



The effects of *Aspergillus fumigatus* challenge on exhaled and nasal NO levels

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ABSTRACT: Several studies have previously shown that exposure to indoor air microbes from moisture-damaged buildings can cause adverse health effects. *Aspergillus fumigatus* is one of the best-documented moulds causing health problems to those exposed.

In this study, inhalation of a commercial *A. fumigatus* solution was assessed, to establish if it would have effects on fractional exhaled (F_eNO) and nasal (F_nNO) nitric oxide levels and on lung function. The results were compared with placebo challenge.

A total of 28 subjects were divided into three study groups: group 1 had been exposed to occupational mould; group 2 consisted of atopic subjects; and group 3 was a control group. Some 3 h after *A. fumigatus* challenge, there was a considerable increase in F_eNO, and a significant difference was observed between the *A. fumigatus* and placebo inhalations. The difference was seen in all study groups. No such differences were found in the levels of F_nNO or nitrite in nasal lavage fluid. Subjects reported significantly more frequent respiratory tract symptoms after the *A. fumigatus* inhalation compared with placebo challenge.

In conclusion, it was shown here that inhalation challenge of *Aspergillus fumigatus* elevated fractional exhaled nitric oxide levels. An increase in fractional exhaled nitric oxide may serve as an indicator of respiratory inflammation of acute mould exposure.

KEYWORDS: Acute mould challenge, *Aspergillus fumigatus*, exhaled nitric oxide, nasal nitric oxide

Worldwide epidemiological studies have indicated that exposure to indoor air microbes present in moisture-damaged buildings is a potential cause of adverse health effects [1]. Although there is a strong association between exposure to mouldy buildings and adverse health effects in the occupants of those buildings, the aetiological factors and mechanisms behind the reported symptoms are still unclear.

An increase in fractional exhaled nitric oxide (F_eNO) concentrations is associated with several inflammatory diseases, such as asthma [2]. Increased fractional nasal nitric oxide (F_nNO) levels have been reported in patients with seasonal rhinitis [3]. In addition, an elevated level of nitric oxide (NO) assessed as nitrite in nasal lavage fluid (NAL) has been detected after occupational exposure to microbes from moisture-damaged buildings [4, 5]. However, no significant differences have been detected in the levels of F_eNO between subjects exposed to microbes from a moisture-damaged building and control subjects [6].

It has been noted that the concentrations of airborne bacteria and fungal spores in moisture-damaged buildings are higher than in reference buildings [7]. Conversely, the cytotoxicity and inflammatory potential of the same microbes can vary between different strains [8], and are also dependent on the conditions that the microbes grow in [9]. Many of the fungi and bacteria isolated from moisture-damaged buildings produce secondary metabolites, which can be hazardous and cause health problems [10]. Among those microbes *Aspergillus* spp. are common toxin producers and they are also opportunistic pathogens, capable of infecting lungs if the host defence of the exposed individual is suppressed [11]. Within *Aspergillus* spp., *Aspergillus fumigatus* has been frequently isolated from the indoor air of moisture-damaged buildings where the occupants have a history of respiratory symptoms [12]. This is of particular interest, as *A. fumigatus* may cause pulmonary aspergillosis with varying degrees of severity [13]. It also produces highly toxic secondary metabolites such as gliotoxin, which can be harmful to health [14].

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The main goal of this study was to assess if a commercial-purified *A. fumigatus* extract had acute effects on FeNO, F_nNO and lung function. In addition, the effects of *A. fumigatus* challenge on the production of NO, assessed as nitrite, in NAL was measured. The results of *A. fumigatus* inhalation were compared with placebo challenge (allergen diluent). Since *A. fumigatus* can cause unexpected health effects, challenging human subjects *via* the inhalation of microbial extracts from moisture-damaged buildings cannot be approved for ethical reasons. Therefore, an extract officially approved by the Finnish National Agency for Medicine was used in this study.

One further area of interest was whether a subject's background was associated with a different response after the challenge. Therefore, the subjects were categorised into three different groups depending on their history: a group with chronic occupational exposure to moisture-damaged building microbes (referred to later as chronic mould exposure), a group of atopic subjects and a control group.

METHODS

Subjects

A total of 28 subjects volunteered to participate in the study. All the subjects were hospital personnel and they were working in two different hospital buildings in eastern Finland. The presence of moisture and mould problems in one of the buildings was confirmed by technical and microbiological investigations. The mean age of the subjects was 45 yrs (27–60). All but one of the subjects were females. The subjects were categorised into three groups as follows. Group 1: 13 subjects working in a moisture-damaged building, who are referred to in the following text as the mould-exposed group; group 2: five atopic nonasthmatic subjects without mould exposure; and group 3: a control group including 10 nonatopic, nonasthmatic subjects with no known mould exposure.

None of the subjects had experienced upper or lower respiratory tract infections during the 6 weeks prior to the study. Those taking any medication were excluded and no hard exercise was permitted during the days when measurements were taken. Smoking was not allowed during the study. Subjects in group 1 had experienced chronic mould exposure for several years. They had normal working days during the study period. Those diagnosed bronchial asthma were excluded. The study was approved by Kuopio University Ethical Committee. The subject characteristics are shown in table 1.

Protocols

Each subject randomly underwent two challenges where they inhaled *A. fumigatus* or placebo solution *via* a nebuliser (Spira elektro 2 flowmeter™; Respiratory Care Center, Hämeenlinna, Finland) using an oronasal mask. The inhalation aerosol was generated from a 1-mL sample reservoir, with the nebulising pressure adjusted to 2 bar (flow rate 7.5 mL·min⁻¹). The nebulisation time was 0.4 s, causing a mean ± SD volume uptake of 6.5 ± 0.3 µL of the solution per breath. The subjects inhaled each solution 60 times using controlled tidal volume respiration during a period of ~5 min, and this was followed by a rest period of 5 min and subsequent spirometric measurements.

Forced expiratory volume in one second (FEV₁) was measured in triplicate before the challenges, and the greatest value was used as the baseline. Four series of 10-fold dilutions of commercial *A. fumigatus* allergen (ALK-ABELLO, Madrid, Spain) were prepared immediately before the challenges and the inhalation was started with the lowest dilution. *A. fumigatus* solution was a purified allergen product accepted to be marketed in Finland by the National Agency for Medicine. The subjects inhaled the allergen at a starting dilution of 0.01 mg·mL⁻¹ until the maximal concentration of

TABLE 1 Subject characteristics

	Group 1 mould exposed	Group 2 atopic	Group 3 controls
Subjects n	13	5	10
Age yrs	48 (40–56)	43 (30–60)	42 (27–57)
Smoking status			
Current smoker	0	1	0
Ex-smoker	2	1	2
Positive skin-prick tests			
Basic series or storage mites	5	5	0
Moulds	1 [#]	0	0
Serum levels IU·L⁻¹			
Total IgE*	57.8 (9.3–112.0)	247.7 (52.2–757.0)	71.4 (6.3–395.0)
ECP levels	14.5 (5.4–26.3)	15.9 (8.0–40.9)	17.9 (7.4–34.4)
Lung function			
FEV ₁ L [*]	2.78 (96)	3.56 (105)	3.44 (108)
FVC L [*]	3.37 (95)	4.16 (100)	3.95 (103)
Diffusion capacity[†] mmol·min⁻¹·kPa⁻¹	8.7 (116)	11.9 (138)	9.3 (120)

Data are presented as mean (range) or mean (%), unless otherwise stated. Ig: immunoglobulin; ECP: eosinophil cationic protein; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity. #: *Geotrichium candidum*; *: p<0.05 between groups (1, 2 and 3) using one-way ANOVA; †: p<0.005 between groups (1, 2 and 3) in one-way ANOVA.

10 mg·mL⁻¹ was administered. FEV₁ was measured 5 min after each inhaled concentration. The difference between the baseline and lowest FEV₁ was observed. The allergen diluent (physiological salt solution) was used as placebo. The placebo challenge protocol was identical to the *A. fumigatus* challenge, although the concentration of the solution did not change during the challenge. The challenges were performed 1 week apart at the same time of the day.

Lung function tests

Standard spirometric values (Medikro 905 Spirometer™; Medikro, Kuopio, Finland) and pulmonary diffusion capacity (Type 2200 Sensor Medics BV™; Sensor Medics, Bilthoven, The Netherlands) were measured according to American Thoracic Society guidelines [15, 16]. Reference values compiled by VILJANEN *et al.* [17] for the Finnish population were used. Baseline levels of spirometry and diffusion capacity were measured before the exposures. Lung function measurements were repeated 24 h after both exposures. The levels of FEV₁ were monitored during the exposures, as described above. Peak expiratory flow (PEF) values were assessed before both challenges. PEF values were observed during the challenges and 3, 6 and 24 h after them.

Symptom questionnaire

After each inhalation of the *A. fumigatus* or placebo solution, the subjects filled in a standardised questionnaire with respect to upper (irritation of eyes, rhinorrhea, blocked nose, sneezing) and lower (cough, phlegm, dyspnoea) airway symptoms. The lungs of the patients were auscultated by a doctor before the exposures and after each inhalation to determine possible wheezing. The patients were asked to report if they had experienced any respiratory symptoms during the subsequent 24 h after the exposures.

Measurement of FeNO and F_nNO

FeNO was measured using a chemiluminescence analyser (Sievers Model 280 NOA™; Sievers Instruments Inc., Boulder, CO, USA), according to the manufacturer's instructions using the same protocol previously described by PUROKIVI *et al.* [18]. When measuring FeNO, the subjects performed a slow vital capacity manoeuvre for 30 s against a fixed expiratory resistance. The pressure level during exhalation was optimised by following the computer screen online to reach a constant flow rate during exhalation. Exhaled air flowed through a nonbreathing valve into a Teflon tubing system connected to the analyser. Recordings were taken using the single-breath program, and they were seen on a computer screen online. The relative SD between three exhaled samples was expected to be <10% and the detection limit for NO was 1 part per billion (ppb). Measurements were made in the same laboratory under constant conditions. The chemiluminescence analyser was calibrated daily by using zero air and a certified concentration of NO.

F_nNO levels were measured by an application of the fixed flow exhalation technique [19]. Two soft well-fitting nose pieces were placed at the entrance to both nostrils. The pieces were attached *via* a "Y" connector to a two-way valve, and a resistor was placed in the exhalation limb, which required a pressure of 10 cmH₂O to produce a flow of 100 mL·s⁻¹. Subjects inhaled

normal room temperature air to total lung capacity *via* their mouths and exhaled nasally while targeting a flow signal displayed on a computer monitor. The expiration was continued until a steady NO plateau, lasting at least 10 s was reached. The contribution of oral NO was excluded. The measurement was repeated three times and the mean value was calculated. FeNO and F_nNO were measured before, and 3, 6 and 24 h after the challenges.

NAL

NAL was performed according to the protocol described by GRAHAM and KORAN [20] with some modifications [4]. A total of 4.5 mL of pre-warmed Hanks' balanced salt solution (37°C) was instilled through a heat-softened catheter into the nare. During the installation process, the subject held his or her chin down toward the chest and held the catheter in place by pinching the nares closed. The cartilaginous bridge of the nose was vibrated with a neonatal percussor (Neo-Cussor™; General Physiotherapy, Inc., St. Louis, MO, USA) while the fluid was refluxed three times. The same protocol was repeated on the opposite nare. The sample was centrifuged (425 × *g* for 10 min) and the cells were resuspended in 2 mL of the supernatant. The remaining cell suspension was incubated for 24 h at 37°C and then centrifuged (425 × *g* for 10 min). The supernatant and cells were frozen at -70°C.

Analysis of NO in NAL

NO in the NAL supernatant was assayed using the Griess reaction, as the stable NO oxidation product nitrite [21], as described in detail previously [4].

Skin prick tests

Atopic status was determined by ALK skin prick tests (ALK laboratories, Copenhagen, Denmark). The prick tests included 14 common environmental allergens (two house dust mites (*Dermatophagoides farinae* and *D. pteronyssinus*), birch, alder, meadow fescue, timothy, meadow grass, mugwort, dandelion, horse, dog, cat, cow and natural rubber), three storage mites (*Lepidoglyphus destructor*, *Acarus siro* and *Tyrophagus putrescentiae*) and 13 mould (*Alternaria alternata*, *Cladosporium herbarum*, *A. fumigatus*, *A. versicolor*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Chaetomium globosum*, *Geotrichium candidum*, *Mucor racemosus*, *Penicillium brevicompactum*, *P. expansum*, *Phoma herbarum* and *Trichoderma viride*). Histamine hydrochloride (10 mg·mL⁻¹) and ALK allergen diluent mediums were used as positive and negative controls, respectively. Atopy was defined as at least one positive reaction with a diameter ≥3 mm.

Blood samples

From serum samples, total immunoglobulin (Ig)E (IU·L⁻¹) was determined by EIA (Immulite total IgE®; Diagnostic Products Corporation, Los Angeles, CA, USA), ECP (IU·L⁻¹) by radioimmunoassay (RIA®; Pharmacia et Upjohn, Uppsala, Sweden) and *Aspergillus fumigatus* IgE (IU·L⁻¹) by UniCAP-FEIA (Model Unicap 100®; Pharmacia AB, Uppsala, Sweden).

Statistical methods

Differences in changes from baseline on the course of time (*e.g.* FeNO, F_nNO, nitrate in NAL and spirometric values) between the *A. fumigatus* and placebo exposures were compared by

variance analysis for repeated measures. Logarithmic transformations were used for variables not normally distributed (FeNO, nitrite in NAL). Means between the groups were compared using one-way ANOVA and mean ranks using the Kruskal-Wallis test. The percentages were compared by using the Chi-squared test. Data are presented as arithmetic means with mean values if not otherwise stated. A p-value of <0.05 was considered significant in all analyses.

RESULTS

Symptoms

Symptoms of both upper (blocked nose, rhinorrhea, sneezing) and lower (cough, phlegm, dyspnoea) airways were more common during the *A. fumigatus* challenge when compared with placebo inhalation, although the differences were not statistically significant (table 2). Instead, during the next 24 h after the challenges, the subjects reported significantly more frequent airway symptoms after the *A. fumigatus* challenge when compared with placebo inhalation (p=0.014). Typical symptoms after the *A. fumigatus* challenge were blocked nose, phlegm and sneezing. There was no difference in the upper and lower airway symptoms reported during the 24-h follow-up period between the study groups. Two of the subjects also complained of fatigue in addition to respiratory tract symptoms after *A. fumigatus* inhalation.

FeNO and F_nNO oxide levels

The baseline levels of FeNO did not differ significantly between the *A. fumigatus* and placebo inhalations (20.4 and 21.7 ppb, respectively). A significant increase in FeNO levels in all subjects was observed after *A. fumigatus* inhalation (p=0.002). Peak levels of FeNO were achieved 3 h after *A. fumigatus* inhalation. No similar responses were detected after the placebo inhalation and there was a significant difference between the challenges (p<0.05). The difference was not dependent on which group each subject belonged to (fig. 1a). When one subject who experienced an asthmatic reaction during the *A. fumigatus* challenge (25% decrease in FEV₁) was excluded, there was still a tendency, but not a significant difference, in FeNO levels between the challenges (p=0.082).

The baseline levels of F_nNO did not differ significantly between the *A. fumigatus* and placebo inhalations (129.5 and 127.0 ppb, respectively). During the 24-h follow-up after the challenges,

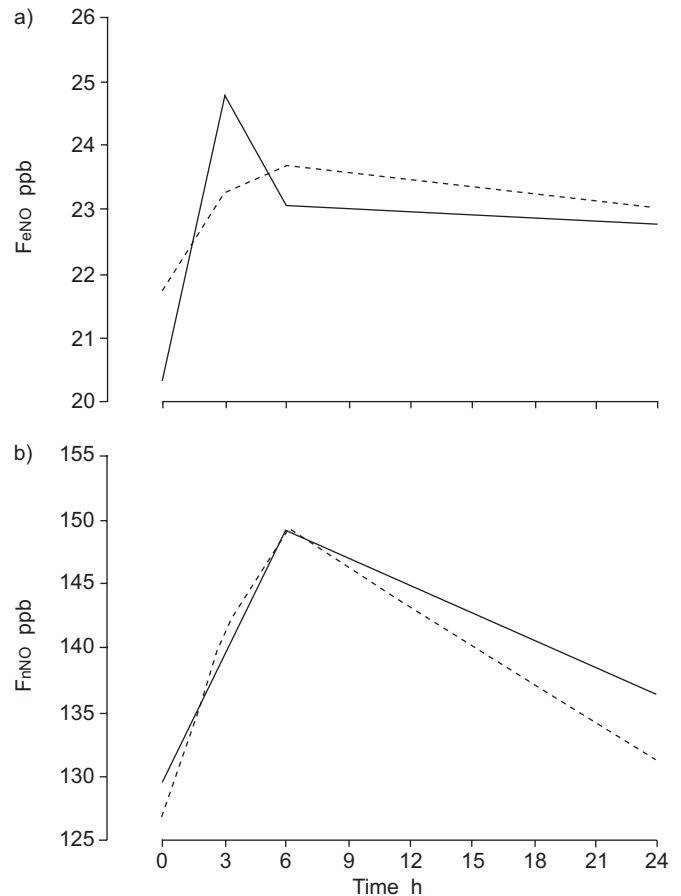


FIGURE 1. The changes in mean levels of a) exhaled (FeNO) and b) nasal (F_nNO) nitric oxide (all of the subjects) after *Aspergillus fumigatus* (—) and placebo (-----) challenges. There was a significant difference between the challenges in FeNO (p<0.05), but not in F_nNO.

the levels F_nNO of all the subjects increased significantly after the placebo inhalation (p=0.003) and there was a tendency to changes in F_nNO concentrations after the *A. fumigatus* challenge (p=0.051). The peak levels of F_nNO after both challenges were measured 6 h after the inhalations. The F_nNO profiles after the challenges were almost identical and there was no significant difference between the challenges (p=0.81; fig. 1b).

TABLE 2 Airway symptoms reported by all subjects during the inhalation challenges and 24-h follow-up			
	<i>Aspergillus fumigatus</i>	Placebo	p-value
Symptoms during the challenges			
Lower airway	17 (61)	11 (40)	0.11
Upper airway	12 (43)	8 (29)	0.27
Lower and upper airway	18 (64)	14 (50)	0.28
Symptoms during the 24-h follow-up			
Lower and upper airway	16 (57)	7 (25)	0.014

Data are presented as n (%).

Nitrite levels in NAL

Nitrite levels in NAL did not change significantly during the follow-up time after *A. fumigatus* or placebo challenges. Moreover, there were no significant differences between the *A. fumigatus* and placebo challenges. However, there was an upward trend in nitrite levels after the *A. fumigatus* challenge but not after the placebo inhalation. There was a wide range of nitrite levels (table 3).

Lung functions

The baseline forced vital capacity (FVC) and FEV₁ % predicted were significantly lower among the mould-exposed subjects when compared with the other groups (p=0.046 and p=0.004, respectively). The baseline pulmonary diffusion capacities were higher among the atopic individuals when compared

TABLE 3 Nitrite concentrations in nasal lavage fluid before and after inhalation challenges

	Before	After		p-value
		6 h	24 h	
<i>Aspergillus fumigatus</i>	2.34 (0.28–10.23)	3.33 (0.22–51.29)	1.88 (0.60–5.13)	0.519
Placebo	2.78 (0.37–12.02)	2.65 (0.68–24.55)	2.10 (0.60–8.71)	0.673

Data presented as geometric μM mean (range).

with the others ($p=0.033$). The diffusion capacities (% pred) of the mould-exposed subjects were normal (table 1).

FEV₁ decreased significantly during the *A. fumigatus* (3.04 to 2.94 l, $p=0.013$) and placebo (3.03 to 2.98 l, $p=0.029$) challenges. There was also a significant difference in FEV₁ changes between the challenges ($p=0.049$), and the changes were not dependent on the group. However, there was one subject who exhibited a decrease of 25% in FEV₁ after *A. fumigatus* challenge and a 12% decrease after placebo inhalation. When this subject was excluded, the difference in changes of FEV₁ between the inhalations did not reach a statistically significant level ($p=0.320$).

Peak expiratory flow values did not change significantly during or after the challenges, and there were no significant differences between the *A. fumigatus* and placebo exposures. No significant changes in spirometric values and diffusion capacity were found 24 h after the exposures when compared with baseline (data not shown).

DISCUSSION

In the present study, the inhalation of *A. fumigatus* solution evoked a significant increase in FeNO levels. The changes in FeNO levels between the *A. fumigatus* and placebo challenges also differed significantly. These findings are in line with an earlier report demonstrating that *A. fumigatus* can stimulate NO production in rat alveolar macrophages [22]. The results presented are also consistent with the findings that NO is increased in the NAL fluid from subjects with occupational microbial exposure, when compared with control subjects [4]. This is of interest since it is well known that FeNO concentrations increase in various inflammatory respiratory diseases, such as asthma and respiratory infections [2, 23]. In a previous study, an elevation in the levels of pro-inflammatory cytokines in induced sputum was observed among occupants of a moisture-damaged building [24]. However, neither nitrite in induced sputum, nor increased FeNO levels were shown to be associated with occupational exposure to indoor air bioaerosols in another moisture-damaged building [6].

Because of the potential unexpected health effects, the extracts from moisture-damaged buildings cannot be used to challenge human subjects. The microbes isolated from moisture-damaged buildings produce secondary metabolites, such as endotoxins, which are known to mediate their inflammatory effects, but may also be cytotoxic or even mutagenic [10]. Therefore, in this study, the subjects were challenged by a commercial *A. fumigatus* solution officially approved by the Finnish National Agency for Medicine. Approval by officials

was considered to be a guarantee of the safety of the inhalation extract.

PAREDI *et al.* [25] have previously shown a late, but not immediate, increase in FeNO concentrations among asthmatic subjects after allergen challenge. In that study, the peak levels of FeNO were achieved 10 h after the challenge. The first measurement point in the present study was 3 h after the challenges, but considering the results of PAREDI *et al.* [25], no earlier FeNO increase could have been anticipated. However, it is worth noting that there were no nonasthmatic subjects in the study by PAREDI *et al.* [25], which was not the case in the current study.

In a previous study, it was stated that NO alone is an insufficient biomarker of exposure to microbes in a moisture-damaged building [6]. In that and several other studies [4, 6, 24], the effects of chronic exposure to moisture-damaged building microbes on NO levels have been assessed. For instance, differences after vacation and occupational exposure have been compared. In the current study, NO levels were measured for 1 day after an acute exposure to only one mould often found in moisture-damaged buildings. Even though NO alone seems to be an insufficient biomarker to assess chronic mould exposure, FeNO may be appropriate for the detection of acute mould exposure.

Baseline FeNO levels were a little higher when compared with studies performed according to European Respiratory Society recommendations [26]. Conversely, the levels did not differ from previous measurements in the same laboratory [18] and the differences may be explained by the ambient NO.

The group which the subjects belonged to was not associated with the changes in FeNO. Atopic status is connected to increased FeNO levels [23], but in this study the baseline FeNO levels of the atopic subjects were not increased compared with the others. Conversely, the baseline FeNO concentrations between the groups are poorly comparable because of the small group sizes in this study. In addition, in an earlier report, those with higher baseline FeNO levels had a minor increase after allergen challenge (including *A. fumigatus*) compared to those with lower baseline values [27]. Thus, a different reaction among atopics could have been anticipated, but it was not found in this study. It is not known if chronic exposure to moulds has any effect on allergen challenge response and no association was indicated.

One of the atopic subjects had an asthmatic reaction during the *A. fumigatus* challenge (25% decrease in FEV₁). After exclusion

of this subject there was not any longer a significant difference in FeNO levels between the challenges, but a distinct tendency was still to be established. The same subject had also a relatively large decrease in FEV₁ after placebo challenge (12%). This refers to bronchial hyperresponsiveness not related only to *A. fumigatus*; however, the decrease in FEV₁ still doubled after the *A. fumigatus* challenge. This subject belonged to the atopic group. It is well-known that some nonasthmatic atopic subjects may have a subclinical asthma not previously detected.

In this study, F_nNO levels increased clearly after both *A. fumigatus* and placebo challenges, and the peak levels were achieved 6 h after the challenges. The F_nNO profiles were very similar after these two inhalations and there was no significant difference between the challenges. The changes in F_nNO can be explained by normal diurnal variation [28]. It has been shown that almost all NO detected in exhaled air originates from the upper airways [29]. The intrinsic level of NO production in lower airways is much smaller and, therefore, FeNO might more easily reflect the possible changes caused by indoor air microbes. Altogether, the inflammatory changes caused by *A. fumigatus* cannot be detected by measuring F_nNO.

In concordance with the detected F_nNO levels, no statistically significant differences in production of nitrite in NAL were found between the *A. fumigatus* and placebo challenges. However, it is worth noting that the nitrite levels in NAL were increased after the *A. fumigatus* inhalation, but not after the placebo challenge, even though the difference in the changes was not statistically significant. There were also some individuals with strong responses after *A. fumigatus* challenge. The ranges in nitrite production of NAL were very large. Possibly the difference in NO of NAL between the challenges could have been detected with a larger number of subjects.

In line with increased FeNO concentrations, respiratory tract symptoms during the 24-h follow-up were significantly more common after the *A. fumigatus* inhalation when compared with placebo challenge in all the studied groups. This finding is also supported by previous studies pointing out that subjects exposed to indoor air microbes more frequently report airway symptoms than their control counterparts [5, 24].

In the current study, the FEV₁ decrease during the *A. fumigatus* challenge was minor, even though there was a significant difference between the challenges in variance analysis. After exclusion of the asthmatic subject, there was no difference in FEV₁ decrease between the challenges. This is in line with a previous study where no significant differences in lung functions were found between occupants with respiratory or rheumatic disorders and their controls, all working in the same moisture-damaged building [5]. Conversely, in this study, the baseline FVC and FEV₁ were significantly lower among the mould-exposed subjects when compared with their controls. Furthermore, it has been noted that among waste collectors exposed to various bioaerosols, the FEV₁ decreased significantly after the 4th working day after vacation [30]. However, because of the small group sizes, the baseline FVC and FEV₁ levels are not comparable in this study.

It has been suggested that exposure to indoor air fungi of moisture-damaged buildings can cause an IgE-mediated

allergic reaction [31]. In the present study, none of the subjects had increased *A. fumigatus*-specific IgE levels, and only one subject in the group of moisture-exposed individuals exhibited a positive reaction to fungus *G. candidum* in skin prick tests. It has been reported that fungal allergy among those exposed in moisture-damaged schools is rare [32]. This suggests that there are also mechanisms other than allergy evoking the respiratory symptoms after exposure to indoor air fungi of moisture-damaged buildings. However, only a few fungal allergens have been accurately characterised with respect to their various morphology and antigenicity, as well as prevalent antigenic cross-reactivity [33].

There was one current smoker and four ex-smokers among the subjects in the present study. Smoking is known to decrease exhaled and nasal NO [34], but after smoking cessation oral NO rises again to normal levels [35]. The smoking subject did not smoke during the days of measurements. Thus, smoking did not have any effects on the results.

In conclusion, the current results support the assumption that inhalation of *Aspergillus fumigatus* solution may cause inflammatory changes in the lower airways. The rapid increase in nasal levels of nitric oxide after *Aspergillus fumigatus* inhalation was not dependent on whether the subjects were atopic or nonatopic or if they were exposed to indoor air microbes of a moisture-damaged building. It is possible that nasal nitric oxide measurement could potentially be applied in the assessment of acute mould exposure.

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