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Exhaled nitric oxide dynamics in asthmatic reactions induced by diisocyanates

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Abbreviations list:

ADMA = asymmetric dimethylarginine; ADMA/TYR = asymmetric dimethylarginine measurements expressed as a ratio to tyrosine; ATS = American Thoracic Society; CaNO = small airway/alveolar nitric oxide concentration; CaNOcorr = small airway/alveolar nitric oxide concentration corrected for axial back diffusion; CawNO = airway wall nitric oxide concentration; DawNO = airway nitric oxide diffusing capacity; DLCO = transfer factor; ERS = European Respiratory Society; EBC = exhaled breath condensate; FVC = forced vital capacity; FeNO50 = fractional exhaled nitric oxide measured at expiratory flow of 50 mL/s; FEV1 = forced expiratory volume in 1 second; HDI = hexamethylene diisocyanate; JawNO = airway wall nitric oxide flux; JawNOcorr = airway wall nitric oxide flux corrected for axial back diffusion; Kco = transfer factor over alveolar volume; MCH = methacholine challenge; MDI = methylene diisocyanate; NO = nitric oxide; OA = occupational asthma; PD₂₀ = provocative dose of methacholine causing a 20% fall from baseline in FEV 1; ppb = parts per billion; SE = standard error; SIC = specific inhalation challenge; TDI = toluene diisocyanate.

ABSTRACT

BACKGROUND. Isocyanate-induced asthmatic reactions are associated with delayed increase in fractional exhaled nitric oxide measured at expiratory flow of 50mL/s (FeNO50), a biomarker of airway inflammation. The time-course of FeNO increase is compatible with activation of NO synthase, but the origin of NO production in the lung is undetermined.

OBJECTIVE. The aim of this study was to define the dynamics of airway and alveolar NO during specific inhalation challenge (SIC) with isocyanates and the role of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase.

METHODS. Spirometry, exhaled NO parameters (FeNO50, bronchial wall NO concentration, NO airway diffusing capacity, NO flux to luminal space, alveolar NO) and ADMA levels in exhaled breath condensate were measured before and at intervals up to 24 hours after exposure to isocyanates. The results were compared between 17 SIC-positive and 8 SIC-negative subjects.

RESULTS. A significant FeNO50 increase in SIC-positives was detected 24h after exposure and was associated to augmented NO flux from airway wall to the lumen, whereas airway NO diffusion and alveolar NO were not affected. The changes in NO dynamics were specific for the subjects who developed an asthmatic reaction, but were independent from the pattern and magnitude of bronchoconstriction. There was no evidence that exhaled NO is modulated by changes in ADMA concentration.

CONCLUSIONS AND CLINICAL RELEVANCE. Since isocyanate-induced increase in FeNO₅₀ was almost exclusively determined by the increase of NO flux, the use of FeNO₅₀ appears adequate to monitor the exhaled NO dynamics during SIC. FeNO₅₀ measurement may provide additional information to spirometry, since bronchoconstriction and airway inflammatory responses are dissociated.

INTRODUCTION

Diisocyanates represent the major cause of occupational asthma (OA) due to low molecular weight agents in many industrialized countries [1]. Although the mechanisms involved in isocyanate-induced asthma remain unclear, this common type of occupational asthma shares many clinical, functional and morphologic features with allergic asthma [2]. Upon specific inhalation challenge (SIC) with diisocyanates, sensitized subjects exhibit early and/or late bronchoconstriction as it is observed after SIC with allergens in allergic asthma [1,2]. We previously demonstrated that isocyanate-induced asthmatic reactions are associated with influx of eosinophils into the airways between 8 and 24 hours after SIC and with a consistent delayed increase in fractional exhaled nitric oxide (FeNO) between 24 and 48 hours after SIC [3,4]. However, it is undetermined whether the increase of FeNO is due to changes in bronchial wall concentration of nitric oxide, in NO diffusing capacity of the airways or in NO flux to luminal space and whether alveolar NO contributes to FeNO levels. Nitric oxide in the lung is synthesized from L-arginine by constitutive and inducible NO synthases, expressed in resident and inflammatory cells in bronchial and alveolar compartments [5,6]. According to ERS/ATS recommendations, since the levels of FeNO are strongly dependent on expiratory flow, they were measured at constant exhalation flow rate of 50 ml/s [7]. At this relatively low flow rate the signal is weighted toward the bronchial contribution. Therefore, it remains unclear whether there is an alveolar contribution to the increase of FeNO in isocyanate-

induced asthma. Although the involvement of lung alveolar compartment in asthmatic inflammation is controversial, the alveolar component of exhaled NO (CaNO) was elevated in some patients severe asthma and during exacerbation [8,9]. Older studies, using higher concentration of isocyanates (16-20 ppb) during SIC, provided evidence of bronchoalveolar inflammation 2 to 8 h after exposure [10]. Furthermore, combined asthma and alveolitis has been described in workers exposed to diisocyanates [11]. Therefore, the hypothesis that exhaled NO may originate from the airways and the alveolar spaces in isocyanate-induced asthma is reasonable.

The formation of asymmetric dimethyl arginine (ADMA), a product of methylation of L-arginine, could modulate NO production in the lung through its ability to inhibit NO synthase isoforms [12]. Since a significant increase in sputum ADMA was demonstrated after allergen challenge in subjects with allergic asthma, NO detected in exhaled air in this condition results from a balance between up-regulation of inducible NO synthase by allergic inflammation and NO synthases inhibition by ADMA [13]. Whether changes in ADMA occur upon SIC with isocyanates is unknown.

The aim of this study was to define the dynamics of airway and alveolar NO during SIC with isocyanates in sensitized subjects. We applied an extended NO analysis using multiple expiratory flow rates and a mathematical two-compartment model that describes pulmonary NO exchange with four relatively flow-independent parameters: airway wall NO flux (JawNO), airway wall NO concentration (CawNO), airway NO diffusing capacity (DawNO) and alveolar NO concentration (CaNO) [14]. The investigation was completed by the measurement of ADMA concentrations in exhaled breath condensate.

METHODS

Subjects

Twenty-six consecutive subjects exposed to polyurethane referred to our center for suspected occupational asthma, who performed SIC with diisocyanates, were included in the study. The patients were allocated to the isocyanate asthma group on the basis of positive response to SIC (i.e. an early asthmatic reaction, a late asthmatic reaction or both – dual reaction). Seventeen subjects with occupational asthma due to isocyanates (toluene diisocyanate, TDI, methylene diisocyanate, MDI and hexamethylene diisocyanate, HDI) and eight subjects with workplace exposure to isocyanates, but with negative response to SIC, were examined. Exclusion criteria were a history of upper respiratory infection, an asthma exacerbation or use of inhaled or oral corticosteroid in the four weeks prior to the study or long acting bronchodilators within preceding 48 h. Each subject provided written informed consent and the local ethics committee approved the investigation (Comitato Etico per la Sperimentazione, Azienda Ospedaliera di Padova, project approval number 69417).

Study design

Subjects were examined on 4 consecutive days. On day 1, clinical and occupational data were collected, skin prick tests with common aeroallergens and pulmonary function were performed. On day 2, a single-blind sham inhalation challenge with air was performed. On day 3, the subjects underwent SIC with the appropriate isocyanate (*ie*, toluene diisocyanate, TDI; methylene diisocyanate, MDI or 1,6- hexamethylene diisocyanate, HDI). The type of isocyanate to test was chosen according to each subject's occupational exposure and medical history. Lung function and exhaled NO were monitored for 7 hours after sham and occupational agents' exposure. At the end of sham and active challenge airway responsiveness to methacholine was assessed. On day 4 (*i.e.* 24 h after SIC), lung function

and exhaled NO were assessed again. Lung carbon monoxide uptake was evaluated before and after SIC to assess peripheral lung dysfunction due to increased ventilation heterogeneity and/or alveolitis induced by diisocyanate exposure. Subjects did not return to work until the end of the study.

Lung Function and Methacholine Bronchial Challenge

Forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV₁) were measured with a spirometer (ESS system; Biomedin). The predicted normal values of the Communauté Européenne du Charbon et de l'Acier (CECA) were used [15]. Diffusing capacity of the lung was measured by using single-breath carbon monoxide (CO) uptake (ESS system; Biomedin) according to the recommendations of ATS/ERS [16]. The measurements were taken the day before SIC and at 24 h post SIC. The results were expressed as transfer factor (DL_{CO}, mL x min x mmHg) and DL_{CO}/alveolar volume (K_{co}).

Airway responsiveness to methacholine aerosol was assessed as previously described, and results were expressed as the cumulative provocative dose causing a 20% fall in FEV₁ (PD₂₀FEV₁ in milligrams of methacholine) [4]. SICs with isocyanates were performed as described previously [4]. Briefly, subjects were exposed to the isocyanate vapor (mean ± [SE], concentration, 10±3 parts per billion [ppb]) for a maximum of 60 min in an exposure chamber. The concentration of isocyanate was continuously monitored (Single Point Monitor; Zellweger Analytics Inc; Lincolnshire, IL). An asthmatic reaction was considered to occur when FEV₁ decreased by at least 20% from baseline within 1 h (early reaction) or >2 h (late reaction) after exposure to isocyanates.

Exhaled NO Measurement

Online measurement was made according to ATS recommendations using a chemiluminescence analyzer (NIOX Version 2.0; Aerocrine) at expiratory rates of 10, 50, 100, 150, and 250 mL/s by applying resistors to maintain the target flow rates and produce a mouth pressure >10 cm H₂O to avoid nasal NO contamination. Expiration was prolonged up to 20 s with lower flows to obtain a NO plateau. A NO plateau was detectable in all subjects. The mean value of three FeNO measurements performed at each flow rate was used for the analysis. The non-linear Högman method was applied to calculate the 'flow-independent' parameters. The FeNO measurements performed at flow rates of 10, 50, 100, 150 and 250 mL/s have been used in the model [14]. The NO output (FeNO multiplied by expiratory flow) was regressed against the respective flow rate. The slope of the regression line through the flows >50 mL/s was used to determine CaNO. Then, DawNO and CawNO were obtained with the second-order algorithm of Högman et al. [14]. The flux from the airway was calculated from the equation: $\text{JawNO} = \text{DawNO} \times (\text{CawNO} - \text{CaNO})$. We applied the quality controls indicated by Högman et al. [17] to test if the measured set of data points was consistent with the model. Over the 60 measurements performed at each time point, negative CaNO values occurred in 3 to 5 cases according to the time point. Negative CaNO values were assigned an arbitrary value of 0.

Correction for axial back diffusion of NO was made for JawNO and CaNO, using the method of Condorelli et al [18]. The calculated JawNO values (picoliters per second) were multiplied by a factor of 1.7 to obtain corrected JawNO (JawNO_{corr}). Corrected CaNO (CaNO_{corr}) was obtained by subtracting calculated JawNO divided by a correction factor of 740 to corresponding slope values.

Exhaled breath condensate

Exhaled breath condensate (EBC) was collected with the apparatus described by Goldoni et al. [19] during tidal breathing for 15 minutes into the condenser kept at a temperature of -55°C . The subjects had not eaten or drunk at least a hour before the collection. Amylase was measured using an enzymatic colorimetric test (IFCC, Roche Diagnostic Modular, lower detection limit 3U/L) to assess salivary contamination. Samples containing amylase were discarded.

ADMA analysis

An online sample trapping and enrichment system, coupled with an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method, was used for the simultaneous quantification of ADMA and tyrosine, using the isotopic dilution [20]. ADMA measurements were expressed as a ratio to tyrosine (ADMA/Tyr), since the latter is considered a good metabolite for adjusting for EBC dilution.

Statistical Analysis

Data are expressed as median and interquartile range (IQR), median and first and third quartile (Q1, Q3) or mean $\pm$ standard error (SE). The comparison of anthropometric and baseline variables between the two groups was performed with the Mann-Whitney test. The response to SIC was assessed by comparing FeNO50 and flow-independent parameters between sham and active exposure at the same time point in each group with the Mann-Whitney test. Changes in FEV1 after SIC were analyzed with the Wilcoxon signed rank test, separately in SIC-positive and SIC-negative groups. The Bonferroni adjustment for multiple comparisons was applied in this instance (Figure 1). The analysis was performed using a

statistical software package (Statview, version 5.0.1; SAS Institute Inc; Cary, NC). A p value of <0.05 was considered significant.

RESULTS

Table 1 shows the characteristics of the subjects divided into SIC-positive group and SIC-negative group according to their response to the challenge. The two groups exhibited comparable anthropometric and baseline lung function characteristics. Baseline FeNO50 was higher in the SIC-positive group than in the SIC-negative group. The severity of occupational asthma was classified as mild in 12 SIC-positive subjects and as moderate in 5 subjects, according to GINA guidelines [21]. The time course of changes in FEV1 after exposure to isocyanates in the SIC-positive and SIC-negative groups is shown in Figure 1.

Table 2 reports the time course of FeNO50 and flow-independent parameters in SIC-positive (2a) and SIC-negative (2b) groups after active and sham exposures. In the SIC-positive group, FeNO50 increased significantly at 24 h after active exposure (median 111.8 ppb [IQR, 167.5]; $p < 0.05$) compared with sham (58.6 ppb [74.7]). JawNO levels showed a similar time course with a significant increase at 24 h after active exposure (6.6 nL/s [81]; $p < 0.05$) compared with sham (3.3 nL/s [3.7]). No significant changes of the other flow-independent parameters were detected. There was a trend of increased CaNO at 24h after active exposure (5.2 ppb [6.9] vs sham 3.9 [2.7], $p > 0.05$). No significant changes were detectable in FeNO50 and flow-independent parameters in the SIC-negative group (table 2b).

When airway wall NO flux (JawNOcorr) and alveolar NO (CaNOcorr) were corrected for axial back diffusion, JawNOcorr confirmed the significant changes during SIC in the SIC-positive group as shown in Figure 2a (baseline median value 5.6 nL/s [6.4] vs 24 h after SIC

median value 11.2 nL/s [13.9]; $p < 0.05$). No changes were observed in JawNOcorr in the SIC-negative group (Figure 2b) neither in CaNOcorr in both groups (Figure 3a, 3b).

To distinguish whether the changes in FeNO50 and JawNO observed in SIC positive group were related to the pattern of bronchoconstriction, the subjects were separated in those who had an early asthmatic response ($n = 6$) and those who had a late or dual one ($n = 11$) (Figure 4). Both subgroups exhibited similar increase in FeNO50 (Figure 4a) and JawNO (Figure 4b) at 24 h, whereas no changes in CaNO (Figure 4c) were detected.

We confirmed that the late increase in FeNO50 and JawNO was not related with the magnitude of bronchoconstriction, since no correlation between the percentage of late FEV1 fall after isocyanate exposure and the changes of FeNO50 or JawNO at 24h was found.

In SIC-positive patients no changes were detected in lung CO uptake between baseline (DLco 30.2 ± 1.5 mL x min x mmHg; Kco 4.5 ± 0.1) and 24 h post active exposure (DLco 29.1 ± 1.4 mL x min x mmHg; Kco 4.5 ± 0.1).

To verify whether the type of isocyanate tested with SIC affected the time-course of FeNO50 and independent parameters, the subjects exposed to TDI ($n=10$) and MDI ($n=7$) were analyzed separately and no differences were found. Atopic SIC+ patients ($n=5$) tended to exhibit higher FeNO50 (122.9 [33.5]) ppb) than non atopic SIC + ($n=12$) at baseline (54.1 [12.5] ppb). The difference is maintained throughout the time course of SIC (data not shown).

Table 3 reports the data of ADMA/Tyr ratio in breath condensate in SIC-positive group (n=9) and in SIC-negative group (n=6). No significant differences were detected between the two groups at any time point.

DISCUSSION

In this study, we demonstrated that the delayed increase in FeNO₅₀, associated with asthmatic reaction induced by isocyanates, is due to changes in NO dynamics within the airways and not in the alveolar compartment of the lung. This increase is explained by an augmented NO flux from airway wall to the lumen (JawNO), whereas airway NO diffusion capacity was not affected. The changes in NO dynamics are specific for the subjects sensitized to isocyanates, who develop an asthmatic reaction upon SIC, but are independent from the pattern and the magnitude of bronchoconstriction. Since no differences in ADMA were seen upon exposure to isocyanates, it is unlikely that the FeNO differences seen in relation to exposure to isocyanates are mediated through ADMA. Therefore, it is more likely that the mechanism involves airway inflammation with activation of eosinophils [3].

According to Fick's principle, JawNO depends on the difference between NO concentration in airway wall (CawNO) and lumen, and on airway NO diffusion capacity (DawNO). In our study, we did not observed any change in DawNO. Indeed, there was a trend to an increase in CawNO at 24 h after SIC in sensitized subjects, albeit not statistically significant. The findings are consistent with over-production of NO within the airways. This interpretation is supported by previous observations that up-regulation of inducible NO synthase occurs during an inflammatory process [5] and that asthmatic reactions induced by isocyanates are associated with airway influx of inflammatory cells, mainly eosinophils [3,4,22,23]. The inflammatory cells in induced sputum have not been analyzed in the present

study since there were missing data due to inability of some patients to produce adequate samples before and after SIC. No changes in NO dynamics were observed after sham exposure in SIC-positive subjects or after diisocyanate exposure in non-sensitized subjects. Therefore, the NO findings are specific of individuals sensitized to isocyanates, who developed an asthmatic reaction.

Although there was a rationale for considering that part of exhaled NO might originate from alveolar NO production in isocyanate-induced asthma, we reject this hypothesis. CaNO did not differ significantly between the two groups after isocyanate exposure taking into account the effect of axial back-diffusion of NO from proximal to peripheral airways/alveoli against the direction of exhalation. Indeed, the trend of increasing CaNO, detected in SIC-positive group 24 hours post active test, disappeared once the values had been corrected for NO back-diffusion, confirming that NO bronchial production is a determinant of NO alveolar concentration [24]. These observations are consistent with the findings in acute asthma studies. Gelb et al [25] described increased JawNO and CaNO during asthma exacerbation. However, after Condorelli's correction, CaNO was comparable to that of healthy controls. Brindicci et al [26] reported increased JawNO and CaNO during asthma exacerbation in moderate-severe asthmatics, but a correction for NO back-diffusion was not applied. The assessment of alveolar inflammation-NO production using CaNO is not fully validated. In fact, a substantial interdependence has been observed between FeNO and uncorrected CaNO in asthmatic subjects [27]. In addition, there are uncertainties on applicability of correction for axial diffusion in presence of airways obstruction. However, at the 24 h time point after active SIC, when the significant changes in FeNO and JawNO were observed in SIC-positive subjects, FEV1 had returned near to the baseline values.

There are few studies of exhaled NO in work-related asthma during SIC with occupational sensitizers. The majority of the investigations in diisocyanate-induced asthma detected a late increase in FeNO 24-48 h after positive SIC [3,19,28-30]. Therefore, the time course of FeNO during SIC with diisocyanate in sensitized subjects is comparable to that observed in patients with atopic asthma after SIC with allergens [31-32].

There are not previous studies that attempted to localize NO production in asthmatic reactions induced by occupational agents. FeNO50 and CaNO were evaluated during allergen challenge in atopic asthma by Haccuria [32]. The conclusion that the inflammatory process induced by allergens is restricted mainly to conductive airways rather than involving alveolar compartment, is in line with our results in isocyanate-induced asthma. A further finding against a peripheral lung dysfunction due to increased ventilation heterogeneity and/or alveolitis after SIC is the lack of changes in lung carbon monoxide uptake after isocyanate-induced asthmatic reaction.

It has been established that late asthmatic response in both allergen-induced and isocyanate-induced asthma is associated with more inflammatory response than isolated early asthmatic reactions [10,33]. Our data do not confirm this assumption when NO production is used as a surrogate of airway inflammation. Indeed, the changes in JawNO and FeNO50 were comparable in early and late responders. A confirmation that bronchoconstriction and airway inflammation are dissociated is supported by the lack of correlation between the magnitude of FEV1 changes and the magnitude of increase in JawNO and FeNO50 in SIC-positive subjects.

We and others demonstrated an effect of airway calibre on exhaled NO [34-36]. In line with these observations, the findings of Haccuria et al. [32] indicate that FeNO50 values measured during allergen challenge in allergic asthma integrate both airway inflammation and lung function. In our study, a major interference of airway calibre can be excluded since changes in FeNO50 and JawNO were detected at 24 h when FEV1 had returned near to baseline values.

The lung represents a major source of ADMA, a molecule that may be involved in the pathophysiology of respiratory diseases [37]. In murine lung epithelial cells, elevated ADMA concentration results in uncoupling of NO synthase (NOS) activity, leading to a decrease in nitric oxide (NO) synthesis and a promotion of superoxide and peroxynitrite formation [38].

Human data on the role of ADMA in asthma are limited. Carraro et al. [39] found higher ADMA levels in EBC in asthmatic children, than in healthy controls, with no differences related to the use of inhaled corticosteroids. In peripheral blood, ADMA levels did not differ between paediatric patients with or without asthma [40]. In contrast, in adults with severe asthma ADMA levels were higher than those observed in subjects with mild-to moderate asthma [41]. Given the interference of ADMA with NO production and previous findings of an increase in airway ADMA levels after allergen challenge [13], the measurement of ADMA in our experimental setting was reasonable for a sound interpretation of NO dynamics during SIC. The lack of differences in ADMA concentrations between SIC+ and SIC- groups suggests that ADMA has no relevant role on the changes in NO production induced by diisocyanate. We are confident with this conclusion, despite the limited number of subjects examined, since there was almost complete overlap of ADMA data between groups at each time point.

There are some strengths in our work. The design of the study include multiple controls, represented by sham exposure in sensitized patients and active exposure in not sensitized patients. The interference of treatment on airway inflammation and NO synthase activity was excluded since the subjects were free from corticosteroids for at least 4 weeks. We monitored the NO dynamics during an adequate time-course. The airway response was elicited by a single type of sensitizing low-molecular weight agent avoiding the bias of stimuli acting through different mechanisms and the influence of environmental exposure. The concentrations of diisocyanates used in the laboratory (10 ± 3 ppb) to perform SIC is largely below the threshold limit value of 20 ppb, adopted for short-term workplace exposure [42]. Therefore, we can reasonably exclude that unspecific irritant effects were elicited in our experimental setting. The focus on occupational asthma due to a single group of chemical implicated a limitation in the recruitment of larger cohort. However, the number of patients in our study is in line with that in most investigations of occupational SIC [28-30].

In conclusion, our study contributes to the interpretation of FeNO during SIC with isocyanates and probably our findings can be extended to SIC with allergens and other occupational sensitizers. Since in this experimental model the increase of exhaled NO is almost exclusively determined by the increase of JawNO, the use of FeNO50 appears adequate to monitor the exhaled NO response to the specific inhalation challenge in sensitized subjects without the need of the more complex, demanding and time-consuming multi-flow analysis. Our findings that exhaled NO originated from airway may provide the rationale to administer the asthma standard treatment with inhaled steroids even in this form of asthma. FeNO measurement may give additional information to the monitoring of spirometry, since bronchoconstriction and airway inflammatory responses are dissociated. Our findings add support to the statement of the 'ERS Task Force on Specific Inhalation

Challenges with occupational agents' that suggests the measure of FeNO to help in the interpretation of SIC when the changes in FEV₁ are equivocal [43].

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Author contributions: Prof Maestrelli guarantees the entire manuscript.

Dr Mason: contributed to the conception and design of the study, as well as hypotheses delineation; acquired, analyzed, and/or interpreted data; wrote the article; and had substantial involvement in its revision prior to submission.

Dr Scarpa: contributed to the acquisition, analysis, interpretation of data and had substantial involvement in its revision prior to submission.

Dr Guarnieri: contributed to the acquisition, analysis, interpretation of data and had substantial involvement in its revision prior to submission.

Dr Giordano: contributed to the acquisition, analysis, interpretation of data and had substantial involvement in its revision prior to submission.

Prof Baraldi: contributed to the conception and design of the study, as well as hypotheses delineation and had substantial involvement in its revision prior to submission.

Prof Maestrelli: contributed to the conception and design of the study, as well as hypotheses delineation; acquired, analyzed, and interpreted data; wrote the article; and had substantial involvement in its revision prior to submission.

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Figure legends:

Figure 1: Time course of FEV1 after SIC with isocyanates in SIC positive and SIC negative groups. Values are given as mean $\pm$ SE.

Figure 2: Time course of JawNOcorr during active and sham exposure in SIC positive group (2a) and SIC negative group (2b). Values are given as mean $\pm$ SE.

Figure 3: Time course of CaNOcorr during active and sham in SIC positive group (3a) and SIC negative group (3b). Values are given as mean $\pm$ SE.

Figure 4: Changes in FeNO50 (4a), JawNOcorr (4b) and CaNOcorr (4c) between baseline and 24h after isocyanate exposure in early and late responders to SIC. Values are given as mean $\pm$ SE.

Table 1: *Characteristics of the subjects at baseline*

	SIC-POSITIVE GROUP	SIC-NEGATIVE GROUP
Gender, Male/Female	12/5	6/2
Age, yr	42.7±1.9	43±3.4
Smoking status, non smokers-ex smokers/ smokers	14/3	6/2
Atopy, yes/no	5/12	2/6
BMI, kg/m²	24.4±0.8	27.1±1.1
FEV1, L	3.5±0.1	3.4±0.1
FEV1 % predicted	92.1±2.6	89.7±3.7
FEV1/FVC %	74±2	78±1
PD₂₀FEV1, mg	0.7±0.1	0.9±0.1
FeNO50, ppb *	58.6 (24.1, 98.8) *	17.6 (11, 26.8)
Isocyanates tested, N°	10 TDI, 7 MDI	3 TDI, 3 HDI, 2 MDI
Type of asthmatic reaction	6 EARLY, 2 DUAL, 9 LATE -	

Values are given as n°, median and first, third quartile *, or mean ± standard error; BMI = Body Mass Index; PD₂₀ Provocative Dose causing a 20% fall in FEV1; * p < 0.05 vs SIC-negative group

Table 2a: Time course of FeNO50 and uncorrected flow-independent parameters in patients with *positive SIC*

		Baseline	1 h	3 h	5 h	7 h	24 h
FeNO50 (ppb)	active	58.6 (24.1, 98.8)	52.5 (25.2, 88.9)	47 (26.7, 79.7)	52.9 (29.6, 85)	65.8 (39.2, 107.2)	111.8 (68.5, 236)*
	sham	51.2 (25.2, 120.3)	47.7 (27.5, 114.1)	50.4 (27, 117)	38.8 (26.9, 111)	43.6 (21.5, 31.7)	58.6 (24.1, 98.8)
CawNO (ppb)	active	209.5 (85, 296.6)	162.1 (90.5, 247.4)	123.1 (66.9, 222.6)	149.8 (66.9, 247.4)	220.5 (115.9, 293.2)	272.3 (236.5, 535)
	sham	189.2 (77, 315)	189.5 (84.5, 363.2)	155.9 (83.1, 294.1)	218.1 (159.8, 345.2)	147.5 (83, 311)	209.5 (85, 296.6)
DawNO (mL/s)	active	21.5 (16.5, 25.7)	23.3 (15.6, 29.4)	23.9 (13.9, 29.3)	24 (18.2, 31.9)	20.6 (11.9, 29.4)	23.4 (13.7, 30.2)
	sham	20.8 (15.3, 25.7)	23.4 (14, 30.4)	14.7 (11, 33.7)	15.7 (8.4, 31.7)	23.2 (9.6, 31.1)	21.5 (16.5, 25.7)
CaNO (ppb)	active	3.9 (2.8, 5.5)	1.9 (0.1, 4.7)	1.7 (0.4, 4)	3.8 (1.5, 7.1)	3.2 (0.8, 7.1)	5.2 (0.8, 7.7)
	sham	3.6 (1.5, 6.2)	2.9 (1.2, 3.8)	3.4 (1.6, 4.2)	3.1 (1.2, 4.7)	5 (1.8, 6.3)	3.9 (2.8, 5.5)
JawNO (nL/s)	active	3.3 (1.4, 5.2)	2.7 (1.4, 4.8)	2.4 (1.5, 4.7)	2.7 (1.9, 4.7)	3.9 (2, 10)	6.6 (3.5, 11.7) *
	sham	3.5 (1.3, 7.7)	3.4 (1.5, 7.2)	3.1 (1.2, 7.3)	3.1 (1.2, 8.2)	2 (0.9, 5.2)	3.3 (1.4, 5.2)

Values are given as median (Q1, Q3); * p < 0.05 vs sham

Table 2b: Time course of FeNO50 and uncorrected flow-independent parameters in patients with *negative SIC*

		Baseline	1 h	3 h	5 h	7 h	24 h
FeNO50 (ppb)	active	17.6 (11, 26.8)	18 (11.7, 27.8)	16.5 (9.2, 25)	15.5 (7, 25.5)	15.9 (11.1, 23)	19.5 (12, 26.8)
	sham	13.5 (10.9, 19.6)	15.9 (9.5, 22.3)	16.9 (13.1, 22.8)	19.3 (16.1, 20.5)	16.1 (11.2, 21.4)	17.6 (11, 26.8)
CawNO(ppb)	active	121.3 (82.7, 186.4)	102.1 (68.9, 124.3)	72.7 (60.2, 140.3)	62.8 (53.1, 79.3)	74 (49.4, 85.6)	70 (55.1, 239.6)
	sham	93.5 (61.5, 111.6)	90.7 (56.3, 132.8)	65.2 (56.9, 87.6)	68.5 (63.9, 89.1)	55 (39.8, 70.6)	121.3 (82.7, 186.4)
DawNO(mL/s)	active	10 (2.6, 15.4)	9.4 (7.1, 19.4)	6.9 (6, 15)	10.1 (5.9, 14.6)	10.6 (7.8, 20.7)	13.6 (11.4, 26.4)
	sham	9.1 (5.4, 10.9)	11.4 (9, 12.9)	9.9 (7.9, 14.9)	12.3 (6.2, 15.3)	11.4 (7.8, 19.8)	10 (2.6, 15.4)
CaNO(ppb)	active	3.9 (1.7, 5.7)	2.2 (1.4, 3.7)	2.1 (1.7, 2.5)	2.3 (1.3, 3.2)	3.1 (1.7, 4.1)	2.9 (1.6, 5.7)
	sham	2.7 (2.2, 3)	3.3 (2.6, 4)	2.7 (2.7, 3.1)	2.7 (2, 3.9)	2.4 (0.7, 3.5)	3.9 (1.7, 5.7)
JawNO(nL/s)	active	0.7 (0.3, 1.1)	0.7 (0.4, 1.4)	0.7 (0.3, 1.2)	0.7 (0.3, 1.4)	0.7 (0.4, 1.1)	0.7 (0.5, 1.3)
	sham	0.5 (0.4, 0.9)	0.7 (0.4, 1)	0.7 (0.5, 1.1)	0.8 (0.5, 1)	0.7 (0.3, 1.1)	0.7 (0.3, 1.1)

Values are given as median (Q1, Q3)

Table 3: Time course of ADMA/Tyr ratio after isocyanate exposure in SIC-positive and SIC-negative group

	Baseline	3 h	7 h	24h
SIC-positive group	0.04 (0.01, 0.08)	0.05 (0, 0.09)	0.03 (0, 0.08)	0.03 (0.01, 0.08)
SIC-negative group	0.09 (0.05, 0.13)	0.05 (0, 0.09)	0.02 (0.01, 0.03)	0.04 (0, 0.1)

Values are given as median (Q1, Q3)

Figure 1

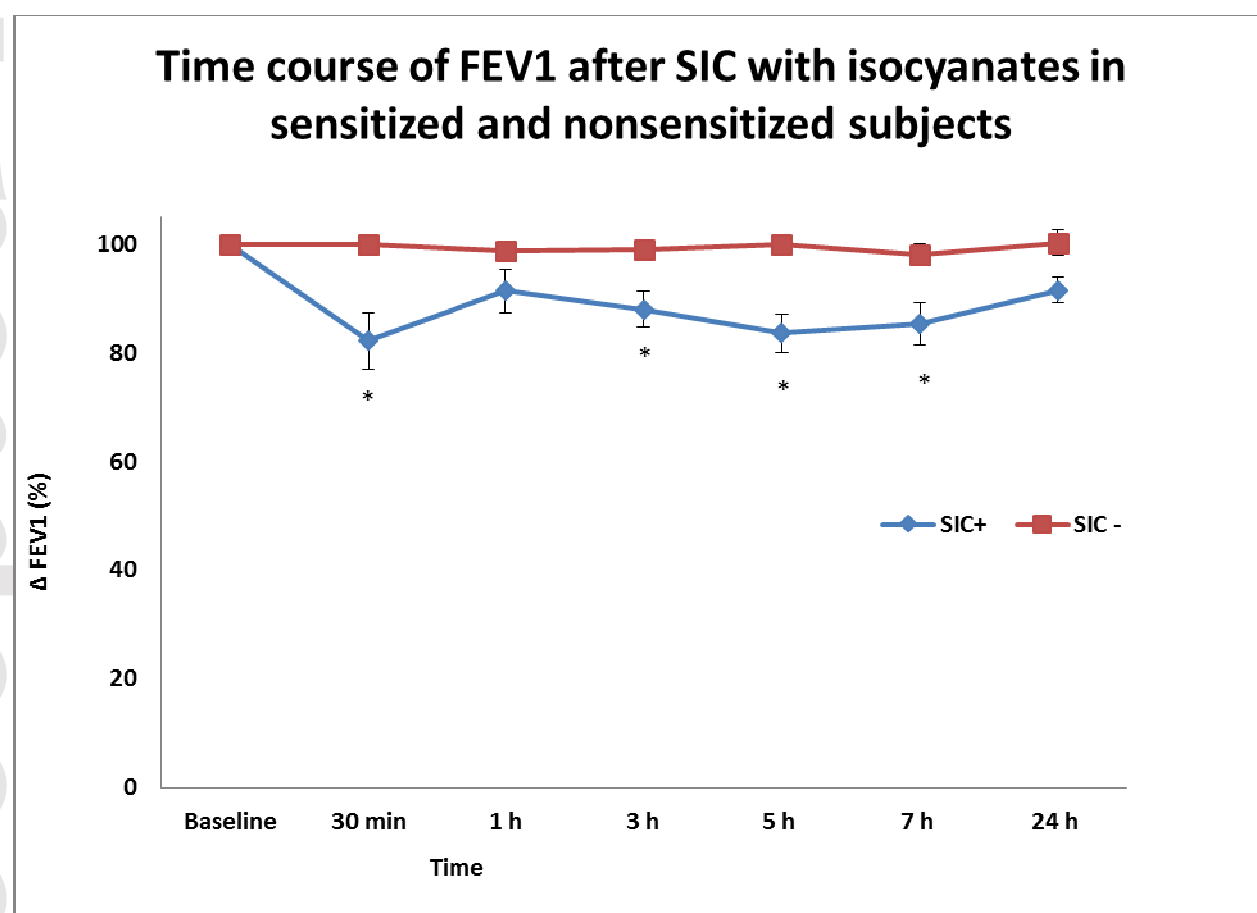


Figure 2a

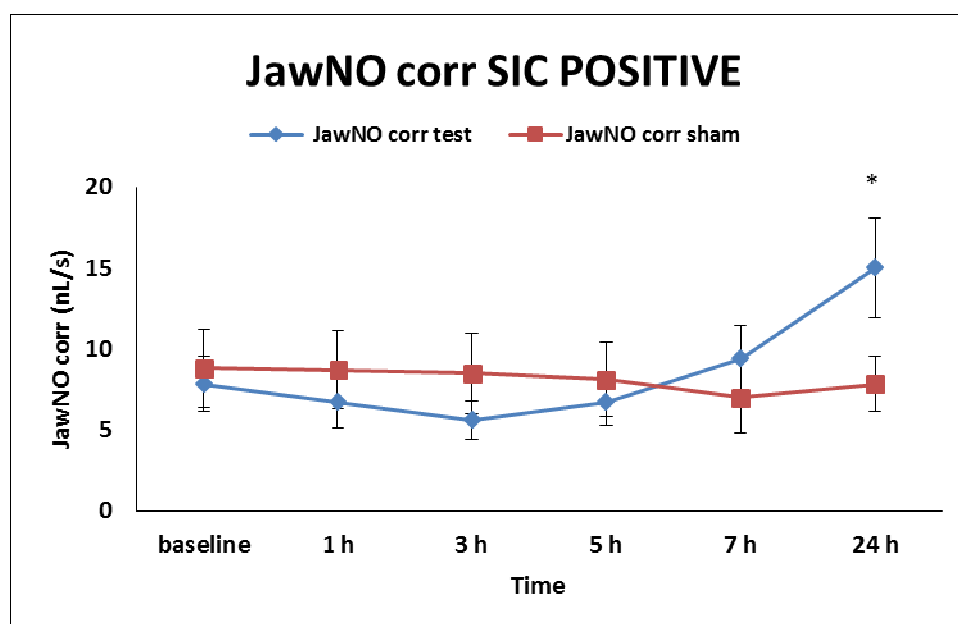


Figure 2b

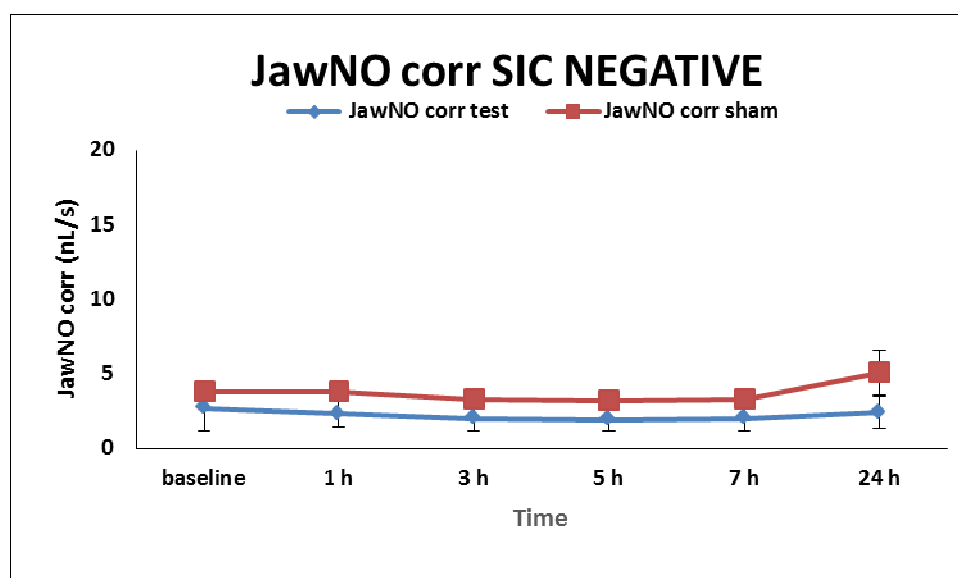


Figure 3a

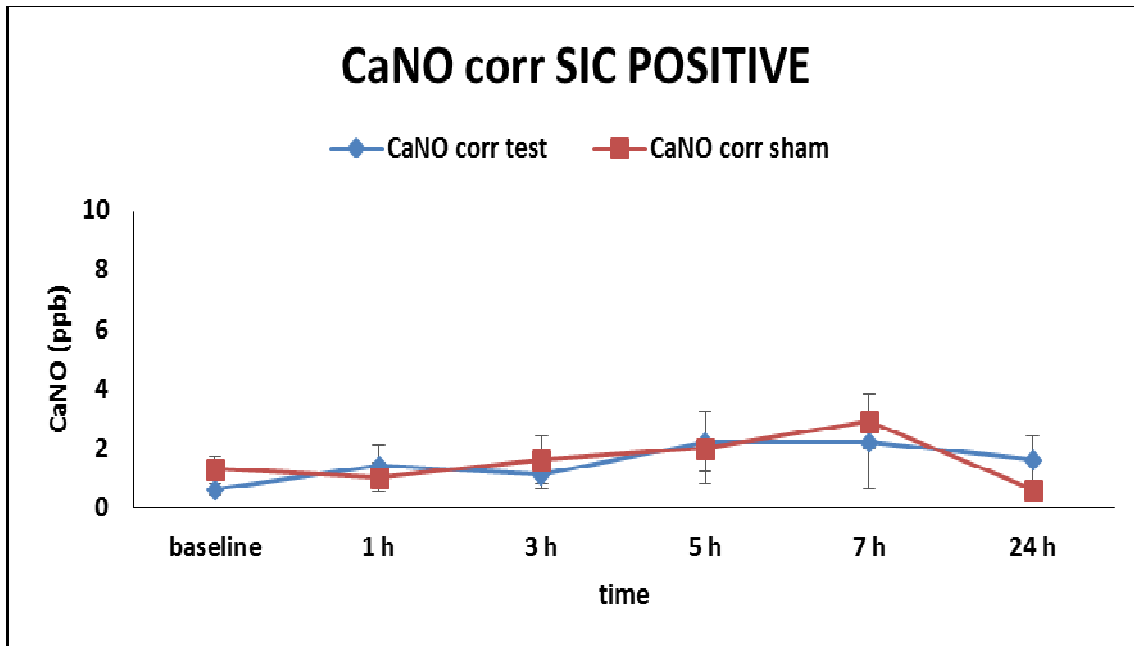


Figure 3b

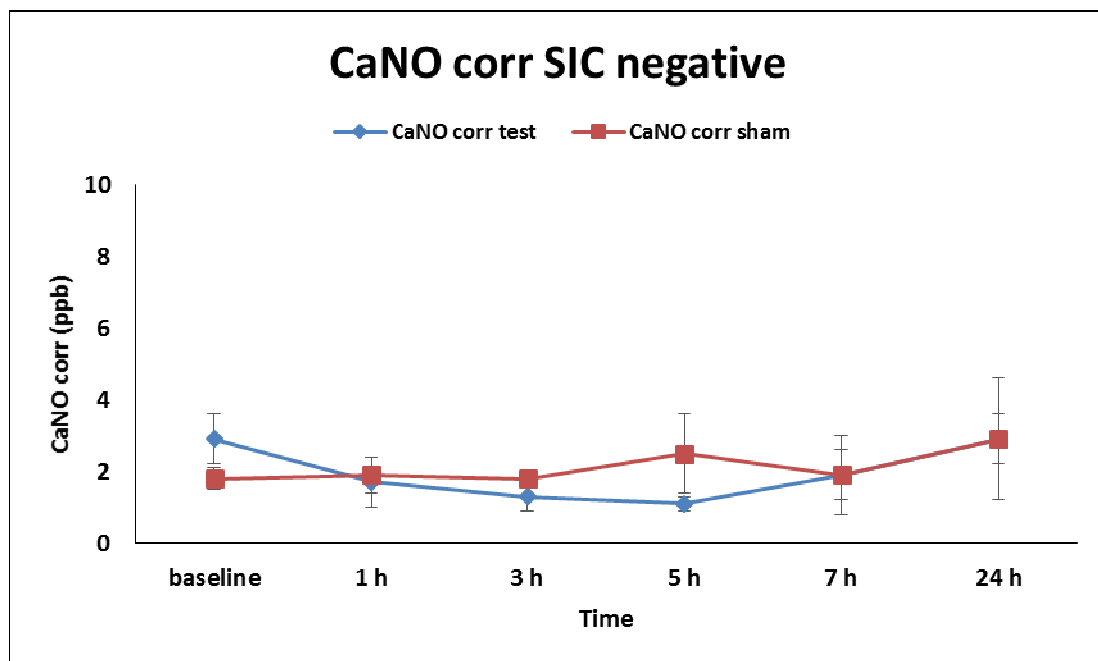


Figure 4a

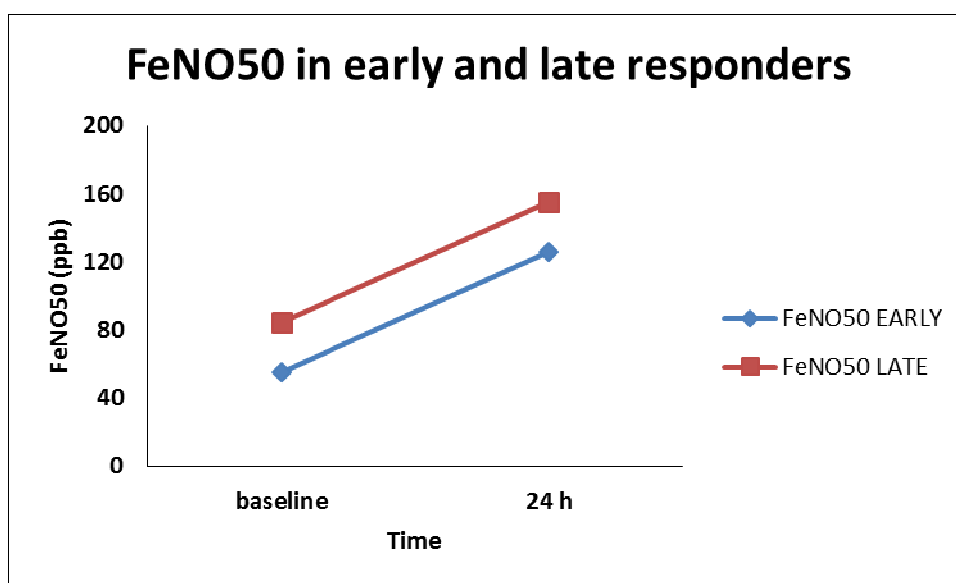


Figure 4b

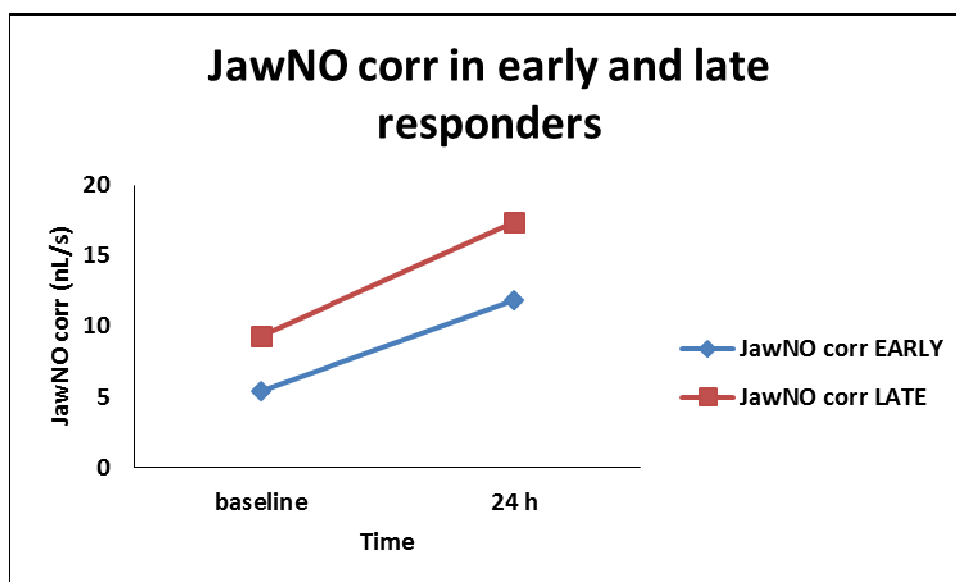


Figure 4c

