

Increase of Exhaled Nitric Oxide (eNO) after Methylene Diphenyl Diisocyanate (MDI) Exposure in Isocyanate Workers with Bronchial Hyperresponsiveness

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ABSTRACT

Background: Isocyanates have become one of the most important causes of occupational asthma in industrialized countries. Increased exhaled nitric oxide (eNO) levels have been shown to be associated with allergic airway inflammation. The objective of this study was to investigate the influence of isocyanate on eNO levels and to elucidate whether the latter are associated with specific sensitization and/or unspecific bronchial hyperresponsiveness (BHR). Additionally, we wanted to compare eNO changes of smokers and non-smokers.

Methods: We determined eNO during diagnostic isocyanate challenges in workers with suspected isocyanate asthma.

Results: Fourteen of 22 symptomatic isocyanate workers showed BHR and five of these 14 developed an asthmatic response upon challenge with methylene diphenyl diisocyanate (MDI). In comparison with the group without BHR, subjects with BHR had higher basal eNO and a significant increase in eNO 22 hrs after MDI challenge. Four of the asthmatic responders and six of the nine MDI non-responders with BHR revealed an eNO increase of > 30%. There was also a positive association between the eNO change and the increase in airway resistance in isocyanate workers with BHR. The highest eNO change was found in subjects with IgE-mediated sensitization to MDI and low MDI thresholds. Only one of the eight MDI non-responders without BHR exhibited an eNO increase of > 30%.

Conclusions: Isocyanate workers with BHR show increased MDI responses both of airway resistance and of the inflammatory marker eNO. eNO measurement is obviously a new suitable tool for monitoring isocyanate workers under respiratory risk.

KEY WORDS

challenge test, inhalation challenge, isocyanates, occupational asthma

INTRODUCTION

Exhaled nitric oxide (eNO) has been identified as a new marker of allergic airway inflammation.¹⁻⁴ Subjects suffering from asthmatic and/or rhinitic symptoms show increased eNO levels^{5,6} and eNO increases were described to precede sputum eosinophilia and lung function deterioration in asthmatics.^{7,8} Since cigarette smoke has a high NO concentration, smokers have decreased eNO values, probably due to the suppression of endogenous NO production.⁹

Several studies demonstrate that inhalative allergen exposure of sensitised subjects results in inflammatory reactions of the airways which are associated with elevated eNO levels.^{1,3,5,9} So far, eNO has scarcely been analysed in workers exposed to isocyanates, worldwide one of the most frequent causes of occupational asthma.¹⁰ Isocyanates are not classic allergens since approximately 80% of affected workers do not show evidence of IgE-mediated sensitization.¹¹⁻¹⁵ To the best of our knowledge, this is the first study dealing with eNO in isocyanate workers

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(with the exception of a preliminary investigation of our group).⁶ In our initial study of 9 subjects,⁶ eNO increased in some subjects 22 hrs after inhalation challenge with methylene diphenyl diisocyanate (MDI). However, statistical analysis and standardization (particularly with regard to the exhalation rate) according to ERS and ATS recommendations published later were not possible in this initial investigation. These deficiencies were overcome in the present study which was designed to evaluate the relationship between isocyanate-induced eNO changes (Δ eNO) and the outcome of the specific challenge test as well as of methacholine challenge testing. Only patients subjected to MDI as well as methacholine challenge tests were included in the present paper.

METHODS

22 subjects consecutively undergoing medical examination for suspected isocyanate asthma in our institute took part in this study. The subjects had worked in chemical plants ($n = 7$), foam production ($n = 3$), coating ($n = 4$), casting ($n = 2$) or in rock consolidation of hard coal mines ($n = 6$) where MDI was used. In all subjects symptoms of cough, wheezing and/or chest tightness had developed after working for at least one year with MDI.

Four patients were current cigarette smokers (2 to 12 packyears; 4 to 25 cigarettes per day), ten were ex-smokers who had smoked for more than two years and eight had never smoked. Smokers were asked to refrain from smoking 1 hr before testing. Five subjects were treated with inhalative corticosteroids at the time of the study.

The subjects were asked not to take any drug 12 hrs before the investigation.

Specific IgE antibodies to the conjugate MDI-HSA were determined by the CAP system (Pharmacia Uppsala, Sweden; 16); values above 0.35 kU/L were considered positive.

Lung function measurement, methacholine challenge testing and occupational-type MDI challenge were performed using a bodyplethysmograph as previously described.¹⁷ Patients were categorized as having bronchial hyperresponsiveness (BHR) if $PD_{40} / 0.5 / (kPa \times s)$ $sGaw$ was < 0.3 mg methacholine.

In the MDI challenge performed in a chamber, an $sRaw$ increase of at least 100% from basal values reaching ≥ 2 kPa*s was considered a positive result. Immediate reactions occurred up to 1 hr, late reactions 2 to 22 hrs post challenge. We started the occupational-type MDI challenge with 3 ppb for 30 min followed by 5 ppb for 90 min if no asthmatic reaction occurred.¹³ Because of ethical reasons, the German occupational exposure limit (OEL) of 5 ppb was not exceeded. In two subjects, MDI challenge was stopped at 3 ppb due to strong immediate asthmatic reactions, but all others underwent the complete challenge test proto-

col.

$sRaw$ as well as eNO were determined before, 1, 2, 4, 6 and 22 hrs post MDI challenge.

eNO measurements were performed according to ATS recommendations¹⁸ with the exception of an expiratory flow of 100 mL/s with a chemiluminescence analyser (CLD-88; ECO-Physics, Dürnten, Switzerland) modified for on-line recording of eNO concentrations. The analyser has a sensitivity to NO between 0.1 ppb and 500 ppb, a resolution of 0.1 ppb, and a response time of 0.5 s. The normal range of eNO was determined in 25 healthy subjects with regular lung function parameters (FVC, FEV1, $sRaw$); their mean value at an expiratory flow of 100 mL/s was 7.7 ppb; the 95% CI was 6.4–9.4 ppb. Regarding this range, we consider basal eNO concentrations ≥ 9.5 ppb elevated values.

At the individual level, an eNO increase of $> 30\%$ from basal values was interpreted as a preliminary cut-off level of eNO changes after MDI challenge in order to categorically differentiate between a remarkable eNO increase and no substantial changes.

STATISTICAL METHODS

We used the Wilcoxon-signed rank test to determine the significance of eNO changes after MDI challenges. The unpaired *t*-test or Kruskal-Wallis-test was used to compare eNO values and lung function data between groups.

Categorical variables were compared with chi-square tests. Regression analysis was performed by Spearman's rank correlation coefficients to determine the relationship between changes in $sRaw$ and changes in eNO during/after MDI challenge. All results were expressed as means $\pm$ SEM. A value of $p < 0.05$ was considered significant.

RESULTS

Table 1 summarizes subjects' characteristics and results of methacholine as well as of MDI challenge tests.

14 of the 22 investigated MDI workers showed BHR as evaluated by methacholine challenge testing. Five of these 14 demonstrated an asthmatic response during or post MDI challenge (MDI responders); three had a dual response, two a late one. MDI concentrations eliciting these reactions were 3 ppb in two subjects (both IgE-positive) and 5 ppb in three subjects (Fig. 1). In nine subjects with BHR, $sRaw$ changes did not fulfill the defined positive criteria (Fig. 2). None of the eight subjects without BHR revealed a remarkable change in $sRaw$ after MDI challenge (Fig. 3).

According to the outcome of methacholine provocation testing and MDI challenge, we could analyse the time course of eNO in the following groups:

I. with BHR ($n = 14$)

-MDI responders ($n = 5$);

Table 1 Smoking habit and results of methacholine as well as MDI challenge tests

Isocyanate worker no.	Smoking habit	Bronchial hyperresponsiveness	Specific challenge test with MDI	MDI-HSA-specific IgE	Inhalative corticosteroid therapy	eNO basal value (ppb)	eNO 22 h post MDI challenge (ppb)	Changes eNO (% of basal value)
Workers with asthmatic response to MDI								
1 (◆)	ex-smoker	yes	pos	neg	yes	29.1	23.5	− 19
2 (◆)	smoker	yes	pos	neg	no	10.7	14.0	31
3 (△)	ex-smoker	yes	pos	pos	no	17.2	32.2	88
4 (■)	smoker	yes	pos	pos	no	2.0	10.3	415
5 (*)	ex-smoker	yes	pos	pos	no	11.9	71.4	502
Workers non-responding to MDI with BHR								
6	ex-smoker	yes	neg	neg	no	17.8	11.8	− 34
7	ex-smoker	yes	neg	neg	no	5.0	4.5	− 10
8 (◆)	ex-smoker	yes	neg	neg	yes	3.5	14.0	300
9	non-smoker	yes	neg	neg	no	76.7	111.9	46
10	non-smoker	yes	neg	neg	no	29.4	64.4	119
11 (●)	non-smoker	yes	neg	neg	yes	15.9	30.1	89
12 (○)	ex-smoker	yes	neg	neg	yes	6.8	8.9	31
13 (◆)	smoker	yes	neg	neg	no	2.5	4.6	83
14	ex-smoker	yes	neg	neg	no	8.2	8.4	H2
Workers non-responding to MDI without BHR								
15	non-smoker	no	neg	neg	no	11.0	9.5	− 14
16	non-smoker	no	neg	neg	no	6.3	6.3	0
17	non-smoker	no	neg	neg	no	5.4	10.0	85
18	non-smoker	no	neg	neg	no	9.6	8.7	− 9
19 (*)	non-smoker	no	neg	neg	yes	5.2	4.4	− 15
20 (◆)	smoker	no	neg	neg	no	4.7	3.8	− 19
21	ex-smoker	no	neg	neg	no	8.9	9.0	1
22	ex-smoker	no	neg	neg	no	41	32.8	− 20

pos = positive result; neg = negative result.

-MDI non-responders with BHR ($n = 9$) ;II. without BHR ($n = 8$)-MDI non-responders ($n = 8$).

With regard to baseline eNO, there was no significant tendency towards higher values in the two groups with BHR compared with the group without BHR (16.9 ± 5.7 ppb *vs.* 11.5 ± 4.3 ppb). Baseline eNO was elevated in all but one of the MDI responders (the exceptional case was a smoker), in four of the nine MDI non-responders with BHR and in three of the eight workers without BHR (Fig. 4). Of the four smokers, three had normal baseline eNO values (the exceptional case with a borderline eNO belonged to the asthmatic group).

No significant changes in eNO occurred in the three groups during the first four hrs post MDI challenge. 22 hrs post MDI challenge, there was a significant increase in eNO only in subjects with BHR when compared with their baseline eNO. Furthermore, the eNO change was significantly higher in the BHR group than in subjects without BHR (12.4 ± 5 ppb *vs.*

- 1 ± 1.2 ppb ; $P = 0.02$) (Fig. 5).

Although there was no significant Δ eNO difference between the two groups with BHR (MDI responders *vs.* MDI non-responders with BHR), the five asthmatic responders showed on average a higher eNO increase 22 hrs post MDI challenge than MDI non-responders with BHR (16.2 ± 5 ppb *vs.* 10.3 ± 5 ppb ; n.s.). The highest Δ eNO was seen in the three MDI responders with MDI-HSA-specific IgE antibodies (27.7 ± 13.7 ppb ; range 8 – 60) (Fig. 6). The differences between the three groups were significant with borderline P -values ($p = 0.05$), indicating a tendency of increasing eNO changes post MDI challenge from subjects without BHR to MDI non-responders with BHR and so forth up to MDI responders. The differences were even more pronounced if eNO changes were expressed in % from baseline (Fig. 7).

According to the preliminary cut-off of 30% of eNO increase, 4/5 MDI responders, 6/9 of those with BHR without MDI response, but only 1/8 of those without BHR showed such an increase in eNO 22 hrs

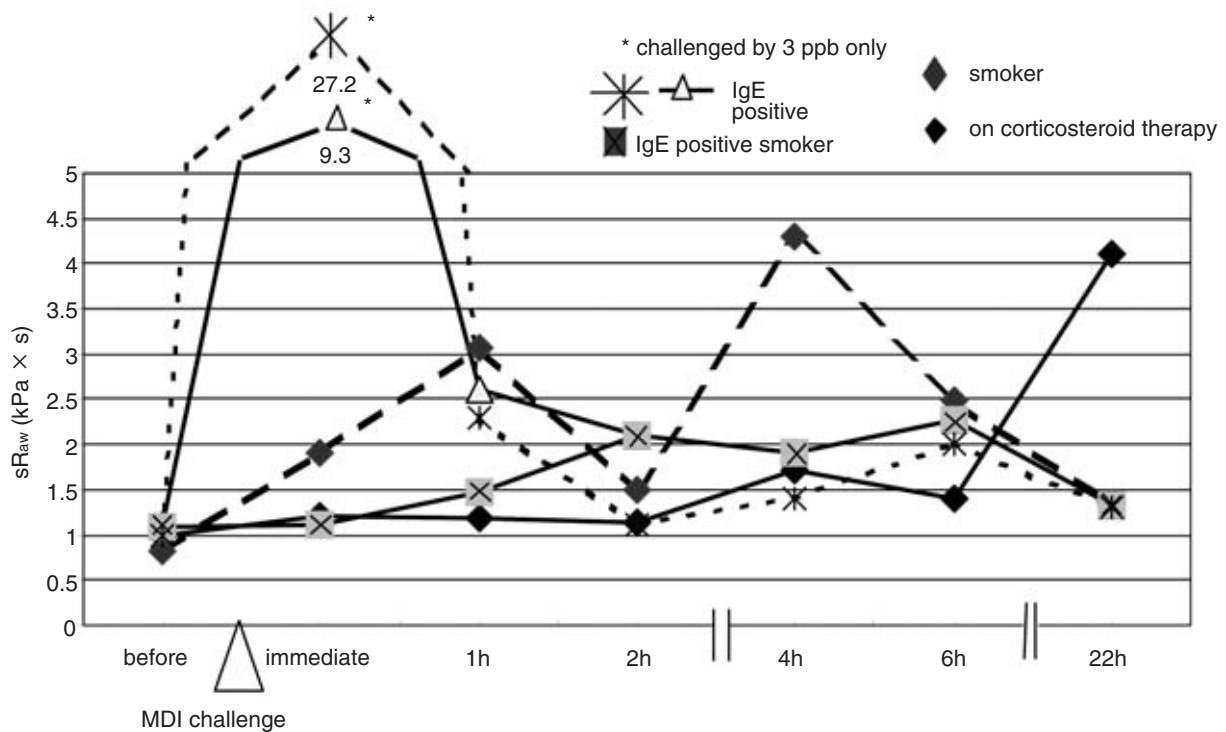


Fig. 1 Individual course of sRt post MDI challenge test in isocyanate workers with asthmatic reactions ($n = 5$)

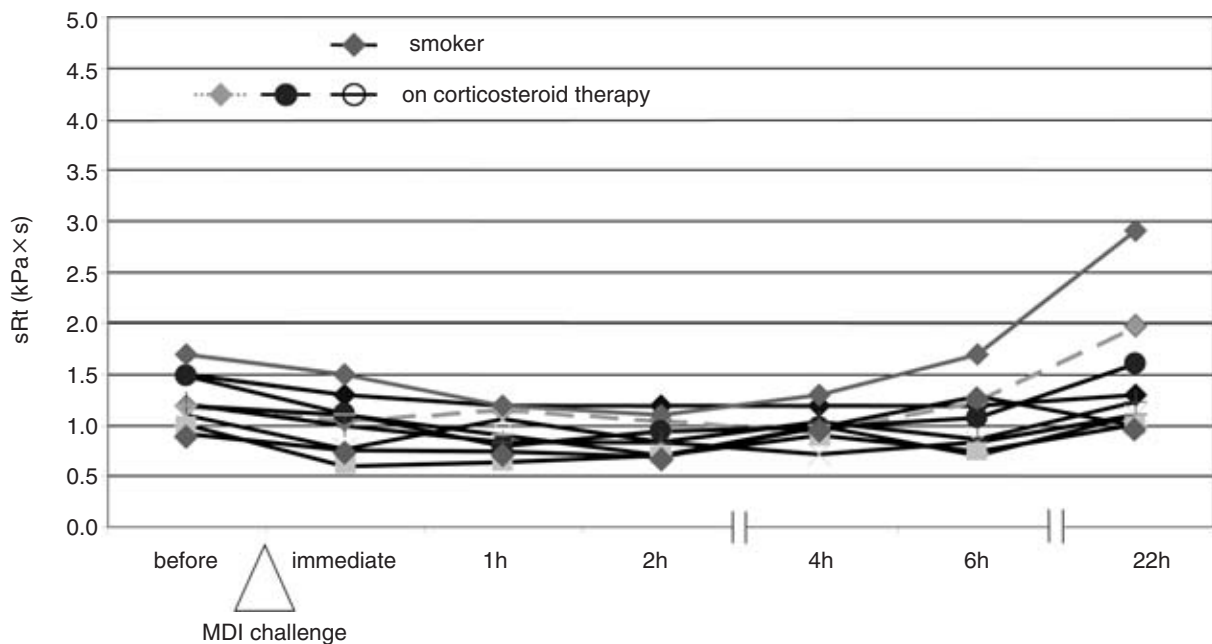


Fig. 2 Individual course of sRt post MDI challenge test in MDI non-responders with BHR ($n = 9$)

post MDI challenge.

Smoking did not seem to influence eNO changes 22 hrs post MDI challenge (Fig. 7) as opposed to its well documented effect on baseline eNO values.^{19,20}

In subjects with BHR, a significant positive correla-

tion was found between eNO changes (% from baseline) 22 hrs post challenge and the maximal changes in sRaw (% from baseline) during the period 0–22 hrs post MDI challenge ($r_s = 0.54$, $p = 0.04$). Such an association was not seen in workers without BHR.

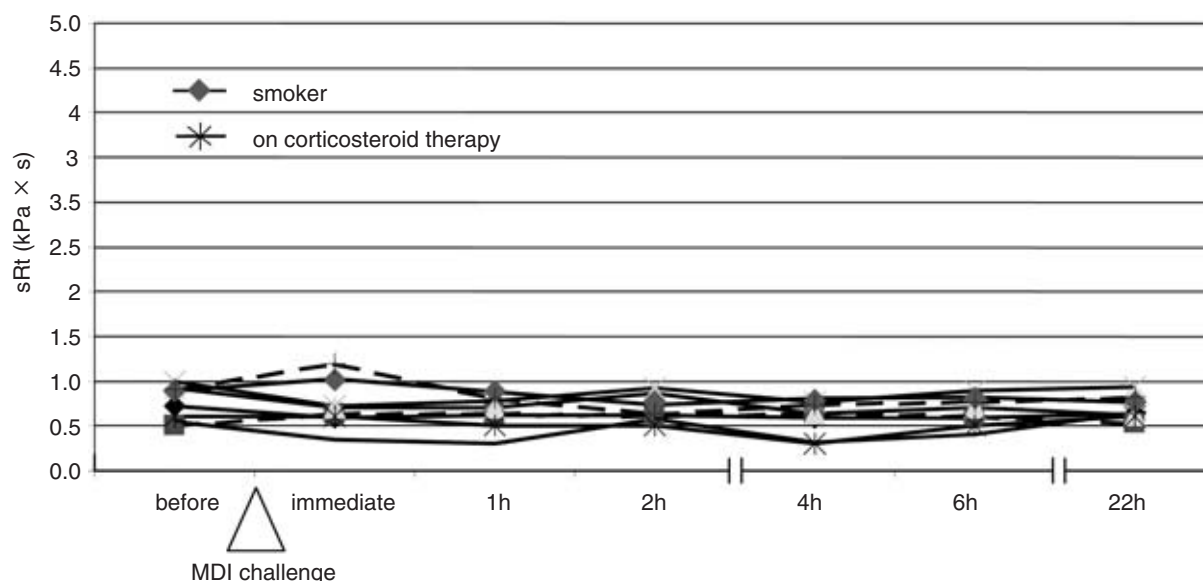


Fig. 3 Individual course of sRt post MDI challenge test in MDI non-responders without BHR ($n = 8$)

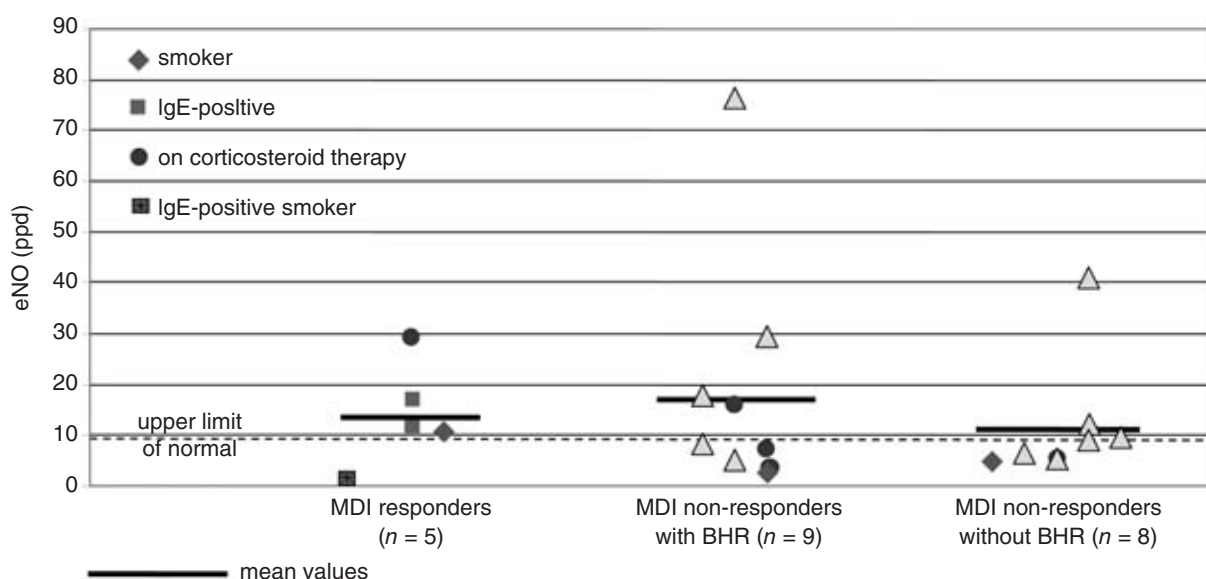


Fig. 4 Comparison of basal eNO values in the three groups of isocyanate workers

DISCUSSION

The significant eNO increase after occupational-type challenge test with MDI in most symptomatic workers with BHR, which rarely occurs in the absence of BHR, is remarkable, since isocyanates are thought to have mainly irritative effects on the airways. The eNO increase is more pronounced when MDI concentrations below OEL elicit an asthmatic response and when IgE-mediated sensitization to isocyanate is present.

The strong eNO increases in IgE-sensitized MDI

workers are in accordance with findings in subjects sensitized to environmental or occupational allergens^{3,21-23} indicating allergic inflammation.

Interestingly, smoking isocyanate workers mostly revealed normal baseline eNO values and always demonstrated an eNO increase upon MDI challenge in the presence of BHR; *i.e.*, they did not give evidence for an impaired eNO response to MDI exposure.

An eNO increase after exposure to occupational substances is a more relevant parameter in occupational settings than an elevated baseline eNO value.

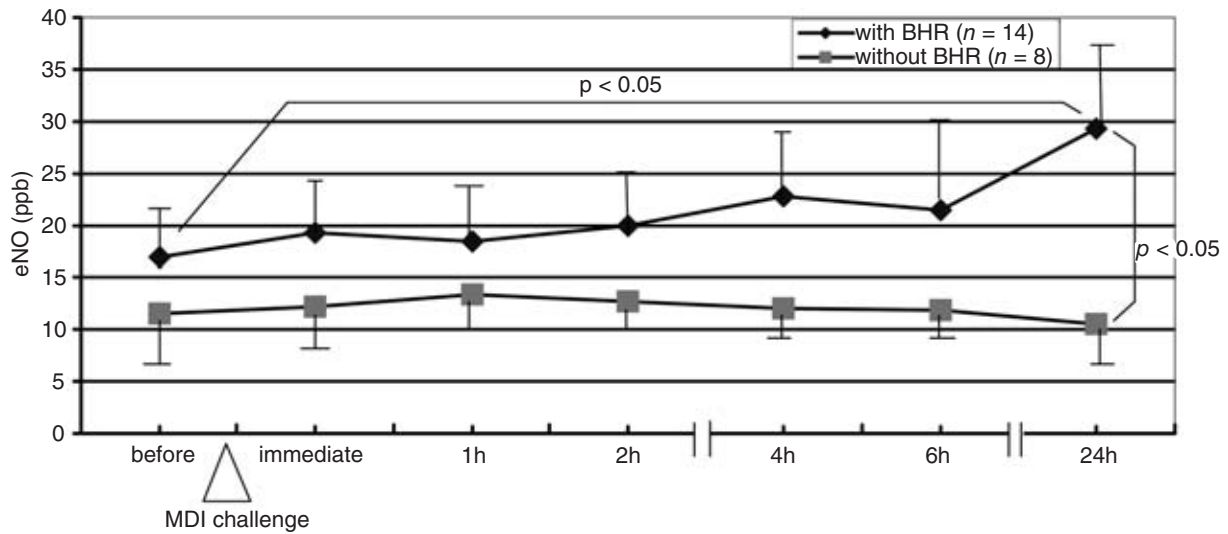


Fig. 5 Changes in eNO post MDI challenge test in subjects with BHR (MDI responders and MDI non-responders with BHR) and in the group without BHR (means $\pm$ SEM)

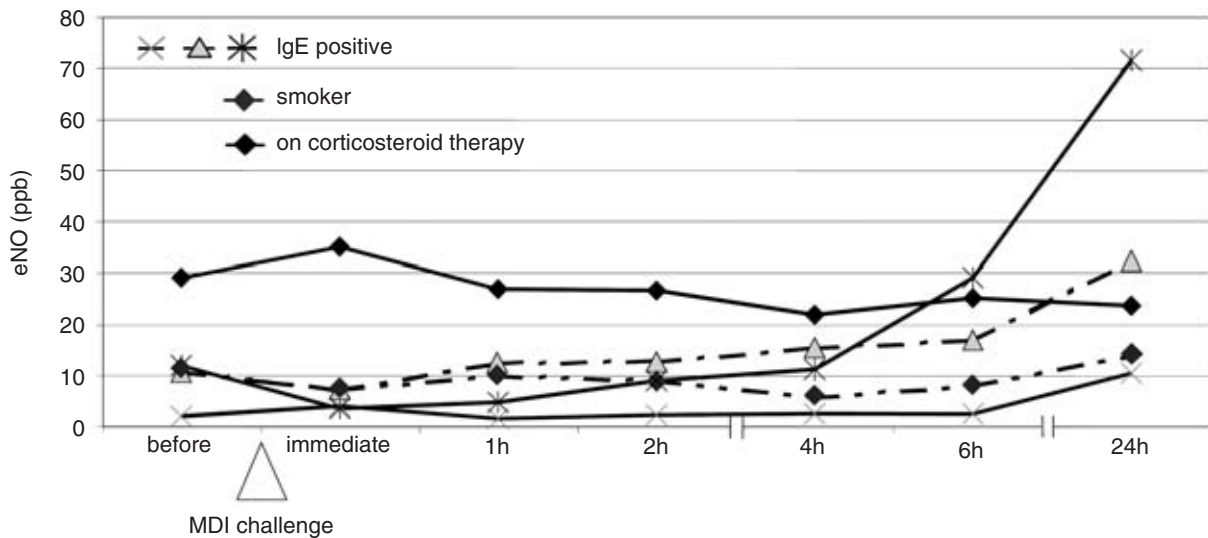


Fig. 6 Individual eNO course post MDI challenge test in isocyanate workers with asthmatic reactions ($n = 5$)

In our study, ten of the 14 subjects with BHR were on the basis of the preliminary cut-off of 30% correctly classified and only one of the nine workers without BHR was misclassified. Respective data of elevated baseline eNO were 8/14 and 3/9. Thus, in addition to the gold standard, the specific challenge test accomplished by lung function measurement, measurement of eNO after defined inhalative exposures may be a valuable tool to identify and monitor isocyanate workers under respiratory risk, *i. e.* with occupational asthma and/or BHR. However, further studies with a larger number of subjects are necessary to clarify the prognostic value of eNO in such populations.

The results of our study are partly in accordance

with the report by Obata *et al.*²⁴ who found an eNO increase during late asthmatic reactions after plicatic acid challenge in symptomatic responders as well as in symptomatic non-responders. Surprisingly, in their study a significant increase occurred in symptomatic non-responders only. These authors also demonstrated a significant correlation between the degree of sputum eosinophilia and eNO levels after plicatic acid challenges in subjects with suspected red cedar asthma. They did not find a correlation between BHR and eNO increase after challenge, however. In our study, an association between BHR and a Δ eNO of $> 30\%$ was observed in 10/14 subjects and an association between corresponding negative findings in 7/8

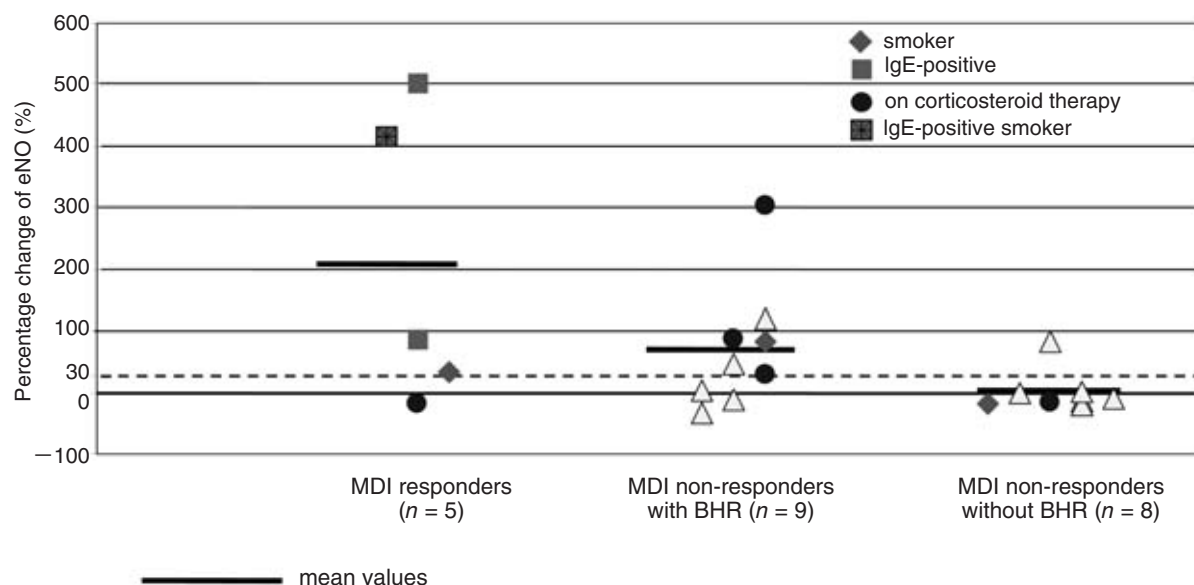


Fig. 7 Comparison of eNO changes 22 hrs post MDI challenge in the three groups of isocyanate workers. There is a significant trend of eNO changes among the three groups ($p < 0.05$; Kruskal-Wallis test)

subjects without BHR. Dupont *et al.*²⁵ and Henriksen *et al.*²⁶ reported a significant positive association between BHR and elevated baseline eNO. Interestingly and even more importantly, the latter authors observed that a combination of these two tools is a very specific finding in atopic asthma. Our study shows that this also applies to proven isocyanate asthma and to nearly half of the symptomatic isocyanate workers with BHR without asthmatic response in the applied challenge test. The latter group may include true cases of isocyanate asthma showing “false negative” MDI challenge test results due to cumulative effects or much higher isocyanate concentrations in the workplace than used in our challenge tests. It may also include pre-stages of isocyanate asthma.

The findings of elevated baseline eNO and Δ eNO 22 hrs post MDI challenge as well as the positive association between Δ eNO 22 hrs and sR_{aw} increases both in MDI responders and in MDI non-responders with BHR suggest that there is no clear borderline between these two groups.

We hypothesize that in susceptible individuals isocyanate recruitment of inflammatory cells²⁷ is associated with cellular activation in the airways resulting in increased local NO production as well as BHR and is followed by the development of MDI asthma. However, this hypothesis needs follow-up studies as well as experimental investigations such as measurements of bronchial NO production and diffusion, immunocytochemical/histochemical analyses of BAL components as well as of biopsy specimens.

REFERENCES

1. Lopuhaä CE, Koopmans JG, Jansen HM, van der Zee JS. Similar levels of nitric oxide in exhaled air in non-asthmatic rhinitis and asthma after bronchial allergen challenge. *Allergy* 2003;**58**:300-305.
2. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am. J. Respir. Crit. Care Med.* 2001;**164**:1376-1381.
3. Kharitonov SA, O'Connor BJ, Evans DJ, Barnes PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 1995;**153**:454-457.
4. Gratziau C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur. Respir. J.* 1999;**14**:897-901.
5. Thomassen MJ, Raychaudhuri B, Dweik RA *et al.* Nitric oxide regulation of asthmatic airway inflammation with segmental allergen challenge. *J. Allergy Clin. Immunol.* 1999;**104**:1174-1182.
6. Allmers H, Chen Z, Barbinova L, Marczyński B, Kirschmann V, Baur X. Challenge from methacholine, natural rubber latex, or 4,4-diphenylmethane-diisocyanate in workers with suspected sensitisation affects exhaled nitric oxide (change in exhaled NO levels after allergen challenges). *Int. Arch. Occup. Environ. Health* 2000;**73**: 181-186.
7. Bates CA, Silkoff PE. Exhaled nitric oxide in asthma : From bench to bedside. *J. Allergy Clin. Immunol.* 2003; **111**:256-262.
8. Henriksen AH, Sue-Chu M, Holmen TL, Langhammer A, Bjerrum L. Exhaled and nasal NO levels in allergic rhinitis : relation to sensitisation, pollen season and bronchial hyperresponsiveness. *Eur. Respir. J.* 1999;**13**:301-306.
9. Gratziau C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis : influ-

- ence of pollen season and therapy. *Clin. Exp. Allergy* 2001; **31**:409-416.
10. Latza U, Baur X, Malo JL. Isocyanate-induced health effects. In : JV Bakke , Norén JO , Thorud S (eds) . *International consensus report on : Isocyanates—Risk assessment and management*, Appendices 10.2.1. Gjøvik:Norwegian Labour Inspection Authority, 2002;37-51. Available from: URL: <http://www.arbeidstilsynet.no/publikasjoner/rapporter/rapport1eng.html>.
 11. Bernstein DI, Korbek L, Stauder T *et al.* The low prevalence of occupational asthma and antibody-dependent sensitization to diphenylmethane diisocyanate in a plant engineered for minimal exposure to diisocyanates. *J. Allergy Clin. Immunol.* 1993;**92**:387-396.
 12. Malo JL, Ghezzi H, Élie R. Occupational asthma caused by isocyanates. *Am. J. Respir. Crit. Care Med.* 1999;**159**:1879-1883.
 13. Baur X, Marek W, Ammon J *et al.* Respiratory and other hazards of isocyanates. *Int. Arch. Occup. Environ. Health* 1994;**66**:141-152.
 14. Redlich CA, Karol MH. Diisocyanate asthma : clinical aspects and immunopathogenesis. *Int. Immunopharmacol.* 2002;**2**:213-224.
 15. Wisniewski AV, Redlich CA. Recent developments in diisocyanate asthma. *Curr. Opin. Allergy Clin. Immunol.* 2001;**1**:169-175.
 16. Baur X, Chen Z, Flagge A, Posch A, Raulf-Heimsoth M. EAST and CAP specificity for the evaluation of IgE and IgG antibodies to diisocyanate-HSA conjugates. *Int. Arch. Allergy Immunol.* 1996;**110**:332-338.
 17. Baur X, Huber H, Degens P, Allmers H, Ammon J. Relation between occupational asthma case history, bronchial methacholine challenge, and specific test in patients with suspected occupational asthma. *Am. J. Ind. Med.* 1998;**33**:114-122.
 18. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children — 1999. American Thoracic Society. *Am. J. Respir. Crit. Care Med.* 1999;**160**:2104-2117.
 19. Kharitonov SA, Robbins RA, Yates DH, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am. J. Respir. Crit. Care Med.* 1995; **152**:609-612.
 20. Robbins RA, Floreani AA, von Essen SG *et al.* Measurement of exhaled nitric oxide by three different techniques. *Am. J. Respir. Crit. Care Med.* 1996;**153**:1631-1635.
 21. Henriksen AH, Sue-Chu M, Holmen TL, Langhammer A, Bjerner L. Exhaled and nasal NO levels in allergic rhinitis : relation to sensitisation, pollen season and bronchial hyperresponsiveness. *Eur. Respir. J.* 1999;**13**:301-306.
 22. Gratzou C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis : influence of pollen season and therapy. *Clin. Exp. Allergy* 2001; **31**:409-416.
 23. Gratzou C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma. *Eur. Respir. J.* 1999;**14**:897-901.
 24. Obata H, Dittrick M, Chan H, Chan-Yeung M. Sputum eosinophils and exhaled nitric oxide during late asthmatic reactions in patients with western red cedar asthma. *Eur. Respir. J.* 1999;**13**:489-495.
 25. Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naïve patients with mild asthma. *Am. J. Respir. Crit. Care Med.* 1998;**157**:894-898.
 26. Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjerner L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma cases in a large population survey. *Eur. Respir. J.* 2000;**15**:849-855.
 27. Mapp CE, Saetta P, Maestrelli P *et al.* Mechanism and pathology of occupational asthma. *Eur. Respir. J.* 1994;**7**:544-554.