ACOUSTIC RHINOMETRY AFTER A CONTROLLED NASAL CHALLENGE OF WASTE HANDLING WORKERS WITH WASTE AND WASTE COMPONENTS

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Introduction

Occupational exposure to organic dust has been known to induce a wide range of symptoms from the respiratory tract. These symptoms have been reported from industries spanning from agriculture to cotton milling {244, 241, 1367, 553, 461, 687, 128}.

In 1990 a report from a waste processing plant with a high prevalence of respiratory symptoms was published {46}. A high number of molds and airborne bacteria exceeding $10^4/m^3$ was reported. From a plant for recycling of household and industrial waste, 15 workers were examined before work started and one and two years later. During the first year, the number of mold spores was $1.2*10^5/m^3$. Within this time period, two workers developed symptoms of airway inflammation with asthma {201}. A survey of Danish recycling workers included 20 workers from a paper recycling plant, eight compost workers and 44 garbage handling workers {555}. Airway inflammation and toxic pneumonitis were found among 20% of the garbage handling workers. The numbers of airborne microorganisms in the garbage and composting areas were around $10^5/m^3$ as compared to $5*10^3$ in the paper recycling areas. The corresponding numbers of Gram-negative bacteria were $5*10^3$ and $10^1/m^3$.

Laboratory studies with exposure of cell cultures of human lung epithelial cells (A459) to extracts of compost and waste have shown a marked effect in a cytotoxic assay {227}. However, no effect has been demonstrated after exposure to pure endotoxin or β-1-3-D-glucan. Hence, the causative agent in the pathogenesis of the organic dust induced respiratory inflammation has not yet been identified. In a study of the effect of extracts of compost it has been shown that the effect of these extracts increases with the composting cycle up to 5 weeks of composting {282}. This might be an indication of an involvement of moulds in the pathogenesis of these symptoms.

This study is a randomised double blinded cross-over experiment with installation of aqueous extracts of waste and waste dust components into the nasal cavity. The outcome of the exposure is mucosal swelling measured with acoustic rhinometry.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:229-232 (1998)
National Cotton Council, Memphis TN

Materials and Methods

Material

Study subjects:
Earlier studies of recycling workers in Denmark identified workers with and without respiratory symptoms during work at recycling facilities. From each of these groups we enrolled 5 participants. The study subjects were well characterized prior to the experiment. Never the less they were reexamined on a introductory session 14 days prior to the first challenge. Their personal characteristics are reported in Table 1a & 1b.

Exposures

The exposures in this study were 5 ml aqueous extracts of LPS, β-1,3-D-Glucan (Curdlan), *Aspergillus fumigatus* and compost, all in a final concentration of 1 mg/ml. As a sham exposure 5 ml of saline was used. The extracts were stored in tubes with an identification code. This code was known only to the person who blinded the samples. The persons were randomised to the exposures according to the time of entry as seen in Table 2.

Skin prick test

A skin prick test (SPT) was performed to evaluate immediate allergic reactions to a panel of 10 common inhalant allergens (Soluprick®ALK). The panel was extended with allergens from storage mites (Tyrophagus putrescentia, Accarus sciro and Lepidoglyphus destructor), moulds (*Rhizopus nigricans* and *Aspergillus niger*), cow, pig and horse. The extracts were placed on the skin of the forearm in two columns 6 cm's apart. The skin was penetrated with a lancet, and after 10 min the test was read as the greatest diameter. Tests < 3mm in diameter are reported as negative.

Pulmonary Function Testing

Pulmonary function was tested in each worker using a dry spirometer (Vitalograph, Buckingham, UK.). Forced expiratory volume in first sec (FEV_1) and forced vital capacity (FVC) were measured. The testing was performed in accordance with the methods of the American Thoracic Society {433}.

Predicted values of FEV_1 and FVC were calculated using the standards supplied by the Danish Society of Lung Physicians {515}.

Acoustic Rhinometry (NADAR)

Measurements of nasal airway dimensions can be done by a methods that applies acoustic reflections from the nasal cavity, the cross-sectional area of the cavity as a function of the distance into the cavity can be calculated (NADAR: Nasal Area Distance by Acoustic Reflections). This method {1150} has successfully been used to measure the effect on mucosal swelling of Histamine and allergens {1421}.

The total nasal volume was calculated as the sum of the two

nasal cavities, in order to minimize the bias by the cycle of the mucosal swelling.

Challenge Sessions

The study was planned as a randomized double blinded crossing-over study with 5 experimental days, in a controlled environment. Each experimental day was separated at least 14 days from the previous one.

On each day 5 persons were exposed to different exposures. The experiments were performed in our climate chamber. The conditions in the chamber were 20°C, 50% humidity of clean filtered air. At each experimental day the persons were in placed in the chamber 30 minutes before the start in order to get the nasal mucosa acclimatised, Fig 1. At time zero NADAR was performed and a catheter with a cut of tip, Fig 2, was placed in each nostril and inflated until no air escaped during a forced expiration with closed mouth. Nasal lavage was performed and immediately after 5 ml of exposure 35°C was installed into each nasal cavity. The exposures were left for 15 min and thereafter the cuffing was removed and the liquid allowed to escape into a cup and discarded. NADAR was measured at time 0, 30, 60, 120, 180, 240, 300, 360, 660 and 690 minutes. The last NADAR-measurement was preceeded by an installation of Otrivin® at 660 minutes.

Statistics

Tabulation, graphical analysis and Mann-Whitneys tests were carried out with SPSS statistical package {532}. For categorised data, χ^2 test or Fishers exact test were performed.

Results

The total nasal volume (TNV) changed a little after the control challenge with a slight decrease in TNV during the first 3 hours post challenge. However, after 5 hours the TNV was back to normal again. No qualitatively difference was found between the different measures of TNV. However the smallest variation was found for V05T, so this is used for the rest of the analyses, Fig 3.

After challenge with LPS a small non-significant increase in the total nasal volume was seen within the first hour post-exposure. From 2 hours and onwards the TNV was equal to the control, Fig 4. For aspergillus and compost extracts we observed a small non-significant increase in TNV from 4 hours and onwards, Fig 5 and 6. The most pronounced effect was seen after challenge with curdlan where we observed an increase of the TNV during the whole session, with a significant increase after 6 hours, Fig 7.

When we divided the participants into persons with and without work-related respiratory symptoms, we did not find any difference in the TNV-response towards the exposures, Fig 8.

Of the persons in this study all but two experienced som type of symptoms from the nose post challenge. No one experienced more than one incident. The symptoms were mostly a feeling of a pressure in the maxillar sinuses. As can be seen from Table 3 there was a significant increase after challenge with LPS where 50% experienced symptoms compared to 10% after the other challenges.

Discussion

The results show, that after a decrease in the cross sectional area the first 2-3 hours post chalenge, the TNV returns to normal. This initial decrease in TNV might be caused by a hyperaemia occurring after the pressure induced upon the nasal mucosa by the cuffing during the 15 minutes long challenge session. LPS seems only to induce slight changes in the TNV. Curdlan and Aspergillus as well as compost were able to induce an increase in TNV with a maximum after 4- 6 hours. This is contrary to what has been seen after to seasonal allergens among rhinitis patients, where a decrease in TNV has been observed during the season {779}. However, this could be a phenomenon brougth upon by the release of vasoactive cytokines. Possibly these cytokines would lead to an influx of neutrofils during the following 12-18 hours and consequently a lower TNV, as seen in BAL after challenge with organic dusts {776}. During the first 6 hours of the experimental day the participants stayed in the climate chamber to avoid disturbance of the measurements {1420, 778}. Hence, the effects seen after 6 hours could be considered an effect of the exposure.

The exposure in total at each session is comparable to 10 mg which is a high dose compared to the deposited amount of dust components during a normal working day in the normal inhaled volume of 3,84 m^3. With an airborne concentration of 700 ng/m^3 this would mean a total deposition in the nose of 3 µg during the day. However we appllied the dose topically for 15 min only, allowing the bulk of the exposure to leave the nose again meaning that the effect should be considerably lower compared to "normal working conditions". We calculate this effect to be equal to the time of exposure = 15/480 = 0.03. This estimate gives a total comparable exposure to the persons in this study of 300 µg. which is 70 times higher compared to known peak exposures during garbage handling {46}.

In this study we did not find any association between the changes in nasal volume and the symptoms experienced by the participants. Nor did we observe any difference in the prevalence of symptoms between persons with and without work-related respiratory symptoms.

Workers who have contracted symptoms during their work in garbage-handling plants might represent a more susceptible subgroup of the general population and hence they will probably elicit a greater response to exposure to components of organic dust. This was not reflected in the

changes in TNV, since we did not find any significant differences between these groups, Fig 3. This might be different for the release of cytokines from the mucosa, and we are currently investigating the IL1, IL8 & TNFα released into the nasal cavity during the experimental sessions.

In conclusion, this study shows only minor changes in the TNV after installation of extracts of organic dust and dust components. After exposure to curdlan a small, however significant increase is seen in TNV with maximum after 6 hours. For none of the exposures a decrease in TNV was seen as has been described after exposures to known allergens among rhinitis patients.

Acknowledgements

This study has been made possible by grants from the European Biomed Program.

Table 1a. Personal characteristics of the study persons.

Study * persons	Age	Sex	Smoker	Allergy** Prick test
1 LH	44	f	**Yes**	-
2 LM	55	m	**Yes**	-
3 URP	39	f	**No**	-
4 PC	41	m	**Yes**	-
5 ON	62	m	**Yes**	-
6 KB	56	m	**Ex**	-
7 FC	42	m	**Yes**	-
8 EM	46	f	**Yes**	-
9 MO	47	f	**Yes**	-
10 VH	39	f	**Yes**	-

*1-5 :Respiratory symptoms; 6-10: No symptoms
*: Any weal > 2 mm against 12 common aeroallergens (see text).

Table 1b. Personal characteristics of the study persons.

Study * persons	Height m	FEV_1 l	FVC l	PD_{20} mg histamine
1	165	1.95	2.42	-
2	174	3.20	3.95	-
3	167	2.85	3.23	1.00
4	180	4.19	5.02	-
5	176	2.91	3.65	-
6	178	2.52	3.40	0.36
7	183	4.20	5.27	-
8	158	1.40	2.28	0.91
9	167	2.40	3.65	-
10	172	3.10	4.35	-

*1-5 :Respiratory symptoms; 6-10: No symptoms

Table 2. Randomization of the persons under study to the exposures

Session / Study person*	1	2	3	4	5
1 & 6	I	II	III	IV	V
2 & 7	II	III	IV	V	I
3 & 8	III	IV	V	I	II
4 & 9	IV	V	I	II	III
5 & 10	V	I	II	III	IV

*1-5 :Respiratory symptoms; 6-10: No symptoms

Table 3. Nasal symptoms post-challenge according to the exposures.

Exposure	Respiratory symptoms	No symptoms
Control*	0	1
LPS	3	2
Curdlan**	0	0
*A. fumigatus**	0	1
Compost*	0	1

*p < 0.05; * p < 0.1 χ^2 group vs LPS\

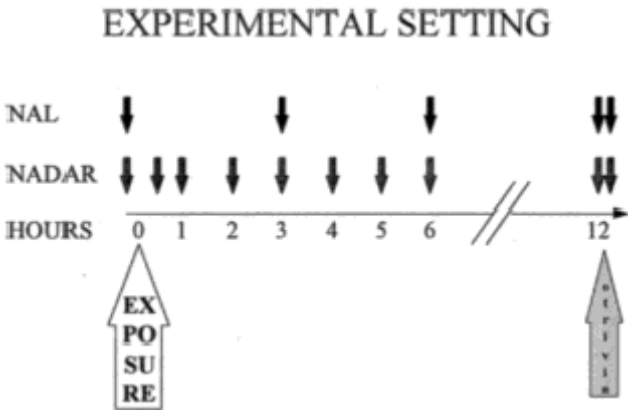

Figure 1. Overview of a day in the climate chamber.

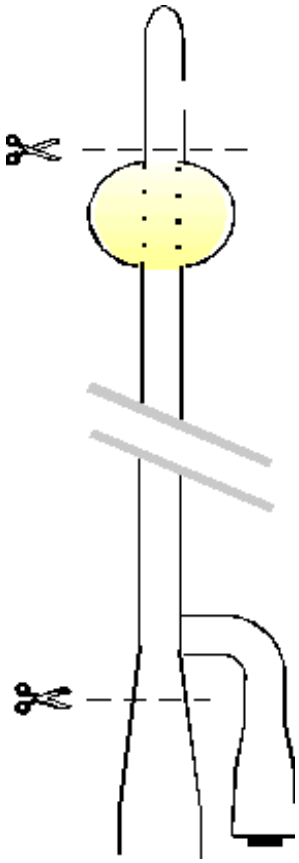

Figure 2. Preparation of the katheter for the nasal installation.

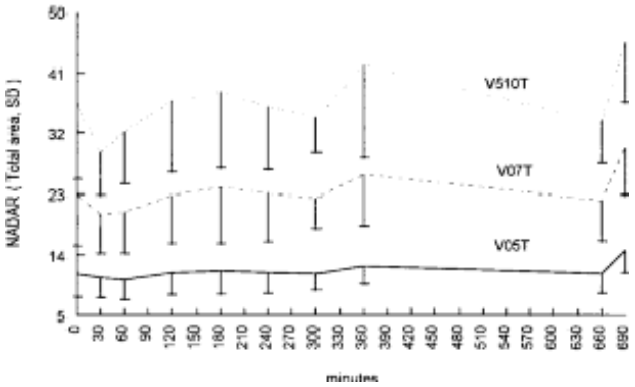

Figure 3. Different measures of total nasal volume

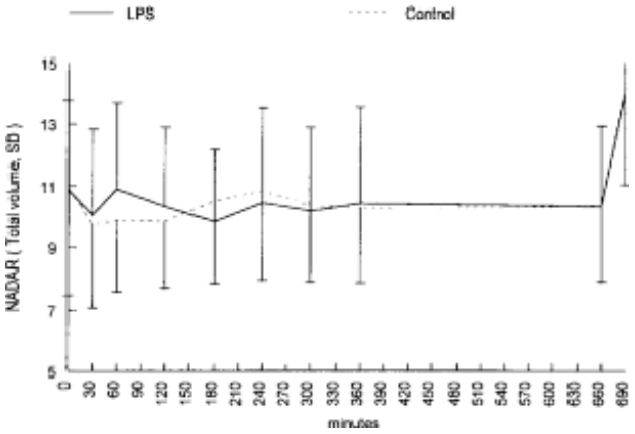

Figure 4. NADAR after exposure to LPS

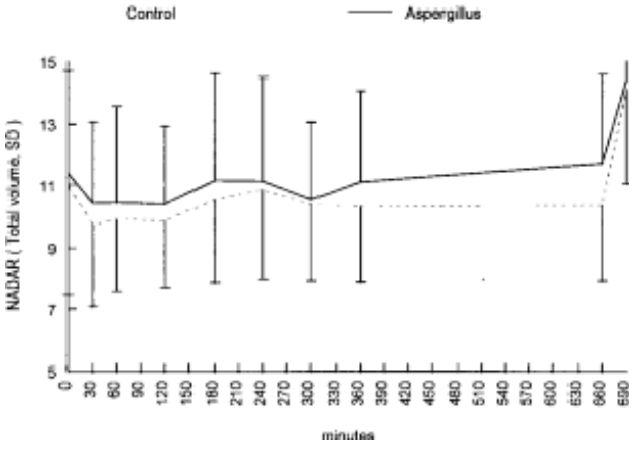

Figure 5. NADAR after exposure to *Aspergillus fumigatus.*

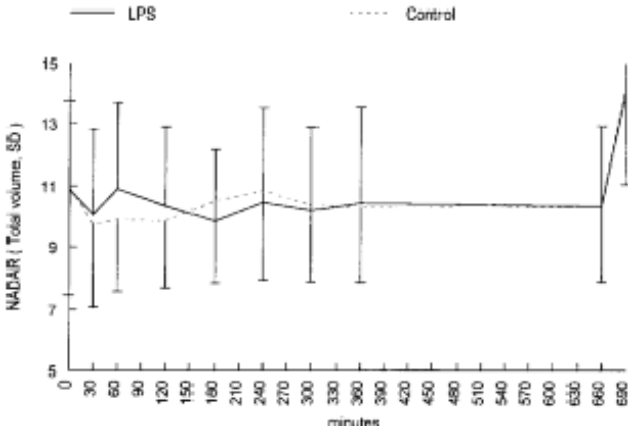

Figure 6. NADAR after exposure to compost.

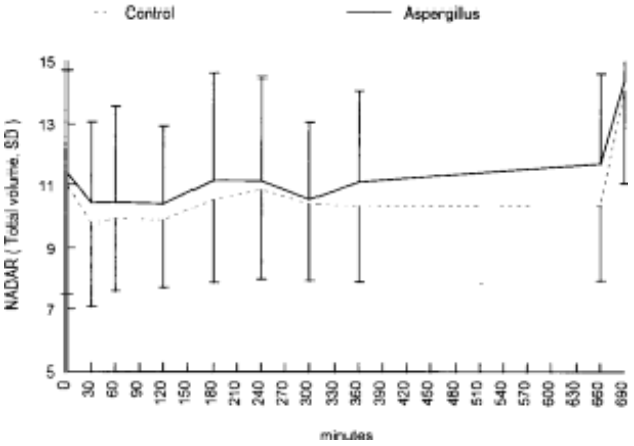

Figure 7. NADAR after exposure to β-1,3-d-Glucan.

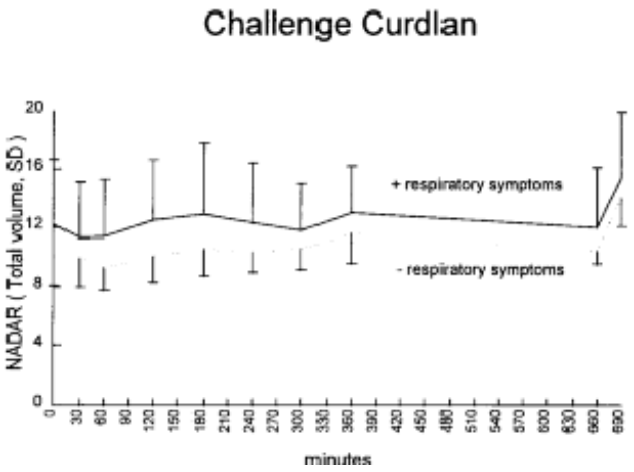

Figure 8. The difference in TNV after challenge with β-1,3-d-Glucan between persons with and without occupationally related asthmatic symptoms.