxon's test was used for paired samples, and Spearman's rho test for correlation analysis. GraphPad Instat3 was used for statistical analysis, and a p-value of less than 0,05 was considered significant.

Results

Fractional NO

Among the 25 rhinitis patients with no concomitant asthma, 16 were bronchial hyperresponsive to methacholine. (Though, they had no symptoms suggestive of asthma.)

There was a significant difference (p=0,001) in CA_{NO} between the groups (Fig. 1). A trend of increased CA_{NO} can be seen from healthy controls (1,6 (0,2-2,6) ppb), patients with rhinitis only (1,9 (0,5-3,6) ppb), rhinitis and BHR (2,4 (0,8-4,5) ppb), to rhinitis and concomitant clinical asthma (3,3 (1,0-5,9) ppb). Moreover, patients with asthma differed significantly from patients with rhinitis and BHR (p=0,04).

Similarly, there was a significant difference (p=0,007) in $J'aw_{NO}$ (Fig. 2) between the groups. Interestingly, in contrast to CA_{NO}, no trend could be found for $J'aw_{NO}$ from healthy controls (1,1 (0,6-2,2) nl/s), patients with rhinitis only (1,0 (0,4-2,3) nl/s) to patients with rhinitis and bronchial hyperresponsiveness (1,1 (0,5-3,8) nl/s), but $J'aw_{NO}$ was increased in the asthmatic group (2,5 (0,5-9,0) nl/s), which was distinguished from the other groups (p=0,02 between patients with rhinitis and BHR and patients with rhinitis and concomitant asthma).

Correlation analysis

Between CA_{NO} and $J'aw_{NO}$ of NO there was a significant correlation (p=0,02; Table 1). CA_{NO} also correlated to several airway resistance parameters (Table 1), including Slope-ΔR5-$R20^{MCh}$ (Fig 3), Slope-$Fres^{MCh}$ and Slope-AX^{MCh} that reflect peripheral airway reactivity, as obstruction in response to methacholine. In contrast, proximal flux did not correlate to any of these IOS parameters.

Only three patients with clinical asthma were treated with inhalation steroids. None of them had decreased CA_{NO} (Fig 1), and all three had indication of high peripheral obstruction in response to methacholine (Fig 3).

On season versus off season

CA_{NO} was significantly reduced from pollen season to off season in the group of patients with rhinitis and concomitant clinical asthma (p=0,03). This could not be seen in the other patient groups (patients with rhinitis with or without BHR). Similarly, $J'aw_{NO}$ was reduced (p=0,005) from pollen season to off season in the group of patients with rhinitis and concomitant asthma, but not the other groups. Interestingly the significant differences in CA_{NO} between the groups remained off season (p=0,007). This was not the case for proximal flux of NO.

Discussion

The major finding of this study was that the peripheral NO concentration (CA_{NO}) was increased in patients with rhinitis and concomitant asthma in comparison to patients with rhinitis only, while patients with rhinitis and BHR represented an intermediate step between those with rhinitis only and those with rhinitis and asthma. CA_{NO} in patients with rhinitis only are at the same level as controls, which are in accordance with a previous study (22). This

may reflect absence of inflammation in the lower airways. However, a small increase in CA_{NO} in patients with rhinitis and BHR may reflect an initial inflammatory process in the lower airways.

Another interesting finding was that proximal flux of NO ($J'aw_{NO}$) was significantly increased merely in patients with rhinitis and asthma. The finding that $J'aw_{NO}$ was increased in patients with asthma is consistent with previous findings (13;14;23). In contrast, the increase in $J'aw_{NO}$ characterized in patients with rhinitis and asthma was not seen in either patients with rhinitis only, nor in patients with rhinitis and BHR.

The increase in CA_{NO} is consistent with our hypothesis that asthma represents a geographically more extensive inflammation involving the peripheral, small airways. The correlation between CA_{NO} and several of the peripheral obstruction parameters supports the concept of asthma being an inflammatory disease involving not only the central but also the peripheral airways (4).

There was a small but significant correlation between $J'aw_{NO}$ and CA_{NO}. This correlation was stronger in the asthmatic group, indicating a more widespread and increased inflammation. However, no correlation was found between $J'aw_{NO}$ and any of the peripheral obstruction parameters.

We have used a linear regression model where plotting NO-output against exhalation flow at exhalation flows > 50 ml/s is approximately linear in adults (20). There are additional models(18), including a non-linear regression model described by Silkoff et al. (24) using nine different flows. Due to our instrumentation, we are limited to 400 ml/s as the highest flow, making Silkoff's model less suitable for our purpose. Despite these differences we had an agreement between the two models with the exception in patients with high $J'aw_{NO}$ (predominantly asthmatics). Among these patients, CA_{NO} was less than zero in several cases

when using the non-linear model, suggesting that this model may be inadequate for these purposes.

Another newly discussed area for NO exchange is axial diffusion and the trumpet shape of the airways (25;26). Axial diffusion of NO transports NO from the proximal region of the lungs to the peripheral region. When this model was used for analysis, several patients with high $J'aw_{NO}$ have a negative CA_{NO}. This finding may represent noise in the estimation of the parameters, or may suggest that the model is not adequate for the asthmatic lung. Nonetheless, the current model used in the analysis does not consider axial diffusion and the trumpet shape of the airway tree, and thus, our predicted CA_{NO} may be falsely elevated in patients with high $J'aw_{NO}$.

A potential weakness of our analysis is the use of only two flows. However, it should be noted that inclusion of the exhaled concentration at 50 ml/s decreased the intercept and increased the slope suggesting a non-linear response in NO-output with flow. It has been previously suggested that a cut-off of 50 ml/s for the low end of the linear region of NO-output versus flow is only an estimate in adults (9;19). Furthermore, in additional studies we have demonstrated that the linear relationship holds when additional flows are used between 100 ml/s and 400 ml/s (e.g., 150, 200, 250 , and 300 ml/s). While the use of only two flows lessens our statistical power, it is easier and faster for the subject to complete and thus may offer clinical advantages.

Using the non-linear regression model it is also possible to approximate the diffusing capacity of NO in the airways (24). The pattern was similar to $J'aw_{NO}$ (Fig 2), with a higher level in the group with patients with rhinitis and concomitant asthma in comparison to the other patient groups. Additionally, the diffusing capacity was reduced off season (data not shown). The diffusing capacity is proportional to the total NO-producing surface area (9;27) thus, an increase suggests a more widespread distribution of the inflammation in the airways.

Due to the low number of patients with inhalation steroid treatment, it was not possible to draw any conclusions. However, no decrease in CA_{NO} could be seen, which is in accordance with previously published data (23;28). Interestingly, it should be noted that all three patients had high levels of both CA_{NO} as well as peripheral obstruction in response to methacholine, despite treatment with inhalation steroids.

The NO in the airways is thought to be produced mainly by airway epithelial cells and inflammatory cells, such as macrophages (29), and the increased amount of exhaled NO in the airways in asthmatics may be due to increased amount of inducible NO synthase (iNOS) as a response to increased amount of inflammatory cytokines (30;31). Little is known about the pattern of distribution of iNOS in the peripheral airways, which would be of great interest.

The involvement of peripheral airways has great implications on how to interpret proximal biopsy studies, quantifying the degree of inflammation in a very small part of the central airways. Our results indicate that the geographical distribution (i.e., the extent of inflammation) is even more important.

We have compared different mathematical models and can only conclude that the linear model selected give us a little bit more consistent results. The clinical usefulness is however not yet satisfactory, as none of the mathematical models used today are optimal. One limitation is the necessary discrepancy between a static trumpet shaped model and the dynamic morphology of the lungs with different sized airways and flow turbulence. Moreover, the unsatisfactory NO-sensors sensitivity and the variability of NO at high flow and low NO-concentrations, is another critical task that need further improvements. Thus we both need new mathematical models and better instruments to make the estimations more reliable and clinically useful.

In this study we have found that patients with asthma in comparison to rhinitis only, even with BHR, have evidence of geographically more widespread and increased inflammation as

assessed by peripheral NO concentration, which is correlated with an increase in airway resistance. This finding supports our hypothesis that asthma is associated with more peripheral, small airway involvement. In addition, rhinitic patients with BHR may suggest a transitional stage between rhinitis only and rhinitis with asthma.

Acknowledgement

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Figure legends

Figure 1. Peripheral NO concentration. Concentration of peripheral NO (CA_{NO}) assessed by measuring exhaled NO at several exhalation flow rates in patients with rhinitis (R), rhinitis with bronchial hyperresponsiveness (R+BHR), rhinitis and concomitant asthma (R+A) and healthy controls (Ctrl). Data are expressed as individual scores and median values. Open symbols indicate patients treated with inhaled corticosteroids.

Figure 2. Proximal NO flux. Proximal maximum NO flux (J'_{awNO}) assessed by measuring exhaled NO at several exhalation flow rates in patients with rhinitis (R), rhinitis with bronchial hyperresponsiveness (R+BHR), rhinitis and concomitant asthma (R+A) and healthy controls (Ctrl). Data are expressed as individual scores and median values. Open symbols indicate patients treated with inhaled corticosteroids.

Fig 3. Correlation between peripheral NO and peripheral resistance. Figure showing correlation between concentration of peripheral NO (CA_{NO}) and Slope-ΔR5-R20^{MCh} for all patients as well as healthy controls. Data are expressed as individual scores. Open symbols indicate patients treated with inhaled corticosteroids.

Fig 4. Peripheral NO concentration in season vs off season. Concentration of peripheral NO (CA_{NO}) during pollen season (s) as well as off season (off s) assessed by measuring exhaled NO at several exhalation flow rates in patients with seasonal rhinitis (R), rhinitis with bronchial hyperresponsiveness (R+BHR) and rhinitis and concomitant asthma (R+A). Data are expressed as paired individual scores.

Fig 5. Proximal NO flux in season vs off season. Proximal NO flux (J'_{awNO}) during pollen season (s) as well as off season (off s) assessed by measuring exhaled NO at several exhalation flow rates in patients with seasonal rhinitis (R), rhinitis with bronchial hyperresponsiveness (R+BHR) and rhinitis and concomitant asthma (R+A). Data are expressed as paired individual scores.

Fig 1

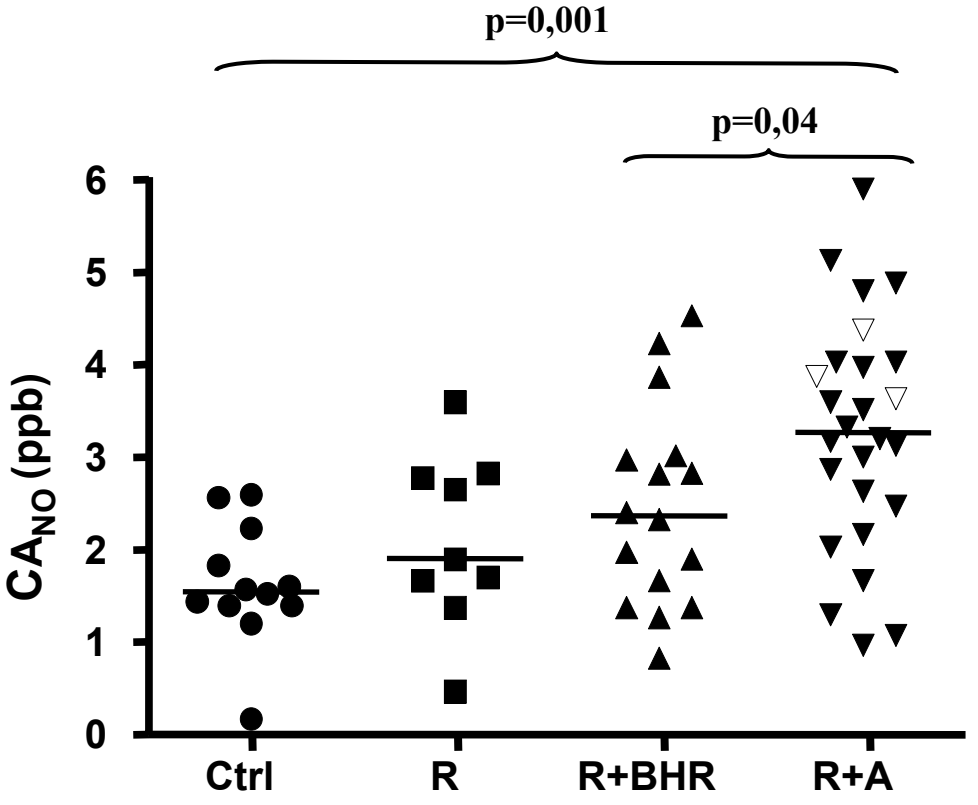

Fig 2

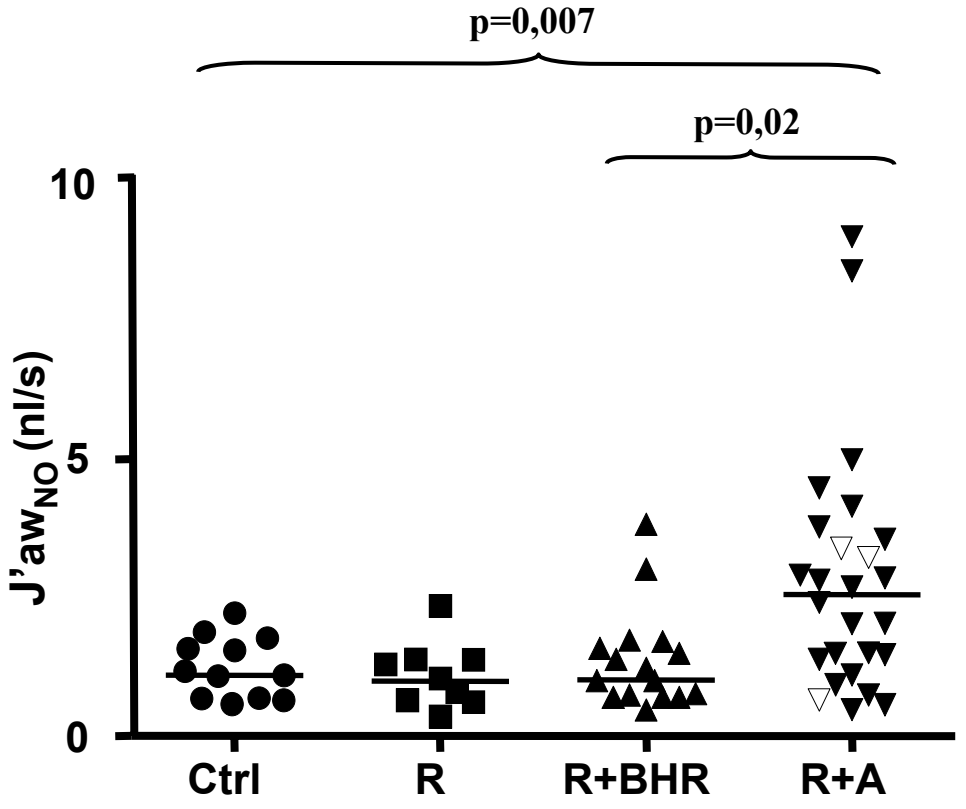

Fig 3

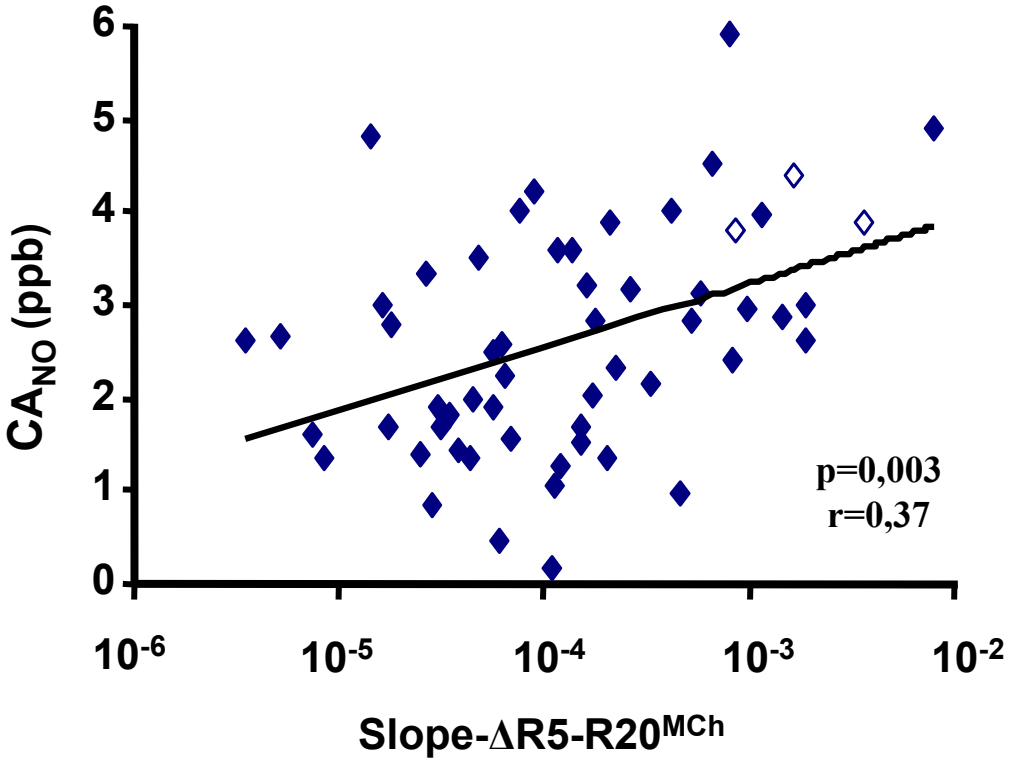
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Fig 4

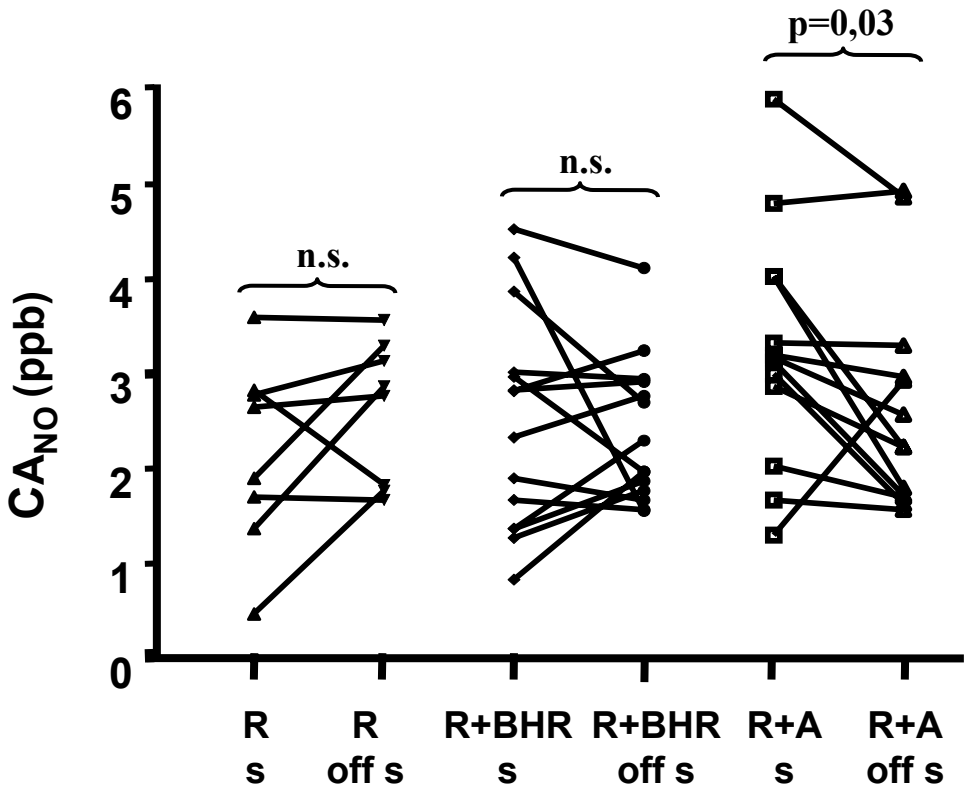
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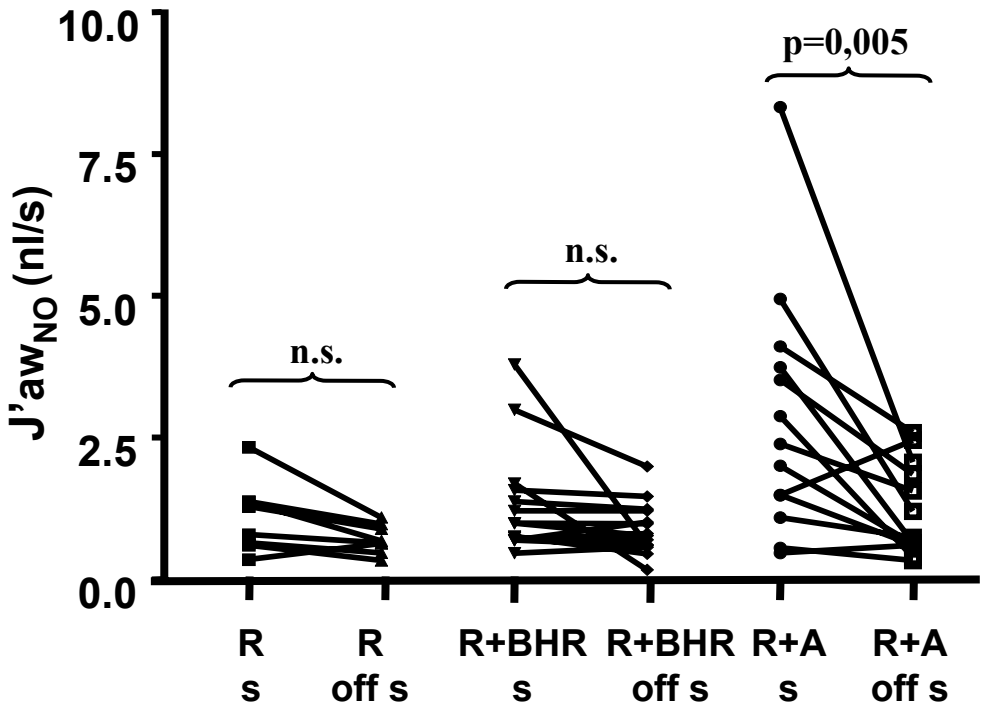
Fig 5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J'aw$_{NO}$ (nl/s)
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R s
R off s
R+BHR s
R+BHR off s
R+A s
R+A off s

Table 1. Correlations between peripheral NO (CA_{NO}), proximal NO flux ($J'aw_{NO}$) and airway resistance parameters (expressed as slope/metacholine dose) in all subjects. Values are shown as p-values (Spearman r). n.s.=not significant

	CA_{NO}	$J'aw_{NO}$
J'awNO	0,018 (0,30)	-
R5	0,0014 (0,40)	n.s.
R20	0,021 (0,29)	n.s.
ΔR5-R20	0,0031 (0,37)	n.s.
Fres	0,0008 (0,41)	n.s.
AX	0,0022 (0,39)	n.s.
X5	0,0012 (-0,41)	n.s.

Conflict of interest statement

ET, DA, JA and LB have no conflict of interest. SG has issued US patents which are licensed by Aerocrine, Ltd (Sweden).