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β-N-Acetylhexosaminidase (NAHA) as a Marker of Fungal Cell Biomass – Storage Stability and Relation to β-Glucan

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Abstract: Background. Laboratory and field studies have demonstrated that the enzyme β-N-acetylhexosaminidase (NAHA) is a marker of fungal biomass. The purposes of this study was to determine, 1) the stability of the NAHA enzyme on stored filters, 2) the effect of air movement during the sampling of particles and resulting NAHA enzyme activity, and 3) the relationship between enzyme activity and β-glucan concentration. Methods. Replicate air, filtered (0.8 µm pore, cellulose acetate) samples were obtained and stored at room temperature. Then 7 to 12 samples were analysed at 0, 10, 20, 30 or 360 days. Air samples were collected in rooms with no activity, walking or with fan use. The NAHA activity on the filters was measured by adding an enzyme activator and the fluorescence was measured. The β-glucan concentrations were measured using a Limulus-based test with and without solubilisation with NaOH. Results. Storage of filters up to 360 days did not influence the content of NAHA. Movements in the room increased the NAHA values but agitation in terms of fan blowing did not increase the potential to detect differences between rooms with or without fungal growth. There was a relation between NAHA and the non-soluble fraction of β-glucan. Comments: Enzyme measurements of fungal biomass are rapid and easy to perform. The sensitivity and specificity of the method is high which makes it suitable for field use. Incorporation of the small fungal fractions in exposure assessment is important from a health point of view as they have a higher penetration rate into the deep parts of the lung. Summary: The evaluation of the enzyme method to determine fungal growth further supports the relevance of this method to relate to medical effects of fungal exposure.

Keywords: β-Glucan, β-N-Acetylhexosaminidase, Fungi, Inflammation

1. Introduction

Fungi are present everywhere in the environment and will grow as soon as the air humidity exceeds 70% or the material relative humidity exceeds 23%. The risk factor for fungal growth is water damage, either in terms of flooding from the outside or leaking water pipes [1]. Condensation of water in air ducts or on surfaces in humid environments may also increase the risk of growth and hence dispersion of fungi in the indoor environment. A build-up of fungi in a humid room will increase the risk of transfer to adjacent rooms, particularly during the drying period, when the fungal hyphae and spores crack and small, submicron particles are formed.

Exposure to fungi may cause medical problems in terms of irritation in the airways and eyes, difficulties to breathe, itching, and an abnormal tiredness [2, 3, 4]. Some persons may develop an allergy to fungi but this is quite rare. There is a large difference between individuals – some persons may exhibit strong reactions in a location where others may be without symptoms.

Methods to measure fungal contamination are important for house owners, building entrepreneurs, and inhabitants of buildings. Traditional measures of fungal contamination are total spore counts, counting spores on spore traps or air filter samples, or determination of viable fungi on surfaces, air filters or sedimentation plates, using nutrient agar. PCR techniques have also been used, particularly for species determination. Studies have demonstrated important differences between floor dust and air sampling [5]. As the major exposure route is by inhalation, air samples are more representative for risk assessment.

Early data on indoor fungi indicated that outdoor levels were often higher than those measured inside in suspect houses. In those studies the dynamics of indoor air was not taken into account. If one places the measuring equipment in a room and leaves it there during the measuring period, the air is still and the particles sediment to the ground. Outdoors

there is always wind movements, not surprisingly causing higher levels. Appropriate measuring conditions for indoor sampling should thus comprise some activity in the room during the sampling.

There are several biologically active agents in the fungal cell wall which account for the medical effects relating to fungal exposure. β-glucan is a major cell wall constituent and may affect the functioning of the immune system [6]. Chitin and mannan are other agents causing similar effects. Chitin and other agents are allergenic and some fungi produce toxins. Of importance is that the biological activity of these agents remains when the fungal cell is dead. They are also present in fragments of fungal cells that are formed when cells are dried or agitated. The proportion of bioactive agents in such fractions may constitute up 50% of the portion found in larger particles such as whole spores or viable cells [7, 8]. This means that determination of viable fungi may have severe limitations in terms of identifying a risk exposure. Techniques to determine total cell biomass are more relevant for dose estimations.

Several laboratory and field studies demonstrate that the enzyme β-N-acetylhexosaminidase (NAHA) is a marker of fungal cell biomass. Significant correlations to total spore counts were found in air samples and in dust generated from biomass in a bio fuel plant [9]. Strong correlations were found between fungal biomass (gravimetric weight) and NAHA in fungal species grown on nutrient agar and between ergosterol and NAHA activity on gypsum boards [10]. NAHA is also present in certain bacteria and in cells from humans. Data from a field study demonstrated that indoor air levels were closely related to the presence of mould damage in the buildings [11]. At a cut-off value of 20 U/m^3, the presence of fungal growth was identified with a sensitivity of 95% and a specificity of 85%. Above 30 U/m^3 the specificity was 100%.

No data are available on the influence of storage on NAHA on air sampling filters. Several early studies show the need to maintain some activity in the room during sampling and agitated sampling through the blowing with a fan has been suggested [12]. The relation between NAHA, a substance without an effect after inhalation, and β-glucan, a fungal cell wall agents with important effects on the immune system has not been studied. The present project was set up to answer these questions.

2. Material and Methods

2.1. Sample Analysis

Air samples (300 L, flow rate 15 L/min) were taken using open filter holders, preloaded with cellulose acetate filters (Mixed Cellulose Esters, 25 mm PCM Cassettes, 0.8μm pore size, Zefon International, Inc., Ocala, FL, USA). The filters were analysed for NAHA using a fluorogenic enzyme substrate (4-methylumbelliferyl N-acetyl-β-D-glucosaminide, Mycometer A/S, Horsholm, Denmark) which is added to the filter. After an incubation period of around 30 minutes, set by room temperature, a developer is added, and the fluorescence of the liquid is read in a fluorimeter (Picofluor, Turner Designs, Sunnyvale, CA, USA). The units (U) read are divided by 10 to diminish methodological scatter, rounded off to whole units, and expressed as NAHA U/m^3. For the β-glucan analysis samples were taken with Millipore filters (Millipore 0,8 μm, ATTP, Millipore, Mass USA) 300 L with a flow rate of 15 L/min.

2.2. Storage

Parallel filters were sampled in different rooms and the amount of NAHA was determined on one filter. The remainder were stored indoors in room temperature and humidity around 30% under darkness and were analysed after 10, 20, 30, and 360 days. The results were calculated as percentage of the amount on the filter on day 0.

2.3. Air Movements

Sampling was performed in different rooms in villas and apartments with no mechanical ventilation. It was first done under quiet conditions with no movements in the room, then when a person walked around in the room 5-6 times during the sampling period, and finally after agitation. Agitation was performed by blowing air with a fan (Makita BUB 182, 18 V, 0,043 m^3/second, Makita Corp, Anjo, Aichi, Japan). The blower, inducing air velocities of 3.3 meters/sec, was directed towards the floor, the ceiling and the walls in the room for two minutes. After a one minute waiting period to let larger particle sediment, air samples were taken. Sampling was also done before and after cleaning of the rooms.

2.4. β-Glucan Determinations

Parallel filters (n=3) were taken in different rooms in villa and apartment buildings to determine NAHA and β-glucan. For determination of β-glucan the filters were placed in a holder and 1 mL endotoxin free water (LAL, Charles River, USA) was added to one filter. To the other filters NaOH (0.05 or 0.3 M) was added to dissolve the non-soluble fraction of β-glucan. After 10 minutes the fluid was sucked through the filter and added to 2 mL LAL. 25 μL of this fluid was placed into a cartridge for measurement of β-glucan, using a Limulus based analysis equipment (Endosafe Pico, Charles River USA).

2.5. Statistical Analysis

The data were stored and evaluated on SPSS. Differences between groups were evaluated using the chi^2- test and relationships between the groups using Spearman's test.

3. Results

3.1. Time After Sampling

Table 1 reports the amount of NAHA in filters at different times after sampling.

Table 1. *The amount of NAHA (% of the value day 0) in filters at different times after sampling (n = number of samples, SEM = Standard error of mean).*

Day	10	20	30 /	360
n	7	12	11	9
%	101.9	100.1	110.6	95.3
SEM	3.2	7.5	12.6	15.2

There were no indications of a systematic decrease in the NAHA values over time during the first month and a small decline at one year. The variation between different sampling times is probably due to differences in the amount of NAHA on parallel filters – the aerosol is not mono-disperse.

3.2. Air Movements and Cleaning

The NAHA levels during different degrees of movement and the results of cleaning are illustrated in Figure 1.

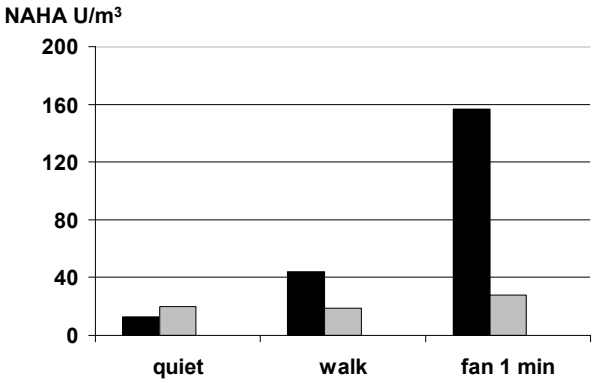

Figure 1. *Airborne NAHA at different degrees of activity in a room during the sampling period, before (black bars) and after (grey bars) cleaning. (Mean values, quiet n=19, walk and fan n= 46).*

When there was no activity in the room, the values were low without significant difference before-after cleaning. Walking increased the amount of NAHA compared to still conditions (p=0.001, paired sample test) and the use of a fan increased the values in relation to walking (p=0.001, paired sample test).The figure also illustrates that ordinary cleaning is an efficient means to reduce the amount of airborne fungi. After cleaning there was no significant increase in the NAHA values when comparing walking with still and fan with walking.

3.3. Fan Agitation During Sampling

Figure 2 shows the per cent increase in NAHA after fan agitation in relation to the values after walking.

The figure illustrates that there was no relation. Amounts lower than 20 U/ m^3, which has been suggested as threshold value for the presence of fungi [11], had the same proportional increase as values above 30 U/m^3. At high values there was a relatively small increase after agitation. Agitation thus yields a higher value than walking, but does not improve the possibility to distinguish between rooms with or without fungi.

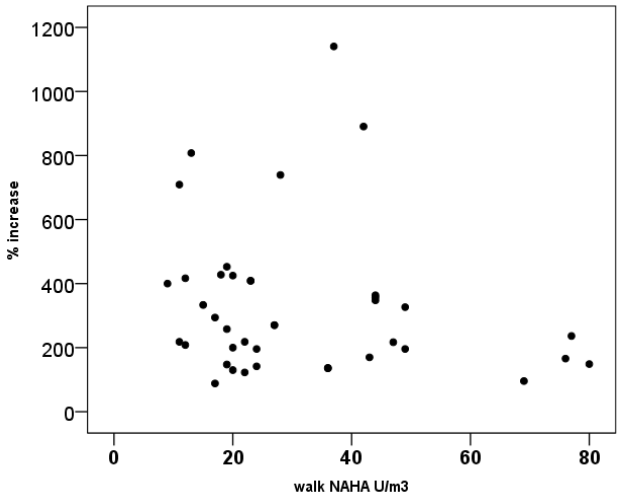

Figure 2. *The percent increase in the amount of NAHA after agitation in comparison to NAHA values measured during walking.*

3.4. NAHA and β-Glucan

The amounts of soluble and non-soluble β-glucan are shown in Table 2.

Table 2. *Soluble and non-soluble β-glucan (µg/mL) in airborne dust samples.*

Fraction	n	mean	SEM
Soluble	18	849	122
0.05 N	12	4509	877
0.3 N	6	60476	19972

These were no statistically significant relationships between the amount of NAHA and the amount of soluble or with 0.05 M NaOH extracted β-glucan. There was a relationship with the β-glucan extracted with 0.3 M NaOH (p = 0.006, Spearman's test). The relationship is illustrated in Figure 3.

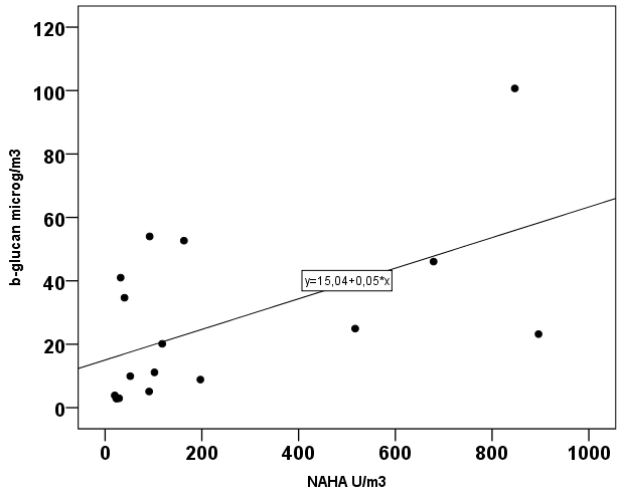

Figure 3. *Relation between airborne NAHA (U/m^3) and non-soluble β-glucan (microg/m^3) extracted with 0.3 M NaOH (p = 0.006, Spearman's test).*

The figure illustrates the relationship which was also present if the results with the high values were deleted (p = 0.018). There were large variations between the different

measurements, probably related to the non mono-disperse aerosol as earlier referred to, and to methodological variations in the method to determine β-glucan.

4. Comments

The main results from the study were the stability of NAHA during prolonged storage, the need to have some air movement during measuring, the absence of an advantage by applying strong agitation during sampling, and a relation between NAHA and the amount of non-soluble β-glucan.

The effect of cleaning on the NAHA levels is interesting from an exposure point of view. It is well known that neglected cleaning may induce airway symptoms among inhabitants. Previously this has been referred to as "dust allergy" but the underlying mechanism is probably an inflammatory response induced by β-glucan from fungi accumulated in the house dust.

The additional information obtained in this study illustrates the potential of the NAHA method to sample fungal contamination in a building with a high accuracy. Enzyme measurements of fungal biomass are rapid and easy to perform. As with all methods measuring fungi in air, there are certain limitations. The data obtained relate to conditions at the moment of measurement. From a building construction point of view, the method will not detect hidden sources of fungi which are without direct connection to the room where the sampling is performed. Measurements in rooms with a high humidity can also give low values even if there is visible fungal growth. This is due to the adherence of the fungal particles to the surface due to humidity, contrary to the conditions in dry rooms or where air dryers have been used. This will increase the proportion of fungal particles that can be aerosolized.

The relation between NAHA and β-glucan is important as it justifies the use of NAHA measurements to investigate the presence of symptoms related to fungal exposure. The results demonstrate that solubilisation with NaOH must be performed to obtain the correct value for β-glucan. Several previous studies have investigated the relation between the presence of symptoms among children and the amounts of β-glucan in the dust. Some of these studies reported the exposure in terms of soluble β-glucan [13]. This represents a severe underestimation of the exposure which can explain the weak relation between exposure and symptoms reported.

Previous studies have found relationships between NAHA and medical parameters. Higher levels of NAHA have been found in homes of patients with sarcoidosis [14] and nocturnal asthma [15] in comparison to control subjects. A relation has been reported between NAHA levels in bedrooms in bedrooms and the spontaneous secretion of IL-12 from peripheral blood mononuclear cells from patients with sarcoidosis [16]. Another study found a relation between NAHA levels in bedrooms and the amount of β-glucan in broncho-alveolar lavage [17].

In conclusion air sampled NAHA does not decrease in activity during storage of the filters. Agitating room air does not improve the possibility to detect high levels of NAHA, and there is a relation between NAHA and the amount of non-soluble β-glucan.

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References

[1] Singh J. Building mycology. E&FN Spoon London 1994, pp 1 – 326.

[2] Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. Env Health Persp 2011,119,748-756.

[3] Sauni R, Uitti J, Jauhiainen M, Kreiss K, Sigsgaard T, Verbeek JH. Remediating buildings damaged by dampness and mould for preventing or reducing respiratory tract symptoms, infections and asthma (Review) Evid Based Child Health 2013; 3:944-1000.

[4] Quansah R, Jaakkola MS, Hugg TT, Heikkinen SA, Jaakkola JJ. Residential dampness and molds and the risk of developing asthma: systematic review and meta-analysis. PLoS One 2012; 11: e47526.doi:10.137 t/

[5] Reponen T, Seo S-C, Grimsley F et al. Fungal fragments in moldy houses: a field study in homes in New Orleans and Southern Ohio. Atmos Environ 2007,41,8140-8149 .

[6] Rylander R. Organic dust induced pulmonary disease – the role of mould derived β-glucan. Ann Agr Env Med 2010,17,9-13 .

[7] Seo S-C, Reponen T, Levin L, Grinshpur SA. Size-fractionated (1-3)-β-D-glucan concentrations aerosolized from different moldy building materials. Sci Tot Env 2009,407,806-810 .

[8] Adhikan A, Reponen T. Rylander R. Airborne fungal cell fragments in homes in relation to total fungal biomass. Indoor Air 2013, 23,142-147 .

[9] Madsen AM. NAGase activity in airborne biomass dust and relationship between NAGase concentration and fungal spores. Aerobiol 2003, 9,97-105.

[10] Reeslev M, Miller M, Nielsen KF. Quantifying mold biomass on gypsum board: comparison of ergosterol and beta-N-acetylhexosaminidase as mold biomass parameters. Appl Environ Microbiol 2003, 69,3996-3998.

[11] Rylander R, Reeslev M, Hulander T J. Airborne enzyme measurements to detect indoor air mould exposure. Environ Monit 2010,12,2161-2164.

[12] Reeslev M, Nielsen JC, Miller M, Rogers L. Aggressive sampling – improving the predicted value of air sampling for fungal aerosols. Manuscript

[13] Holst G, Host A, Doekes G, Meyer HW, Madsen AM, Sigsgaard T. Determinants of house dust endotoxin and β-(1-3)-D-glucan in houses of Danish children. Indoor Air 2015; 25:245-249.

[14] Terčelj M, Salobir B, Rylander R. Fungal exposure in homes of patients with sarcoidosis – an environmental exposure study. Env Health 2011,10,8-13.

[15] Terčelj M, Salobir B, Narancsik Z, Kriznar K, Grezetic-Romcevic T, Matos T, Rylander R. Nocturnal asthma and domestic exposure to fungi. Indoor+Built Env 2013,22,876-880.

[16] Terčelj M, Salobir B, Zupancic M, Wraber B. Rylander R. Inflammatory markers and pulmonary granuloma infiltration in sarcoidosis. Respirology 2014,19,225- 230 .

[17] Terčelj M, Stopinšek S, Ihan A, Salobir B, Simčič S, Rylander R. Serum IL-10 in patients with sarcoidosis is supressed at high levels of fungal exposure. Pulm Med, 2014,dx.doi.org/10.1155)2014/164565.