Nasal mucosal reactivity after long-time exposure to building dampness

STIG RUDBLAD
Photo on the front page:
Measurement of nasal mucosal swelling with rhinostereometry after histamine provocation.
ABSTRACT

An association between working and/or residing in damp buildings and respiratory health has been reported in a number of studies. A major limitation has been difficulty in objectively verifying any effects on the mucous membranes of the respiratory tract in order to explain symptoms of irritated eyes, nasal congestion and cough that are often reported by occupants in buildings with indoor air problems. The main aim with this thesis was to objectively study changes in the nasal mucosal reactivity after long-time exposure to a deteriorated indoor climate.

Twenty-eight teachers who had worked for at least five years in a recently renovated school, which for years had had severe moisture problems, were randomly selected to participate in this study. Eighteen teachers randomly selected from another school, with no known moisture problems, formed the control group (in 1995). Although remedial measures had been taken, an increased prevalence of mucous membrane irritations was still reported by the teachers from the target school. A nasal challenge test with three concentrations of histamine (1, 2 and 4 mg/ml) was used. Recordings of the swelling of the nasal mucosa were made using rhinostereometry. The analysis of the mucosal swelling induced by the three concentrations of histamine showed a significant difference in the growth curves of the two groups, indicating that long-time exposure to indoor environments with moisture problems may contribute to mucosal hyperreactivity of the upper airways.

A study comparing students who began their high-school studies at both schools in 1995 and the teachers was performed regarding mucosal reactivity, frequency of atopy and symptoms. A nasal histamine provocation test and a skin-prick test were administered to 45 students from each school. They also answered a standardized questionnaire. The teachers had significantly greater mucosal histamine reactivity than the students, compatible with an age-related pattern of mucosal reactivity. The students had significantly higher frequency of allergic sensitization. In 1997 the nasal histamine provocation test was repeated among the teachers. This showed that the teachers from the repaired water-damaged school still demonstrated an increased reactivity to histamine compared to those in the control school, but the differences between the growth curves of the provocation tests were less than in 1995. No major differences were observed in the technical investigation between the two schools and the measurements were all within the range of values usually seen in schools in northern countries.

In a longitudinal study the students were followed during their high school studies. They underwent a nasal histamine provocation test and answered a questionnaire on three occasions, in 1995, 1996 and 1997. No significant differences in the nasal histamine provocation curves between the students at the target school and those at the control school could be shown from the start to the end of the study period. Nor were there any differences concerning perceived indoor air or mucosal symptoms between the target group and the control group. Based on both technical and objective medical measures, this study indicated that the indoor air in the remediated moisture-damaged school did not exert an irritant effect on the upper airway mucosa of the students.

In 2000, six years after remedial measures had been taken, the teachers underwent a nasal histamine provocation test. In addition to using mucosal swelling as a measure of mucosal reactivity, we also examined the mucosal microcircular reaction to histamine provocation with Laser-Doppler flowmetry (LDF). We found that the difference in nasal histamine reactivity between the two study groups, measured as mucosal swelling, was no longer significant. However, Laser-Doppler flowmetry showed a significant difference between the two teacher groups regarding microcircular perfusion and CMBC (concentration of moving bloodcells), indicating a more pronounced plasma leakage and oedema from the nasal mucosa upon histamine provocation among the target school teachers.
In conclusion, we found a restored nasal histamine reactivity, measured as the mucosal swelling reaction, among the teachers six years after long-time exposure to building dampness. LDF showed remaining changes in the microcircular pattern of the target school teachers. Consequently, long-time exposure to building dampness may increase the risk for hyperreactivity of the upper air-ways. This acquired hyperreactivity may last for years and decrease only slowly, even after the indoor climate has been properly improved. A possible explanation for this slowly decreasing reactivity might be a slow but ongoing restoring process in the mucosa of the upper air-ways.

It is of importance to determine if residing in bad indoor environment implies a risk of future health problems. Following a group of people exposed to building dampness with objective mucosal tests over several years provides knowledge about how long and in what way the increased mucosal reactivity persists. It is important to identify both predisposed people and particular risk environments.

**Key words:** indoor environment, histamine provocation, nasal mucosa, mucosal reactivity, rhinostereometry, moisture, Laser-Doppler.
The difficulty in most scientific work lies in framing the questions rather than in finding the answers.
(A.E. Boycott)

We should make things as simple as possible, but not simpler.

(Albert Einstein)
LIST OF PAPERS

This dissertation is based on the following original publications, which will be referred to in the text by their Roman numerals:


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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CMBC</td>
<td>Concentration of moving blood cells</td>
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<td>FEV$_1$</td>
<td>Forced expiratory volume (during 1 second)</td>
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<td>LDF</td>
<td>Laser Doppler flowmetry</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
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<td>RSM</td>
<td>Rhinostereometry</td>
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<td>SAR</td>
<td>Seasonal allergic rhinitis</td>
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<td>SBS</td>
<td>Sick building syndrome</td>
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<td>SPT+</td>
<td>Skin prick test positivity</td>
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<tr>
<td>SPT-</td>
<td>Skin prick test negativity</td>
</tr>
<tr>
<td>TVOC</td>
<td>Total concentration of volatile organic compounds</td>
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<td>VOC</td>
<td>Concentration of volatile organic compounds</td>
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INTRODUCTION

Background

Poor indoor air quality has been discussed, but also rejected by some authors, as one of the factors related to the increased prevalence of allergies and asthma in the Western world [1, 2, 3]. Allergens from furry pets are ubiquitous in public buildings, including schools [4, 5], and the amount of allergens in school dust is sufficient to cause increased symptoms in sensitized persons [6]. However, there are no obvious indications that exposure to these environments actually increases the incidence of atopic diseases [1, 3].

General symptoms such as headache, mental fatigue and difficulties concentrating as well as non-specific airway symptoms are frequently reported by residents and/or employees in polls regarding indoor environments. This variety of inconveniences is often referred to as symptoms of “sick building syndrome” (SBS) [7, 8].

Many occupants of buildings attribute irritative mucosal airway symptoms, such as eye irritation, nasal blockage and cough, to a bad indoor climate [9]. These complaints frequently emanate from people working in non-industrial buildings such as offices, schools and day-care centres [10, 11, 12]. In an overhaul of 220 Swedish schools during the period 1978-1997, obvious maintenance deficiencies were demonstrated, with poorly functioning and polluted ventilation systems, water leakages and building dampness [13]. Taking measures to rectify verified insufficient indoor ventilation when SBS-symptoms have been reported has sometimes resulted in a rapid reduction of perceptions and symptoms attributed to SBS [14]. However, there are also indications that an increased nasal mucosal reactivity due to long time exposure to a deteriorated indoor climate may last for years even after moving out of the problem area, although residents no longer complain of irritative symptoms [15, 16]. An association between working and/or residing in damp buildings and symptoms of mucosal irritation from the upper and lower airways has been reported in a number of studies [17, 18, 19, 20].

Symptoms included in SBS are common in the general population, and are of multifactorial origin related to personal, occupational, and residential factors [21]. However, in many of these environments with reported SBS-symptoms, there have been difficulties in verifying a relationship between measured low-level indoor air pollution and reported symptoms [22, 23, 24]. The indoor climate is complex and symptoms are influenced by physical factors, such as emissions from damp building materials, ventilation, and variation in temperature, as well as by different psychosocial factors [21, 25, 26]. The prevalence of reported
symptoms is also related to age, sex and allergic constitution of the occupants [27].

Studies on work-related symptoms in non-industrial buildings have i.a. shown a high frequency of reported nasal mucosal symptoms [28, 29, 30]. Nasal symptoms, such as nasal irritation, stuffiness and runny nose are, however, common among otherwise healthy individuals, and there is no clear-cut boundary between normal physiology and inflammatory disease such as rhinitis [31].

There is a growing interest in objectively verifying the effects of a deteriorated indoor climate on the mucous membranes of the respiratory tract. Furthermore, there seems to be a need for increased knowledge about the physiological and pathophysiological changes that take place in the mucosa of the airways during these conditions. The nose, as compared to the lungs, is easily accessible, but it is also a quite complicated organ.

**Nasal mucosal reactivity**

Behind the nasal valve, the site with the narrowest cross-sectional area of the nose, the cavity of the nose expands and the turbinates hang down from the lateral wall (figure 1). Approximately 12 m³ of air passes through the nose each day. The turbinates increase the mucosal surface and, together with the narrow valve, contribute to a turbulent flow of inspired air that results in extensive contact between the inspired air and the mucosal surface. This contact makes it possible for inspired air to reach proper moisture and temperature conditions on its way into the lower airways. The nose also has the function of a filter where inhaled foreign particles are deposited (mainly >10µm) and prevented from passing to the lower airways [32].

Upon exposure to irritants, the nasal mucosa react with symptoms like itching, sneezing, nasal discharge and/or nasal congestion. Irritant stimuli give the experience of itching, propagated afferently by trigeminal nerves and resulting in a spasmodic inhalation that is followed by an explosive expiration caused by contraction of the accessory respiratory muscles [33]. Sneezing clears the throat, nose and mouth. Nasal discharge is primarily caused by a mixture of plasma exudation and glandular secretion and serves as an important defence mechanism upon inflammatory stimulation in the airway mucosa [34, 35].

An inflammatory reaction in the nasal mucosa also results in nasal congestion due to dilatation of the deeper situated sinusoidal vessels, which increases the blood volume in the nasal mucosa [36, 37]. Another factor that contributes to nasal congestion might be extravasation of fluid and the consequent interstitial oedema
Studies on nasal inflammatory conditions have predominately focused on allergic rhinitis [39, 40], which is the most common allergic manifestation [41]. However, it was found in a questionnaire study, that 21% of a rural and urban population in Sweden was suffering from non-allergic nasal complaints [42]. Furthermore, among persons who develop symptoms of chronic rhinitis the proportion with a non-allergic aetiology increases steadily with age and exceeds 60% after the fifth decade of life [43]. Besides the obvious association between allergic rhinitis and asthma there also seems to be a strong association between non-allergic rhinitis and asthma [44, 45, 46].

The term nasal hyperreactivity is often used to describe hyperreactive symptoms caused by various non-specific irritants such as smoke, odours and dust. Hyperreactivity is of central importance and is usually present in allergic as well as non-allergic rhinitis, although with variable intensity [47]. The pathogenesis of nasal hyperreactivity is not known but a greater permeability of the nasal mucosa has been discussed as a contributing factor [48]. An increased sensitivity of the mucosal sensory nerve endings may also play a role [49, 50].

**Figure 1.** Schematic drawing of the lateral wall of the nasal cavity showing the narrow nasal valve and the extension of the turbinates.
Assessment of nasal mucosal reactivity

Inflammation of the nasal mucosa evokes nasal obstruction, and measurements of nasal patency constitute an objective measure of how open the nose is. Rhinomanometry, a method which indirectly measures nasal airway resistance, has previously been the main objective method of quantitating nasal obstruction [51]. Acoustic rhinometry, which uses acoustic reflection to assess the geometry of the nasal cavity, is a method that has been increasingly used in environmental studies [52, 53]. This method has, for example, been applied to show that a low air exchange rate in schools may affect the airways and cause a swelling of the nasal mucosa [54].

An airway-provocation test is a method to study the dynamic course of mucosal reactivity when exposed to allergens or non-specific irritants. In the field of bronchial pulmonology clinical methods are well established for estimating the degree of bronchial hyperreactivity. These methods include provocation tests with histamine and methacholine followed by spirometric recordings of forced expiratory volume (FEV\textsubscript{1}) or measurement of peak expiratory flow (PEF) [55, 56].

Nasal provocation tests, using histamine or methacholine, have been used in different surveys to study nasal mucosal reactivity [57, 58]. However, these provocation tests have not been particularly suitable for routine clinical work because the nasal mucosa is very sensitive and easily affected by internal and external disturbances. Nevertheless they have been helpful as a research tool in selected investigative set-ups [59, 60].

Histamine, a major mediator in the allergic reaction, exerts a broad action on the nasal mucosa causing congestion, rhinorrhea and sneezing in atopic as well as non-atopic subjects [61]. Histamine acts directly on cellular histamine receptors, which cause vasodilation, oedema formation and exudation of plasma to the airway lumen and indirectly, via reflexes, which accounts for sneezing and watery hypersecretion [62]. In one study it was shown that unilateral nasal histamine provocation caused ipsi-lateral obstruction and increased the patency of the contra-lateral nostril, the latter possibly by a neural reflex [63]. However, in other studies the nasal mucosal swelling reaction upon histamine provocation has been shown to be ipsi-lateral with no effect on the contra-lateral side [15, 64, 65].

Nasal hyperreactivity can be demonstrated by a nasal histamine provocation test. However, these tests cannot differentiate a normal from a diseased population as efficiently as an inhalation test in asthma. Therefore, the descriptive term increased nasal reactivity seems more adequate than nasal hyperreactivity in
order to differentiate a more reactive group of subjects from a control group. The main problem is the recording of the mucosal reaction. Recordings have been made using symptom scores, counts of sneezes, or quantitative or qualitative analyses of nasal discharge [60, 61].

Rhinomanometry and acoustic rhinometry have also been used in nasal histamine provocation tests measuring nasal obstruction as an indirect measure of mucosal reactivity [58, 59]. In a study of methods for assessment of nasal histamine reactivity these two methods were found comparable for measuring mucosal changes [66]. In the same study nasal peak flowmetry, a simple and clinically useful method, was found more sensitive to mucosal changes than the other methods studied.

Another method, rhinostereometry, has lately been used to record the degree of mucosal swelling after nasal histamine provocation. It is an optical, non-invasive and direct method that exclusively studies changes in the nasal mucosa [67, 68]. In our studies rhinostereometry has been used for recordings of mucosal swelling after histamine provocation.

**Nasal mucosal blood flow**

Arterial blood enters the nasal mucosa via arteries forming anastomoses along their course [69, material and methods: fig 4.]. The arteries ramify into smaller arterioles controlling the blood flow of the mucosa. [70] Because they control nasal blood flow, the arteries are referred to as resistance vessels. The arterioles end in a capillary network arranged in subepithelial and glandular zones.

The blood from the capillaries is drained via postcapillary venules, which seem to be the site of the inflammatory, mediator induced, increase in permeability to macromolecules [71]. Larger collecting veins then drain into the deeper situated sinusoids, which are especially well developed in the mucosa of the turbinates [69]. The venous sinusoids drains primarily through specialized throttle veins in bony canals in the turbinate bone. Arterial blood can also reach the sinusoids by way of arteriovenous anastomoses, and there are indications that control of nasal mucosal congestion may depend on the balance between filling of the tissue via arteriovenous anastomoses and drainage of the tissue through the throttle veins [72].
Nasal mucosal blood flow during histamine provocation

Vascular changes in the nasal mucosa occur during various pathophysiological conditions. Histamine locally injected into the mucosa of the inferior turbinate increases the blood flow, measured with the 133 Xe wash-out method, in allergic as well as non-allergic subjects [73]. Histamine solution applied locally on the nasal mucosa also increases the blood flow in healthy volunteers, as recorded with laser Doppler flowmetry (LDF), extending the previously established vasodilating properties of the substance [74].

Another effect of histamine on the nasal mucosa is to increase the permeability of microvessels [75]. The sites of plasma leakage have been determined to be in the small postcapillary venules. In an animal study using the hamster cheek pouch model, histamine provocation was found to provide a rapid onset of increased vascular permeability peaking at 5 minutes and returning to normal after 25 minutes [76]. These observations roughly correspond to a study where allergic and healthy subjects were exposed out of season to nasal histamine provocation [77]. The mucosal microcirculation was recorded with LDF and the vascular leakage (interstitial oedema) was calculated as the fall in the parameter CMBC (= concentration of moving blood cells). Following provocation there was a significant decrease in CMBC in the allergic subjects with the minimum at 8 minutes which was not seen in the normal subjects. This was interpreted as the development of a transient interstitial oedema by the allergic subjects in response to the histamine provocation. In our fifth paper we used the combination of rhinostereometry (RSM, see above) and LDF making it possible to record nasal mucosal congestion and microcirculation simultaneously.

Mucosal signs and symptoms in relation to building dampness

The importance to respiratory health of building dampness and mould growth in houses has been the focus of substantial interest, and it has been concluded that there is a consistently increased risk of respiratory symptoms among people living in houses with dampness problems and mould growth [78, 79, 80]. It is usually possible to classify the severity of the problem simply by local inspection [81]. There is evidence in the literature, that building dampness increases the prevalence of asthmatic symptoms [82, 83, 84]. However, there are no reliable indications that exposure to moisture and/or mould in damp buildings leads to sensitization and development of immunoglobulin E (IgE) mediated mould allergy [85].

Some studies indicate that microbial or chemical exposure related to building dampness could influence the nasal mucosa. Increases in inflammatory
biomarkers in nasal lavage were observed in occupants of a building with pronounced microbial growth in the building structure [86].

The relation between mould exposure in schools and respiratory symptoms has been investigated in some studies. Attending a school with moisture damage and mould growth was found to be related to asthmatic symptoms in the pupils [17, 87]. Among school employees, exposure to building dampness at school has been associated with lower respiratory tract disorders [88, 89] and pathophysiological effects on the nasal mucosa [89, 90]. In a study on school personnel, a lower degree of nasal patency and increases in inflammatory biomarkers in nasal lavage were found when there were higher concentrations of total moulds in classroom air [91].

There are indications that the water content in building materials may have an effect on the emission in the air, either due to microbial growth or to chemical degradation of the material [92, 93]. This is in accordance with an increased risk of respiratory tract symptoms among occupants in houses with moisture problems with or without the presence of mould [94, 95]. Furthermore, a thorough renovation in moisture-damaged schools has resulted in a decrease in the prevalence of respiratory symptoms among personnel and pupils [96, 97].

In summary, there is reliable evidence that exposure to building dampness is associated with mucosal signs and symptoms from the upper and lower respiratory tract.

**Atopy and allergy**

A revised nomenclature for allergy was presented in a position paper [98]. Atopy is defined here as a personal or familial tendency to produce IgE antibodies in response to low doses of allergens and to develop typical symptoms such as asthma and rhino-conjunctivitis. This means that IgE sensitization per se (e.g. skin prick positivity) is not a criterion for atopy. The term atopy should be reserved for the combination of IgE sensitization and typical allergic symptoms. Furthermore, allergy is defined as a hypersensitive reaction initiated by immunological mechanisms and can be either IgE-mediated or non-IgE-mediated.

In our first two papers we used skin prick test positivity and atopy as synonyms, which is frequently the case in many studies [99, 100]. However, this new nomenclature was applied starting with the third paper.
**Hyperreactivity in allergic rhinitis during and out of season**

Nasal non-specific hyperreactivity is an important feature of allergic rhinitis [101]. However, there is some controversy regarding the way in which this hyperreactivity changes during and out of season. Konno et al. found no differences in nasal histamine reactivity among seasonal allergic rhinitis (SAR) subjects during off season compared with normal subjects [102].

In two consecutive studies on SAR patients 19 to 43 years of age, increased and unchanged nasal histamine reactivity was found during and out of season compared to controls [103, 104]. This was interpreted as an indication of continuous mucosal inflammation regardless of season in these patients. In another study, where the degree of nasal mucosal inflammation was estimated by the concentration of exhaled nitric oxide (NO), an increased inflammatory reaction was found during the non-pollen season among allergic rhinitis subjects as compared to controls that further increased during the pollen season [105]. Reasons for the discrepancies in these studies might depend on the sensitivity of the measuring method, the intensity and duration of the allergic disease and/or the inclusion criteria for the allergic subjects. Thus there seems to be a need for further studies in this field.

**Indoor air quality questionnaires**

If information is to be gathered from a large number of employees or residents, a questionnaire is a useful aid. Indoor air problems are of multifactorial origin, and methods facilitating the diagnosis phase are of vital importance in solving indoor air problems. When investigating SBS, questionnaires should be used to collect structured information from occupants concerning their perception of environmental conditions, psychosocial factors and symptoms [106].

A questionnaire widely used in the Nordic countries, which also includes items concerning the psychosocial work environment, is the MM-questionnaire [107]. The MM-questionnaire is used for studying the respondent’s experience of the quality of the indoor air and the conditions at the workplace, as well as SBS symptoms attributed to the work environment. The respondent’s experience of the indoor environment and reported SBS-symptoms can be used to help determine necessary technical measurements as well as remedial measures to improve the environment in buildings with problems. The usefulness of this questionnaire has been confirmed in some recent studies [108, 109]. We used two different versions of the MM-questionnaire in our studies, MM040NA for the teachers and MM060NA for the students. Both versions have identical questions about perceived indoor climate and symptoms.
AIMS OF THE INVESTIGATIONS

The overall aim of this thesis was, by using objective methods, to elucidate if long-time exposure to building dampness may lead to an increased mucosal reactivity of the upper airways. The purpose was also to study the dynamic changes in the nasal mucosa when exposed to repeated histamine provocations.

The specific aims were the following:

1. to study if long-time exposure to building dampness contributes to an increased nasal mucosal reactivity measured as mucosal swelling upon repeated histamine provocations

2. if so, to examine if this increased reactivity remains after remedial measures have been taken

3. to elucidate if nasal mucosal histamine reactivity among students attending senior high school differs from that of adult teachers

4. to determine if there is a difference in the frequency of IgE sensitized (“skin prick test positive”) subjects between the students and the adult teachers

5. to evaluate if nasal mucosal histamine reactivity increases among the students at the target school during their three years of study compared to students at the control school

6. to investigate if there is a difference in nasal histamine reactivity among atopic compared to non-atopic students out of season

7. to study the changes in mucosal microcirculation, measured with laser Doppler flowmetry, in response to nasal histamine provocation and find out if there is a difference between teachers at the target school and teachers at the control school

8. to examine if there is a correlation between the nasal mucosal swelling reaction and the nasal microcirculation, measured with laser Doppler flowmetry, when subjects are exposed to repeated nasal histamine provocations
MATERIAL AND METHODS

The study schools

The target school was constructed in the 1960s as a single storey building with flat roofs and an inlet and exhaust ventilation system and was situated close to a river that usually overflowed in the spring. Moisture damages were reported shortly after the construction period. Technical investigations performed in the beginning of the 1990s showed very severe moisture problems because of inadequate drainage and numerous water leaks from the flat roof causing growth of mould and discolouring of building materials. Measurements from insulation material in the ventilation system showed a substantial growth of mould of different species (e.g. cladosporium, penicillium, alternaria, and paecilomyces).

No systematic measuring programme was followed, but available measurements during the wintertime of relative air humidity (22-27%), airborne microorganisms (low levels) and chemicals in the air (total volatile organic compounds, TVOC $\approx$ 50 $\mu$g/m$^3$) showed no deviations from what is usually seen in Swedish schools [110]. The ventilation flows were within the range stipulated by the Swedish regulations and most CO2-measurements showed concentrations below 1 000 ppm (parts per million).

The control school was composed of four 3-4-storey buildings built between 1890 and 1930. Most classrooms were naturally ventilated during the 1980s, with very low air exchange rates, and carbon dioxide levels during the lessons occasionally exceeded 2 500 ppm. No moisture problems were reported. Extensive remedial measures were taken in both schools in 1993-94.

Study populations

1. Studies I, II and V represent different phases of a longitudinal study of teachers. A random sample of teachers who had worked at least five years at the water-damaged school (target school) before the renovation (39 persons), and all teachers in the control school who fulfilled the same inclusion criteria (30 persons), were invited to participate in the study. Study I was performed in the spring of 1995. Twenty-eight and 18 teachers, respectively, agreed to take part and formed the two study groups. Those who did not participate usually indicated lack of time for the examination during the limited period of the study as their reason for not doing so. In study II, which was performed in the spring of 1997, 26 teachers from the target school (one woman had died and one declined to participate because she was breast-feeding her baby) and all 18 teachers from the control school participated in the investigations. Study V was carried out in the...
spring of 2000. Twenty-four teachers from the target school and 16 from the control school agreed to participate. Reasons for not participating were mainly practical, i.e. a change of school.

2. In study IV a random sample of 180 students (90 from each school) who were beginning their high-school studies were asked to participate in the study. Forty-five students from each school agreed and formed the study groups from the target school and the control school.

In order to estimate the possibility of a selection bias, all of the initially randomly selected 100 students (50 from each school) who refused participation were offered a simplified test procedure. As a result of this measure, a total of only eight students in this initially selected group provided no information at all. The first investigations were performed in the fall of 1995 (about two months after the start of the term). The next two investigations were done in the fall of 1996 (38/38 participated) and the fall of 1997 (38/35 participated). In 13 cases the reason for non-participation was that the students no longer attended the school in question. In no case were health reasons reported as the reason for leaving school or changing schools. One student who had participated in 1995 but not in 1996 also took part in 1997.

Thus, 37 students from the target school (37/45, 82%) and 35 students from the control school (35/45, 78%) participated in all investigations during the study period including nasal histamine provocation tests.

3. Study III comprised a comparison between the study populations of teachers and students in 1995 (presented above).

**Questionnaires**

Surveys of all teachers in the target school and the control school were conducted, using a standardised questionnaire (MM040NA [107]), before (1989) and after (January 1995) the renovation. The same MM-questionnaires were answered in February/March 1995 by all teachers (about six weeks before the start of the study) and used for analysis of the representativity of the test groups. The questionnaire contained questions about the perceived indoor climate, symptoms often referred to in indoor climate research, allergic manifestations, and some background factors.
In the tables in our papers, perception of poor indoor air quality means often troubled (at least once a week) by dry air, stuffy bad air, or unpleasant smell. In some tables we have used the single factors (dry air, stuffy bad air and unpleasant smell).

In January 1995 (Table 1) the teachers in the target school still reported an increased frequency of mucous membrane irritations, while there was a slight decrease in the perception of stuffy bad air and a marked reduction of perceived unpleasant smell and complaints about dust compared to the outcome of the survey of 1989. The prevalence of dry air was high in 1989 and even higher in 1995. The teachers in the control school reported improved indoor air after the ventilation system was changed.

In all our studies, participating subjects answered a questionnaire in direct connection with the investigation. The teachers answered the same standardized questionnaire mentioned above and the students a somewhat modified one (MM060NA, [107]). In 1996 (study IV) the students answered two additional questions in order to determine the current prevalence of allergic symptoms (Have you had allergic eye or nose symptoms (itching, sneezing, runny eyes/nose) during the past twelve months? Have you had asthmatic symptoms during the past twelve months?).

**Table I.** The frequency of perceived indoor air quality, mucosal symptoms and general symptoms among teachers in the target- and control schools in 1989 and in January 1995.

<table>
<thead>
<tr>
<th></th>
<th>Target school</th>
<th>Control school</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 131</td>
<td>n = 138</td>
</tr>
<tr>
<td>Perception of dry air (%)</td>
<td>33</td>
<td>54</td>
</tr>
<tr>
<td>Perception of stuffy bad air (%)</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>Perception of unpleasant smell (%)</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Perception of dust and dirt (%)</td>
<td>47</td>
<td>25</td>
</tr>
<tr>
<td>General symptoms (%)</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Mucous membrane irritation (%)**</td>
<td>28</td>
<td>27</td>
</tr>
</tbody>
</table>

* The frequency of teachers often (every week) troubled by tiredness, feeling heavy-headed, headache, nausea, vertigo or concentration problems.

** The frequency of teachers often troubled by irritation from eyes, nose, throat or cough.
Rhinostereometry

Rhinostereometry (RSM) evolved primarily as a method for direct measurement of changes in nasal mucosal congestion with special reference to the reactivity of the vascular bed of the mucosa [Figure 2, 67]. The method is non-invasive and permits standardization of the nasal swelling reaction, without interference from secretion or anatomical disparities. The rhinostereometer (RHINOMED, Sweden) consists of a surgical microscope, with a small depth of focus, placed on a micrometer table fixed to a frame. The microscope can be moved in three orthogonal directions defining a three-dimensional co-ordinate system. The nasal cavity is placed in the co-ordinate when the subject bites down on an individually adapted tooth splint fixed to the frame. In this way the nasal cavity resumes the same position with a high degree of precision in repeated measurements. The nasal mucosa is examined via the eyepiece of the microscope. The ocular is equipped with a horizontal mm scale making it possible to measure changes in the congestion of the nasal mucosa. The accuracy of the method is 0.18 mm, that is to say the apparatus measures any movement of the mucosa exceeding 0.18 mm.

RSM permits measurements of all visible parts of the nasal mucosa. The structure mainly responsible for the perception of nose blockage is the head of the inferior turbinate which can undergo large variations in the degree of swelling. This region is part of the valve area, which is considered to play a major role in the development of nasal obstruction [111, 112]. Moreover, the inferior turbinate (or choncha) represents a highly reactive part of the nasal mucosa [64, 113].

Figure 2. Rhinostereometer. The surgical microscope in front of the self-retracting nasal speculae and the individually formed tooth-splint.
Laser Doppler flowmetry

Laser Doppler flowmetry admits continuous registrations of relative changes in tissue blood flow in a non-invasive manner [114, 115]. The method utilizes the reflection of laser light from various components of the tissue studied. Light reflected from moving structures, such as blood cells, undergoes a frequency shift (the Doppler effect). By analysing the frequency distribution in the reflected light, one can make relative calculations of blood flow in the tissue. The concentration of moving blood cells (CMBC) can be calculated by analysing the amount of Doppler-shifted light. The average velocity of the moving blood cells can also be calculated from the degree of frequency shift. The product of CMBC and velocity of flow is the perfusion or flow.

The LDF probe, specially designed (PERIMED, Sweden), emitting the laser light and collecting the reflected light, is introduced into the nasal cavity and placed close to the surface (0,1-0,3 mm) of the mucosa of the inferior turbinate (figure 3, figure 4). The measuring volume (depth) is influenced by tissue properties and light source wavelength [116]. The wave-length of the laser beam was 780 nm. LDF was performed using a PERIFLUX 4001 (PERIMED, Sweden) and the signal was fed into an IBM compatible computer using PERISOFT software program.

Figure 3. Rhinostereometry and laser Doppler flowmetry. The probe is introduced into the right nasal cavity (left). The microcircular flow (from top to bottom): perfusion, total back scatter (= a control function), CMBC and velocity is registered on a computer screen (right).
The provocation test

We selected histamine as the provocation substance because of its documented ability to evoke mucosal swelling in rhinostereometric studies of nasal mucosal reactivity [15, 64]. We used a histamine dihydrochloride solution with no preservatives to avoid the potential risk of causing a double challenge effect [117].

The provocation test in study I was performed as a single blind procedure at a near-by hospital. In studies II, IV and V the provocations were carried out in a reception room at the respective schools. The subjects were allowed to acclimatize during 30 minutes, after which they were challenged every 10 minutes with 0.14 ml of histamine dihydrochloride in rising concentrations (1, 2 and 4 mg/ml), using the same standard nasal provocation procedure as described by Hallén and Juto [65, 118]. The challenge substance was applied to the medial side of the right inferior concha with a syringe, while the left inferior concha was unprovoked and served as a control. Only if the initial rhinoscopic investigation
showed pronounced right septal deviation was the left inferior turbinate used for the provocation.

Recordings of the mucosal swelling were made with rhinostereometry in both nasal cavities 5 and 10 minutes after each challenge (in study V recordings were also made 2 minutes after challenge with the lowest histamine concentration and only the right nasal cavity was recorded as earlier studies showed only marginal mucosal changes on the contralateral side during provocation). In study V, directly after histamine provocation and recording of the mucosal congestion with rhinostereometry the LDF probe was placed in position. As soon as there were stable recordings of perfusion, CMBC and velocity on the computer screen, these were saved for later analysis. Each time, a 10 to 20-second recording was saved. Laser Doppler registrations were made 2, 5 and 10 minutes after provocation with the lowest histamine concentration (1mg/ml), and 5 minutes after provocation with the remaining histamine concentrations (2 and 4 mg/ml).

In study I the subjective perceptions of nasal blockage during histamine provocation (not specified to the provocation side) were recorded on a four-point scale where zero meant no blockage and three severe blockage. The amount of secretion observed by the investigator on the provoked side was rated as none, sparse, covering the medial part of the anterior choncae, or covering the choncae with wide septal contact, also on a four-point scale. Number of sneezes were recorded by the investigator.

**Skin prick test**

In 1995 (studies I, III and IV) a skin prick test with a standardized panel of allergens, often used in Scandinavia (ALK, Copenhagen), was performed after the histamine challenge.

The following allergens were tested: pollen (birch, timothy and mugworth), mites (d. pteronyssinus and d. farinae), furry animals (cat, dog and horse) and moulds (claudosporium, alternaria and aspergillus fum.). Skin prick test positivity (SPT+) was defined as a wheal with a diameter of at least 3 mm [119] (for further information see previous heading “Atopy and allergy”).

**Exposure measurements in the study schools**

Exposure measurements were performed, although not systematically, before remedial measures had been taken in the study schools (and also before the start of our studies), and these were mentioned earlier (under the heading “The study schools”).
After the renovations, exposure measurements were performed in both schools on three occasions. To reflect the possible influence of outdoor climate on the indoor conditions technical measurements were done at different times of the year. The first series of measurements were performed in spring 1996, the second in autumn 1996 and the third in winter 1997.

Representative rooms were chosen and the measurements performed were identical on the three occasions. The following factors were studied (described in more details in study II):

- total concentration of airborne dust (sampling time 7 am to 5 pm during three consecutive work-days)
- particle size distribution of airborne dust
- indoor air temperature
- relative humidity in indoor air
- concentration of carbon dioxide (continuously during at least three days)
- concentration of formaldehyde (24 hours)
- concentration of volatile organic compounds (14 days)

**Technical investigations of the students’ home environments**

In the longitudinal student study (study IV) 81 of the 90 homes were examined (39/42, respectively, of the target group and control group environments) by experienced building engineers. Dropouts were due mainly to a recent move to a new home, difficulty in finding a suitable time during the limited time of the investigation or, in a few cases, unwillingness to participate. Moisture- and mould damage in different rooms and spaces was noted, and a question was posed as to whether there was condensation on the inside of bedroom windows in the wintertime, which is an indication of insufficient ventilation.

Room temperatures and relative humidity were measured in 76 of the homes (36/40, respectively). The additional dropout here was because of technical problems. The average indoor temperature in each student’s home was measured during the heating season over a period of approximately 30 days. Relative humidity in the indoor air was determined in parallel with the temperature.

**Statistical methods**

For the continuous outcome variables we used an analysis of variance model for repeated measurements (an ANOVA model) in studies I, II, III, IV and V. The basic formulation of this model aimed at modelling the growth curves for the variables as a function of the provocation concentrations of 1, 2 and 4 mg/ml of the histamine solution. The model was applied with somewhat different
specifications but the core specification had one main exploratory factor or ‘between subjects’ factor, i.e. the study group factor. This factor was school (target school or control school) that classified the individuals (teachers or students) in the two groups. In study III a further classification was used since both school and person group (teachers or students) were analysed simultaneously.

In addition to the group factor there were ‘within subjects’ factors, i.e. factors for which there were repeated measurements for the subjects. These factors were dose (1, 2 and 4 mg/ml of histamine solution), time (5 and 10 minutes after provocation), and in studies II, IV and V also year of investigation (1995, 1997 and in study V 2000).

Some additional factors to control for a potential confounding were also tested in the model: age, sex, smoking, and status of allergy or atopy. To facilitate the understanding of the rather complex ANOVA model we used a slight reformulation that enabled the estimation of the average increase in swelling per \( \log \) mg/ml histamine (actual doses 1, 2 and 4 which give the logarithmic values 0, 1 and 2) in studies II, III and V. This is equivalent to an estimate of the slope in the linear regression of mucosal swelling on \( \log \) provocation level, restricted to the interval 1-4 mg/ml histamine solution.

Correlation analysis of the continuous variables was done with Pearson’s as well as Spearman’s correlation coefficients in study V.

The binary outcome variables were analysed with Fisher’s exact test in study II, the chi-square test in study IV, logistic regression in study IV, and two reformulations of the common logistic regression model into a generalised version with repeated measurements in studies I and V.

The statistical models were implemented in the statistical packages SAS, version 8.1 (modules GENMOD and MIXED), BMDP, version 7 (procedure 5V), EPILOG, version 3 and StatXact, version 5.
RESULTS

The main results in the studies (studies I-V) are briefly summarized as follows.

Nasal hyperreactivity among teachers in a school with a long history of moisture problems (Study I)

Personal characteristics and symptoms
Analysis of personal characteristics (table II) showed that the test groups were fairly representative of the total populations of personnel at the two schools. The teachers at the target school complained more about the quality of the indoor air than those at the control school and also reported a higher prevalence of mucous membrane symptoms.

Table II. Personal characteristics and mucosal and general symptoms in the two groups of teachers and the total populations of employees in the two schools answering the questionnaire in 1995. The frequency of positive skin-prick tests in the two study groups is also shown.

<table>
<thead>
<tr>
<th></th>
<th>Target school</th>
<th>Control school</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study group</td>
<td>Total¹</td>
</tr>
<tr>
<td></td>
<td>n = 28</td>
<td>n = 129</td>
</tr>
<tr>
<td>Sex (% men)</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>51</td>
<td>48</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Allergic disease (%)²</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Employment (years)</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Poor Indoor air quality³</td>
<td>57</td>
<td>66</td>
</tr>
<tr>
<td>General symptoms (%)⁴</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Mucous membrane irritation (%)⁵</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Positive prick test (%)</td>
<td>18</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Total means the whole group of personnel answering the questionnaire in Feb./March1995.
²Reported cumulative incidence of hay fever and asthmatic symptoms.
³The prevalence of teachers often troubled by dry air, stuffy bad air, or unpleasant smell.
⁴The frequency of teachers often troubled by tiredness, headache, feeling heavy-headed, nausea, vertigo or concentration problems
⁵The prevalence of teachers often troubled by irritation from eyes, nose, throat or cough.
The provocation test
The results of the provocation test are shown graphically in figure 5. The mucosal congestion was calculated as the deviation from the baseline. The ANOVA model showed significantly different growth curves in the two study groups (p < 0.01) with respect to the provoked side of the nose. On the control side, only slight mucosal congestion was recorded around the baseline.

![Mucosal Swelling Graph](image)

**Figure 5.** Histamine provocation curves (the mean of net change in mucosal swelling from baseline on provoked and unprovoked sides) in target-school and control school. The significance of the difference in slope between the two provocation curves is shown (p<0.01).

Local reactions
The prevalence of local reactions (nasal blockage, secretion and sneezing) during the histamine provocations was registered. For nasal blockage we obtained no differences in trend but there was a borderline statistical significance for the two groups with respect to the general level of perceived nasal blockage, p=0.06, with higher scores for the target school. With respect to secretion we observed no difference in level of the scores but there was a difference in trend for the target school as compared to the control school, p=0.03. (The scores between 2 and 4 mg/ml did not increase in the control school.) For sneezing, no statistically significant results were found. If the analysis is done on the whole group of teachers there is a statistically significant increase in the perception of nasal
blockage and registered nasal secretion with increasing histamine concentration (p<0.01, not shown in the paper).

**Skin prick test**
Eighteen percent of the teachers at the target school and seventeen percent of those at the control school had a positive skin prick test (table 2). Allergy to birch pollen was most common (13%), while no allergy to mites or moulds was detected in the two schools.

**Slowly decreasing mucosal hyperreactivity years after working in a school with moisture problems (Study II)**

**Perceived indoor climate and symptoms**
Differences in the outcome for the two test groups analysed separately for each year (1995 and 1997) resulted in significance only for perception of dry air in 1995 where the target school had a significantly higher prevalence (Fisher’s exact test, p=0.0002).

There was a significant improvement for the target school compared to the control school (Fisher’s exact test, p=0.03), although 31% of the teachers still complained about perceived dryness of the air. No symptoms showed significant improvement and most individuals reported the same outcome in 1995 and 1997.

**Provocation test**
The mean congestion of the nasal mucosa for the two provocation tests (1995 and 1997) and the two groups are presented graphically in figure 6. Using the ANOVA model we estimated the slope in the regression of mucosal swelling on the provocation level under two different hypotheses: the first assuming that the difference in slopes was constant between 1995 and 1997, and the second that a change had occurred. Based on the results of these two hypotheses we concluded that there was still a difference between the two schools, but the difference appeared to be decreasing. It will later be shown that this finding also fits well into the corresponding findings in study V.
**Figure 6.** The histamine provocation curves (mean values) for the two groups in 1995 and 1997. Readings for 5 as well as 10 minutes after provocation are shown.

**Technical measurements**

The total concentration of airborne dust was found to be low in all classrooms and at the same level as found in dwellings. Continuous measurements during several days showed that the concentration could vary considerably during the day. When the classroom was unoccupied the concentration was very low, less than 5 \( \mu \text{g/m}^3 \), whereas the concentration momentarily could reach values of approx. 100 \( \mu \text{g/m}^3 \) when persons entered or left the classroom.

The concentration of compounds in the air that are generally associated with water-damages in the construction (n-butanol and 2 ethyl-1-hexanol) were below the detection level (1µg/m³).

The ventilation systems were not equipped with humidifiers and therefore the relative humidity indoors was mainly a function of temperature and relative humidity outdoors. The highest values (55%) were recorded in the autumn and the lowest (30%) in the winter. When a classroom was occupied, the steady state concentration of carbon dioxide during a lesson exceeded the recommended highest level, 1000 ppm (AFS, 1993), in 25% of the classrooms in the target school and in 33% of the classrooms in the control school. The concentrations of volatile organic compounds (VOCs), of total volatile organic compounds
(TVOC), and of formaldehyde were low and well within expected levels. Individual organic compounds were also those that would be expected considering the type of construction and surface materials used.

**Nasal mucosal histamine reactivity among young students and teachers, having no or prolonged exposure to a deteriorated indoor climate (Study III)**

**Study population**

Table III shows a few characteristics of the students and teachers in the two schools. The proportion of boys was higher in the target school than in the control school, but this simply reflected the gender ratio in the two schools (47.9% versus 33.4% boys in the target and control schools, respectively).

**Table III.** Some personal characteristics of the students and the teachers at the two schools.

<table>
<thead>
<tr>
<th></th>
<th>Target school (students)</th>
<th>Control school (students)</th>
<th>Target school (teachers)</th>
<th>Control school (teachers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% males)</td>
<td>46.7</td>
<td>28.9</td>
<td>46.4</td>
<td>66.7</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>16.1</td>
<td>16.1</td>
<td>51.4</td>
<td>50.6</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>15.6</td>
<td>13.3</td>
<td>7.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

A description of the randomly selected teachers (study I) and students (study IV) has been reported in the papers. There was no bias in the selection of teachers or students to participate in the histamine provocation study according to reported allergy, mucosal symptoms or positive skin prick test.

**Reported allergy and symptoms**

The reported prevalence of allergic disease was similar in students and teachers in the target school, but the teachers in the control school reported a somewhat lower prevalence. However, the overall difference between students and teachers was not significant, p=0.52. Teachers had a higher frequency of mucosal symptoms (21.9%) than students (10.0%), but the difference was of only borderline significance, p=0.07.
Skin prick test
The frequency of skin prick positivity (SPT+) and the prevalence of mucosal symptoms are shown in table IV. The total frequency of SPT+ among the students was much higher than in the adult teachers (35.6% versus 17.4%, p=0.03) and in good accord with results from other studies of young people [120]. Allergens from pollen and furry animals dominated.

Table IV. The prevalence of symptoms and frequency of atopy (positive skin prick test) in students and teachers at the two schools.

<table>
<thead>
<tr>
<th></th>
<th>Target school (students) n = 45</th>
<th>Control school (students) n = 45</th>
<th>Target school (teachers) n = 28</th>
<th>Control school (teachers) n = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms (according to questionnaire):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma/hayfever (%)</td>
<td>27.9</td>
<td>24.4</td>
<td>25.0</td>
<td>11.1</td>
</tr>
<tr>
<td>Mucosal symptoms (%)</td>
<td>8.9</td>
<td>11.1</td>
<td>25.0</td>
<td>16.7</td>
</tr>
<tr>
<td>SPT+ (%)</td>
<td>35.6</td>
<td>35.6</td>
<td>17.9</td>
<td>16.7</td>
</tr>
</tbody>
</table>

The provocation test
The results of the histamine provocation test on the provoked side of the nasal cavity in the students and teachers are given in figure 7. Only small oscillations around the baseline were seen on the control side. The ANOVA analysis showed a statistically significant difference of the main factor teachers versus students, p=0.0001, with students having much lower values than teachers (on average, about half the teachers’ values).

The first ANOVA model with all main factors and interactions showed that the interaction group*concentration level was highly significant, p=0.008, which indicated that the four groups had different provocation curves. Figure 7 shows that the statistical significance is due to two features, i.e., the curves are different in the two groups of teachers, and those of the students are much lower than those of the teachers.

Since we found no difference in any respect between students in the two schools, they can be viewed as a homogenous group. Our analysis of each school showed that the provocation curve of the target school teachers had consistently higher values than that of the students, p=0.0001, but its slope and shape were similar, p=0.15. In the control school, however, there was a difference between the slopes of the curves that was of borderline significance, interaction (category)* (provocation concentration), p=0.07.
Figure 7. Histamine provocation curves of the teachers and students at both schools.

A separate analysis of differences between atopic and non-atopic teachers as well as between atopic and non-atopic students showed no differences in the provocation curves.

The rhinoscopic examination of the teachers showed that 15 had a dry and crusty mucosa and 31 did not. No significant difference was found between teachers in the target school and those in the control school (10/28 and 5/18 respectively, \( p > 0.50 \)). Among the students less variation was noted in the rhinoscopic findings and only one had such a dry and crusty appearance on rhinoscopic examination.

Among the teachers we found a definite statistical significance for the crusty appearance, \( p = 0.002 \), in the analysis of baseline values and in the analysis of the provocation curves (\( p = 0.0004 \)) (Figure 8). However, and more importantly, the significant difference between the target and control schools in the latter analysis did not disappear with the introduction of a crusty appearance in the statistical model since we obtained \( p = 0.002 \) for difference in slopes. Furthermore, in a later analysis (Fisher’s exact test), not included in the primary study, we found that the teachers with a rhinoscopically dry and crusty mucosal appearance complained more frequently of irritative mucosal symptoms (33%/16% respectively) and skin symptoms (53%/7%, respectively) than those without this appearance. The difference between the two groups is significant regarding skin symptoms (\( p = 0.004 \)).
Figure 8. Histamine provocation curves for teachers with a rhinoscopically dry and crusty appearance compared with those without this appearance.

Nasal histamine reactivity among adolescents in a remediated moisture-damaged school – a longitudinal study (Study IV)

Study population
Of the initially randomly selected 100 students in 1995, 54 took part in the histamine provocation study and 46 declined to participate. Data (skin prick test and questionnaire) were obtained from 38 of these 46 students who refused the provocation test. There was no bias in the selection of students who participated in the histamine provocation study according to reported allergic manifestations (24.5% (13/53) and 28.9% (11/38), respectively), mucosal symptoms (11.1% (6/54) and 7.9% (3/38), respectively) or positive skin prick test (33.3% (18/54) and 28.9% (11/38), respectively). The participation rate was higher for girls than for boys (61% (33/54) and 29% (11/38), respectively).

Technical investigation of home environments
Students at the target school more often came from the suburbs and lived more often in multi-family houses. Their homes less often had natural ventilation, condensation on the bedroom windows in wintertime and a high internal moisture supply (difference between the absolute water content in indoor and outdoor air), all of which are indicators of better ventilation than in the homes of students at the control school.
There were, however, no important differences in the homes of the students at the target and control schools with respect to room temperatures or relative humidity.

**Questionnaires**
Complaints about variable and low room temperatures were more frequent among the students at the target school during the years of the study (1995, 1996 and 1997), while the perception of air quality was similar at the two schools. The prevalence of mucosal and dermal symptoms was low and fairly similar in the two schools and did not change during the study period. No significant differences regarding the perceived indoor air climate or mucus membrane irritation were seen between atopic and non-atopic students, and we did not observe any statistical interaction between school and atopy.

**Skin prick test**
The frequency of skin prick test positivity among the students was 36% at each school (16/45). In 1996, 70% (19/27) of the SPT+ students reported symptoms of nasal allergy and/or asthma during the past year. During the same time, 18% (9/49) of the SPT- students reported symptoms of nasal allergy, while there were no reports of asthma.

**Provocation test**
The results of the provocation tests are shown in figure 9. In the figure, the swellings for 5 and 10 minutes after provocation are averaged so that the figure is easier to interpret, and therefore the time factor is not shown. The ANOVA with outcome values for 1995, 1996 and 1997 gave a borderline significance for school*dose*year (p=0.06), indicating that the provocation curves for the two schools shifted over the three years. This was due to the different curve in 1996 for the target school.

For all factors where school was included (school*dose*time, school*time, school*dose and school), no statistically significant results were found, and all p-values were in fact >0.25. At the end of the three-year study period there seemed to be no substantial difference in mucosal swelling induced by histamine provocation for the students at the two schools when differences at baseline were accounted for.

We could find no evidence for differences between atopic and non-atopic students during these three years of study.
Figure 9. Histamine provocation curves (the mean net change in mucosal swelling from baseline) for students from the target and control schools measured on three occasions, in 1995, 1996 and 1997. The curve for the target school in 1996 deviates somewhat from the other curves (p=0.06).

Nasal mucosal histamine reactivity among teachers six years after working in a moisture damaged school (Study V)

Reported complaints and symptoms
As shown in table V, there were more complaints of varying and low room temperatures among the teachers in the target school as compared to the teachers in the control school. The analysis of varying room temperature and low room temperature showed a significant difference between the two schools, p=0.01 and p=0.04, respectively. The odds ratios for complaints, with the control school teachers as referents, were 5.7 (95% CI 1.2 – 27.7) and 5.4 (95% CI 1.1 – 25.6), respectively, indicating a much higher risk for complaints at the target school.
For poor indoor air quality there was also a significant difference between the two schools, p=0.01, and the odds ratio was 5.3 (95% CI 1.2 – 22.3) for the target school, but no significance for the factor year or interaction school*year.
For the symptoms none of the investigated factors, showed statistical significance. However, there was a decreasing tendency for general, mucosal and skin symptoms during the years of investigation among the teachers in the target school, which was not seen in the control school.

<table>
<thead>
<tr>
<th>Year</th>
<th>Target school (n = 24)</th>
<th>Control school (n = 16)</th>
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<tbody>
<tr>
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<td></td>
<td>Skin symptoms⁴</td>
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<td>1995</td>
<td>5/24 (20.8)</td>
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<td>1997</td>
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<tr>
<td>2000</td>
<td>2/24 (8.3)</td>
<td>3/16 (18.8)</td>
</tr>
</tbody>
</table>

¹The percentage of teachers often troubled (= at least every week) by stuffy bad air, dry air or an unpleasant smell the preceding three-month period.
²The frequency of teachers often troubled by tiredness, feeling heavy-headed, headache, nausea, vertigo or concentration problems.
³The frequency of teachers often troubled by irritated eyes, nose, throat or cough.
⁴The frequency of teachers often troubled by facial dryness- or redness, scaly and itchy scalp/cars or dry itchy skin on the hands (eczema of the hands).
Prohibition test

RSM/mucosal swelling reaction

The ANOVA analysis showed that in 1995 there was a statistically significant difference in the responses of teachers in the two schools to increased provocation levels, (p=0.001 for the 5-minute readings). In 1997 the difference had decreased compared to 1995 (the difference was still statistically significant). In 2000 (figure 10) there were no statistically significant differences (p=0.35). The swelling reaction 2 minutes (in 2000) after provocation with the lowest histamine concentration was more pronounced in the two study groups than after 5 or 10 minutes.

Figure 10. Histamine provocation curves (the mean net change from baseline) for teachers in the target and control schools in 2000.

Laser Doppler flowmetry

An analysis of variance of repeated measurements of perfusion for various dose levels in schools, based on 5-minute readings, showed a significant difference for school*dose (p=0.0022) indicating a difference in reaction patterns between the two teacher groups during the provocation procedure. This is well illustrated in figure 11 where the microcircular perfusion in the nasal mucosa steeply increases from baseline 2 minutes after the first provocation with the lowest histamine
concentration in the two teacher groups. The control group then seems to restore the level of perfusion rapidly and reacts more strongly to the following provocations compared to the weaker reaction from the teachers in the target school. There was also a significant difference over time (2, 5 and 10 minutes) (p=0.0064) when considering the lowest histamine concentration (1mg/ml).

Figure 12 shows the changes in CMBC during the histamine provocation procedure. The teachers in the target school, in contrast to those in the control school, show a decrease in their CMBC after the first histamine provocation and this remains low during the whole provocation procedure. There is a significant difference based on the 5-minute readings for the factor school (p=0.0009), but not for the factor school*dose (p=0.25), indicating that the provocation curves are well separated with a similar reaction pattern during the provocation procedure. As with perfusion, there was also a significant difference over time (p=0.0048) when considering the lowest histamine concentration.

There was no correlation between measured mucosal swelling and Laser-Doppler flowmetry (perfusion/CMBC values) during the histamine provocation procedure, with almost all correlation coefficients in the order of –0.10 to 0.10. On the other hand, correlations within the measured mucosal swelling as well as within flowmetry were much higher, in the order of 0.6 – 0.7, clearly indicating the need for the analysis of these measurements with allowance for correlations between repeated measurements, i.e. our chosen method of analysis.
Figure 11. Perfusion, difference from baseline. Values read at 2, 5, and 10 minutes after provocation for the 1 mg/ml histamine provocation level, and at 5 minutes for the 2 and 4 mg/ml levels.

Figure 12. CMBC, difference from baseline. Values read at 2, 5, and 10 minutes after provocation for the 1 mg/ml histamine provocation level, and at 5 minutes for the 2 and 4 mg/ml levels.
DISCUSSION

Decreasing Nasal histamine reactivity among the teachers in the target school

In 1995, one year after the renovations, the provocation tests showed significantly higher nasal mucosal reactivity to histamine among teachers working at the earlier water-damaged school than among teachers in the control school (study I). In 1997 the difference was less pronounced but still statistically significant (study II) and in 2000, six years after remedial measures had been taken, the statistical analyses showed no significant differences regarding nasal histamine reactivity between the two teacher groups (study V). These findings strengthens the assumption that long-time exposure to building dampness may induce mucosal hyperreactivity of the upper air-ways. This acquired hyperreactivity may then last for years and decrease only slowly even after the indoor climate has been properly improved. A possible explanation for this slowly decreasing reactivity might be a slow but ongoing restoring process in the mucosa of the upper air-ways.

Nasal histamine reactivity among the students

The statistical analysis showed no significant differences regarding nasal histamine reactivity between the previously unexposed students at the target school and the control school from start to endpoint of the time of observation (study IV). Furthermore, there were no differences in nasal histamine reactivity between the usually more sensitive atopic and non-atopic subjects during the years of study. Accordingly, persistent symptoms and increased nasal mucosal reactivity among personnel (teachers) in a remediated moisture-damaged school building does not necessarily imply an inadequate renovation.

This longitudinal study with registration of subjective (questionnaires) and objective (nasal histamine reactivity) data concerning this earlier unexposed group in the same building further contributed to the assumption that the renovation was successful.

Comparison of nasal histamine reactivity between students and teachers

The nasal histamine provocation tests differed markedly between teachers and students as regards nasal mucosal histamine reactivity. The fact that even the teachers from the control school showed more marked mucosal swelling in response to the lowest histamine provocation concentrations than the students suggests that age and/or various types of environmental exposures are of
importance. Such a mucosal reaction pattern may be due to a reduced secretory ability with age [121] and the lack of a protective mucus layer, which would make the mucosal sensory nerve endings more sensitive to non-specific stimulation.

The teachers in the control school had only a moderate increase in mucosal swelling when exposed to the highest histamine concentration as compared to the students. The marked mucosal reaction to the lowest histamine concentration may induce tachyphylaxis of the neural response during repeated stimulation. This is unlike the response of the teachers in the target school who reacted most to the highest histamine concentration. Their curve for mucosal swelling closely resembled that of the students, but at higher levels.

**Exposure measurements before and after the renovation**

The investigations performed by professional consultants before renovation of the target school showed a substantial increase in mould growth in the ventilation system and severe moisture problems caused by water leakage for many years. However, measured indoor air parameters in the target school before renovation, including relative air humidity, concentrations of moulds and volatile organic compounds in indoor air, were not higher than what is usually measured in Swedish schools without indoor climate problems [110]. Indoor concentrations of airborne viable fungi vary extensively, mostly depending on differences in climate conditions, and mainly follows the outdoor concentrations [122, 123, 124]. In colder climates, such as in Scandinavia and Canada, the prevalence of moisture and mould damage in the building construction but “normal” levels of pollutants, including moulds, in the indoor air has been described [125, 126, 127]. Furthermore, the outdoor humidity largely determines the relative humidity levels in the indoor air. In the Nordic countries, the relative humidity indoors is usually low (mostly below 45%), even in mould contaminated buildings [128, 129].

The control school had had severe ventilation problems before renovation. In spite of this, significant differences in nasal histamine provocation curves were seen between the two schools after the renovations, which might indicate that specific pollutants were causing a prolonged effect on the mucous membranes of the exposed teachers in the water-damaged school. Indeed, the relationship between working and/or residing in edifices with building dampness and increased mucosal reactivity seems to be due to emissions in the air from the moisture-damaged foundation by microbial growth or chemical degradation of the material [130, 131, 132]. The lack of remaining signs of water damage in the building construction and the fact that, according to the technical measurements performed after the renovations, the physical environments in both schools were
essentially of the same quality also indicated a successful renovation of the previous moisture-damaged school.

**Complaints and symptoms**

The high frequency of experienced poor indoor air among the teachers in the target school in 1995 only marginally decreased during the years of observation. A contributing factor might be difficulties in maintaining a uniform temperature in a single-storey building, leading to a significantly higher prevalence of complaints of varying and low room temperature among the target school teachers. Uniformity of temperature and well adapted ventilation are important for comfort and seem to be associated with a lower incidence of experiencing air dryness [133], which was a dominating complaint in the experience of poor indoor air climate among the target school teachers.

The frequency of reported mucosal irritative symptoms and skin symptoms decreased among the teachers in the target school during the time of observation. However, there were no statistically significant differences regarding these symptoms over time between the teachers in the target school and the teachers in the control school, probably because the study was not fully dimensioned to evaluate differences in these parameters.

The assumption that the renovation was adequate is also strengthened by the fact that the students at both the target and the control school reported a similar low frequency of perceived bad indoor climate and mucosal symptoms during the time of observation. Furthermore the perception of bad indoor climate as well as general and mucosal symptoms among the students did not differ much from those of the teachers in the control school.

**Sample size**

It should be mentioned that these studies were designed primarily to analyse differences in histamine reactivity and for that purpose the sample size was considered well in line with experiences of earlier investigations [15, study I].

The secondary aim was to analyse the questionnaire data, where the variables were measured on a nominal or ordinal scale with few categories. For that purpose, it would have been better to increase the sample somewhat, but for practical reasons that was not possible. However, the number of participating students in studies III and IV was quite high, allowing for the possibility of differentiating moderate differences in reported complaints and symptoms.
Atopy/skin prick test positivity

The prevalence of IgE sensitization, defined as a positive skin prick test (SPT+), was not higher among the teachers in the target school compared to the teachers in the control school, indicating that the increased reactivity of the nasal mucosa in the target group was of a non-specific type.

The frequency of SPT+ was at least twice as high among the students as compared to the teachers (36% versus17%). This is in accord with the increasing frequency of atopy in the Western world [1, 134]. The exposure measurements did not show any deviation from what is usually seen in Swedish schools [110]. However, exposure to indoor air pollutants in moderate concentrations has been shown to be related to airway symptoms, particularly among atopic individuals [6, 135, 136]. We found no significant differences between SPT+ and SPT- students or between atopic and non-atopic students at the two schools regarding nasal histamine reactivity, perception of indoor climate or mucosal irritative symptoms, indicating a decent indoor air quality in both schools. In 1996, the majority of the SPT+ students (70.4%) reported allergic symptoms during the preceding year. Consequently, a positive skin prick test in this age group seems to indicate an active atopic disease. None of the SPT- students reported asthmatic symptoms, indicating that asthma in this age group (16-18 years) is predominately a manifestation of atopy.

Mucosal appearance (rhinoscopically)

Among the teachers in the two schools with a rhinoscopically dry and crusty appearance the histamine provocation curves for mucosal swelling showed a marked significant increase compared to those for the remaining teachers. The prevalence of this dry and crusty mucosa did not differ significantly between the teachers in the two schools. The development of such a dry mucosa may be constitutional, but it may also be age dependent and predispose to an increase in mucosal reactivity to histamine.

One explanation could be an age dependent lower mucosal water content and the lack of a protecting mucus layer, which would make the mucosal sensory nerve endings more sensitive to unspecific stimulation. However, we think that the dry and crusty mucosal appearance is a constitutional variant that may indicate an increased risk for developing nasal hyperreactivity when exposed to various mucosal irritants. It is also interesting that the teachers with a dry and crusty mucosal appearance and increased mucosal histamine reactivity also presented a higher frequency of mucosal irritative symptoms and skin symptoms. This leads us to believe that there is a relationship between measured mucosal reactivity and
mucosal and skin symptoms, but a larger study population is needed in order to demonstrate this.

**Nasal mucosa/sensitivity/swelling/secretion**

The nasal mucosa is very sensitive and easily affected by external and internal disturbances. By using histamine, a potent substance with a broad action on the nasal mucosa, the influence of these background factors is reduced. Histamine directly affects sensory c-fibres of the nasal mucosa as well as receptors of the vessels [137]. The induced mucosal swelling is apparently due to a combination mainly of dilatation of mucosal vessels and to some extent, leakage from the microcirculation [138, 139].

Both the mucosal swelling and the perception of nasal blockage and nasal secretion increased with increasing histamine concentrations in the provocation test (study 1). This is in accordance with a study by Hallén and Juto, where a significant correlation between symptom score and recorded mucosal swelling was shown [140]. Regarding nasal blockage the scores (symptoms) were higher among the target school teachers, which is in line with an increased mucosal swelling reaction upon histamine provocation. The teachers in the control school did not increase their secretory response to the highest histamine concentration, which might be in accordance with an acquired tachyphylaxis to provocation in this “normal adult group of people” (study III). The appearance of sneezes did not follow this pattern.

**Rhinostereometry**

Rhinostereometry (RSM) permits standardization of the nasal mucosal swelling reaction, without interference from secretion or stenosis [67]. RSM measures the congestive status of the mucosa, probably mainly reflecting the degree of filling of the deeper situated venous sinusoids [141]. Its high sensitivity makes it possible to measure small changes in mucosal congestion, which is probably necessary when studying groups of people with moderate symptoms of mucosal disease.

**Laser Doppler flowmetry (LDF)**

LDF is the method of choice for measuring microcirculation in combination with RSM as it is non-invasive and continuous recordings can be made of the microcirculation.
It is suggested that LDF measures the superficial layers of the nasal mucosa, containing arterioles and a dense capillary network, and does not reflect the status of the deeper situated venous sinusoids [69,142, figure 4].

The combination of RSM and LDF makes it possible to accurately regulate the distance between the probe and mucosal surface.

The CMBC (the concentration of moving blood cells) is probably affected by changes in the amount of interstitial fluid [143]. Therefore, an increase in vascular permeability resulting in oedema would appear in the LDF recordings as a reduction in CMBC.

In our study (study V), both study groups initially reacted to nasal histamine provocation with an increase in perfusion (“blood flow”), but there was a somewhat flatter provocation curve among the teachers in the target school indicating a somewhat slower reaction pattern. This is in accordance with the decreasing CMBC during the provocations among the teachers at the target school which indicates plasma leakage and to some extent interstitial oedema.

The absence of correlation between mucosal congestion and the microcircular pattern was probably due to the measurements of different parameters. The RSM measures changes in mucosal congestion which is due to dilation of the deeper situated sinusoids in the nasal mucosa while LDF measures the superficial microcircular pattern.

Allergic rhinitis in the non-pollen season

The studies were conducted during the non-pollen season and no significant differences in mucosal swelling due to histamine provocation were seen between atopic and non-atopic or between SPT+ and SPT- students.

Although some studies show an increase in nasal mucosal reactivity to histamine challenge among atopic subjects out of season [103, 105], our study indicates that the ongoing mucosal inflammation among young atopic subjects during the pollen season may disappear almost entirely in the non-pollen season.
CONCLUSIONS

In conclusion we have shown:

that long-time exposure to building dampness contributed to an increased nasal mucosal reactivity measured as mucosal swelling upon repeated histamine provocations

that this increased reactivity lasted for years, even after remedial measures had been taken

that there was an increased nasal histamine reactivity among the adult teachers as compared to the adolescent students

that the frequency of IgE sensitization (SPT+) was about twice as high among the students as compared to the teachers

that there was no increase in nasal mucosal reactivity among students in the target school as compared to those in the control school during their three years of study

that there were no significant differences in nasal histamine reactivity among atopic students as compared to non-atopic students in the non-pollen season

that there was a highly significant increase in nasal histamine reactivity among teachers with a dry and crusty nasal mucosa as compared to the remaining teachers

that there were indications of a slower reacting nasal mucosal circulation and increased interstitial vascular leakage among the teachers in the target school upon repeated histamine provocations as compared to those in the control school six years after the renovation of the target school

that there was no correlation between the nasal swelling reaction measured with RSM and nasal microcirculation measured with laser Doppler flowmetry upon repeated nasal histamine provocations
General considerations

The association between residing in moisture damaged buildings and adverse health (especially increased risk for health effects regarding the airways) is apparent, but the factors responsible for the symptoms are not at all clear [144]. To gain more information about possible mechanisms, future research should test new hypotheses such as those dealing with effects of specific chemicals and microbial agents.

Evidence that elevated levels of airborne fungi in school buildings are a causal factor for health complaints remains inconclusive [145, 146]. In a Danish school study there was a positive association between building-related symptoms and viable moulds in floor dust, but not between symptoms and the extent of moisture and mould growth in the school buildings [147]. Furthermore, there are no generally accepted standards for interpretation of fungal levels in indoor and outdoor air [148]. Another possible mechanism resulting in inflammation of the airway mucosa is a synergistic effect between certain emitted chemicals and dust [149].

Although the mechanisms are unclear, the best approach today to indoor microbial control is moisture control in the indoor environment. Assuming that the health effects are reversible, renovation of the moisture damage should lead to improvement in mucosal symptoms, which was seen in one school study from Denmark [150]. In our study the teachers had been exposed to severe building dampness for several years and we found a gradually and slowly decreasing nasal mucosal reactivity after remedial measures had been taken. This was interpreted as a slow but ongoing restoring process in the mucosa of the upper airways. However, six years after the renovation signs of persistent microcircular leakage and oedema upon nasal histamine provocation were shown among the target school teachers. These long-standing effects on the air-way mucosa seem to be mostly subclinical and represent no actual clinical disease.

The role of sensory nerves and psychological factors in the generation and perception of symptoms has been relatively neglected compared to the large amount of research on inflammatory mediators [86, 151, 152]. For instance, subjects can acquire somatic symptoms and altered respiratory behaviour in response to harmless, but odorous chemical substances, if these odours have been associated with a physiological challenge that originally caused these symptoms [153, 154].

There is much evidence that the sensory nervous system in the nasal mucosa plays an important role in normal and pathological nasal reactions [155, 156].
Triggering of sensory-efferent nerves (for instance with histamine provocation) can initiate systemic reflexes such as sneezing, as well as central cholinergic reflexes [157]. These reflexes cause release of mediators that stimulate blood vessels and glands, resulting in vasodilatation and secretion. Local reactions are also caused by release of neuropeptides from the sensory nerves.

Thus neurogenic inflammation involves interaction between nerves and inflammatory cells. The observation that reflex mechanisms of neurogenic inflammatory origin can be relatively easily investigated in the nasal mucosa indicates that the nose is a useful instrument in studying and understanding inflammatory processes in the respiratory tract [158]. It is quite possible that unspecific hyperreactivity of the nasal mucosa can be caused by chemical irritants with a resulting change in the neuropeptide-contents of mucosal nerve fibres [159].

In addition to agents used for provocation, the sensitive nasal mucosa reacts to other stimuli such as odours, temperature changes and touch. RSM allows direct inspection of the nasal mucosa and introduction of probes for stimulation of different receptor areas of the nasal cavity with a very high precision. We think that this technique will be a valuable future tool for learning about the physiological and pathophysiological mechanisms of airway mucosal reactions.
ACKNOWLEDGEMENTS

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Nasal Hyperreactivity among Teachers in a School with a Long History of Moisture Problems

Stig Rudblad, M.D.,* Kjell Andersson, M.D.,# Göran Stridh, Ph.D.,# Lennart Bodin, Ph.D.,§ and Jan-Erik Juto, M.D., Ph.D.¶

ABSTRACT

Upper airway symptoms have frequently been reported in people working or residing in damp buildings. However, little information has been available on objective pathophysiologic findings in relation to these environments. Twenty-eight teachers, who had worked for at least five years in a recently renovated school that had had severe moisture problems for years, were randomly selected for this study. Eighteen teachers, who had worked in another school that had no moisture problems, were randomly selected to serve as the control group. Although remedial measures had been taken, an increase in the prevalence of mucous membrane irritations was still reported by the teachers in the target school. We used a nasal challenge test with three concentrations of histamine (1, 2 and 4 mg/mL). Recordings of swelling of the nasal mucosa were made with rhinostereometry, a very accurate optical non-invasive method. The growth curves of mucosal swelling induced by the three concentrations of histamine differed significantly between the two groups (p < 0.01). The frequencies of atopy, evaluated with the skin-prick test, were almost identical in both groups. The study indicates that long-term exposure to indoor environments with moisture problems may contribute to mucosal hyperreactivity of the upper airways. Such hyperreactivity also seems to persist for at least one year after remedial measures have been taken. (American Journal of Rhinology 15, 135–141, 2001)

Many occupants attribute mucous membrane irritations and general symptoms to the indoor climate in numerous non-industrial buildings, including schools and day-care centers. One association between working and/or residing in damp buildings and respiratory health has been reported in a number of studies. However, it has been difficult to verify a relationship between measured low-level indoor air pollution and reported symptoms. The indoor environment is complex and symptoms are influenced by physical factors, such as emissions from building materials, ventilation, and variations in temperature; psychosocial factors, including work-related stress and social status; and constitutional factors, such as age, sex, and atopy.

A major limitation has been the difficulty of objectively verifying any effects on the mucous membranes of the respiratory tract that might explain, e.g., eye irritation, nasal congestion, and cough, often reported by occupants of buildings with indoor air problems. Rhinomanoetry, a method that indirectly measures nasal airflow resistance, has been used for a long time in many clinical studies to estimate the degree of nasal blockage. Nasal peak flowmetry is a simple and clinically useful method, where instead of the mouthpiece of a bronchial peak flow meter, an anesthetic mask is used to measure nose breathing. The findings seem to correlate well with those obtained by rhinomanometry. During the last decade, new techniques have been introduced to record changes in the mucous membranes of the nose. Acoustic rhinometry, first described by Hilberg in 1989, uses acoustic reflection to assess the geometry of the nasal cavity, showing the cross-sectional
areas of the nasal passageway at various distances from the nostril. It has been used in studies of school environments. More nasal congestion after exposure to vasoconstrictive drugs, as a measure of increased swelling, was found in personnel in a school with a low air-exchange rate and increased relative humidity compared to personnel in a control school, although weekly reports of nasal congestion were similar in both schools. Rhinostereometry, an optical high-precision method, measures small changes in nasal mucosal swelling. By using rhinostereometry, Ohm et al. found an increase in reactivity to histamine, measured as nasal mucosal swelling, among non-atopic tenants living in a residential area with severe indoor climate problems compared to people living in a control residential area without such problems. Hyperreactivity, defined as an increase in swelling of the nasal mucosa after histamine provocation, was common in symptomatic and asymptomatic groups in the domestic area with indoor air problems. The documented increase in nasal reactivity seemed to persist for years after the subject moved from the problem area or after remedial measures had been taken, although the residents no longer complained of mucous membrane irritation.

This study aimed at determining the reaction of the nasal mucosa to histamine in a provocation test among teachers who had worked for a long time in a school with serious moisture problems. Although extensive remedial measures had been taken in the school one year before the study, they continued to complain about mucosal irritation from the respiratory tract.

MATERIALS AND METHODS

The Study Schools

The target school was constructed in the 1960s as a single-story building with flat roofs and an inlet and exhaust ventilation system. It is located on the outskirts of a town in the middle of Sweden close to a river that usually overflows in spring. Moisture damage was reported shortly after its construction. In the late 1980s, there were frequent complaints about the indoor climate and at least five teachers resigned because of health problems, which they ascribed to the school environment. Technical investigations performed in the beginning of the 1990s showed very severe moisture problems because of inadequate drainage and numerous water leaks from the flat roof causing growth of mold and discoloring of building material. Measurements of insulation material in the ventilation system showed a substantial growth of different species of mold (e.g., Cladosporium, Penicillium, Alternaria, and Paecilomyces). No systematic measuring program was followed, but available measurements during the winter season of relative air humidity (22–27%), airborne microorganisms (low levels), and chemicals in the air (total volatile organic compounds (TVOC = 50 μg/m³) were within current limits for what is usually seen in Swedish schools.

The ventilation flows were also within the range recommended in Sweden, and most carbon dioxide concentrations were below 1000 ppm (parts per million).

Another high school was selected as a control school. It is located in the center of the same town and consists of four 3–4-story buildings built between 1890 and 1930. Most classrooms were “naturally” ventilated during the 1980s with very low air-exchange rates, and the carbon dioxide levels during the lessons occasionally exceeded 2500 ppm. No moisture problems were reported.

Extensive remedial measures were taken in both schools in 1993–1994. Surveys, using the same standardized questionnaire (MMQ01NA), were made before (1989) and after (January 1995) the renovations (Table I). In 1995, the teachers in the target school still reported an increased frequency of mucous membrane irritations, while there was a slight reduction in the perception of stuffy bad air and a marked reduction in unpleasant smells and complaints about dust. The prevalence of dry air was high in 1989 and even

<table>
<thead>
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<th>TABLE I</th>
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<tbody>
<tr>
<td><strong>The Frequency of Perceived Indoor Air Quality, Mucous Membrane Irritation, and General Symptoms among Personnel in the Target and Control Schools in 1989 and 1995</strong></td>
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<tr>
<td><strong>Target School (%)</strong></td>
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<tr>
<td><strong>1989</strong></td>
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<tr>
<td></td>
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<tr>
<td>Perception of dry air</td>
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<td>Perception of stuffy bad air</td>
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<tr>
<td>Perception of unpleasant smell</td>
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<tr>
<td>Perception of dust and dirt</td>
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<tr>
<td>General symptoms*</td>
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<td>Mucous membrane irritation#</td>
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</table>

*The frequency of teachers often troubled by fatigue, headache, nausea, vertigo, or concentration problems.

#The frequency of teachers often troubled by cough, irritation from the eyes, nose, or throat.
higher in 1995. The teachers in the control school reported that the indoor air had improved after the ventilation system was changed.

The Study Population

A random sample of teachers, who had worked at least five years in the target school before the renovation (39 persons), and all teachers in the control school, who fulfilled the same inclusion criteria (30 persons), were invited to participate in the study in March and April 1995. Twenty-eight and 18 teachers, respectively, accepted and formed the two study groups (A and B). Most of those who did not take part said that they had no time for the examination during the brief period of the study.

The Questionnaire

During the acclimatization period (see below) a short medical history was taken (not by the same person who made the provocation test) and a standardized questionnaire (MM040NA) was filled in. This contained questions about the perceived indoor climate, symptoms often referred to in indoor climate research, allergic manifestations, and some background factors.

The Provocation Test

The histamine provocation test was performed as a single-blind procedure at a nearby hospital. The investigator did not know which school the test person was working in. The medial side of the inferior turbinate, a highly reactive part of the nasal mucosa, was used as the challenge area. The subjects were allowed to acclimatize for 30 minutes, after which they were challenged every 10 minutes with 0.14 mL of histamine dihydrochloride without preservatives, in increasing concentrations (1, 2, and 4 mg/mL), using the same standard nasal provocation procedure described by Hallén and Juto. The challenge substance was applied to the right inferior concha with a syringe, but not to the left inferior concha, which served as a control. The left inferior turbinate was used for the provocation only if the initial rhinoscopic examination showed marked right septal deviation. Recordings of mucosal swelling were made with rhinostereometry in both nasal cavities 5 and 10 minutes after each challenge. Rhinostereometry, an optical method for measuring changes in congestion of the nasal mucosa, is not invasive and allows direct determinations with high accuracy (±0.18 mm). The rhinostereometer has a surgical microscope with a small depth of focus placed on a micrometer table. The table is fixed to a frame and can be moved in three directions at right angles to one another. The mucosal position of the inferior turbinate is recorded on a horizontally located millimeter scale inserted in the ocular. During the measurement, every fifth minute, the subject sits for approximately one minute in a reproduceable position with the help of an individually formed plastic tooth-splint fixed to the frame of the apparatus.

The subjective perceptions of nasal blockage (not specified to the provocation side) were recorded on a four-point scale where zero meant no blockage and three severe blockage. The amount of secretion, observed by the investigator on the provoked side, was rated as none, sparse, covering the medial part of the anterior concha, or covering the concha with wide septal contact, also on a four-point scale. The number of sneezes was noted by the investigator.

Skin-Prick Test

A skin-prick test with a standardized panel of allergens, often used in Scandinavia (ALK, Copenhagen), was done after the histamine challenge. The following allergens were tested: pollen (birch, timothy, and mugwort), mites (Dermatophagoides pteronyssinus and D. farinae), furry animals (cat, dog, and horse), and molds (Cladosporium, Alternaria, and Aspergillus fum.). Atopy was defined as a wheal with a diameter of at least 3 millimeters.

Statistical Methods

We restricted the formal statistical testing to the two major groups of outcome variables, i.e., swelling of the mucous membrane of the nose and prevalence of local reactions (nasal blockage, secretion and sneezing). In both cases statistical models based on repeated measurements were used. Thus, we analyzed the correlation between measurements taken from the same person at different provocation concentrations. For the continuous variable, mucosal swelling, we used an analysis of variance model for repeated measurements (an ANOVA model). The analysis aimed at modeling the growth curves for mucosal swelling as a function of the provocation concentrations of 1, 2, and 4 mg/mL of the histamine solution. In the ANOVA model, school (with two levels, school A versus school B) was the main explanatory variable, but a set of additional variables was included in the model to control for confounding. These were time (5 and 10 minutes after each provocation), age, sex, smoking habits, and presence of allergy. The primary test in the model was that for interaction between school and provocation concentrations, because a significant result here indicates that the growth curves for mucosal swelling are seen to be essentially different with regard to slope and shape when the two schools are compared.

In the analysis of the prevalence of local reactions we used a generalized logistic regression model for repeated measurements of ordinarily coded outcomes. Contrary to the ANOVA model, the analysis for prevalence of reactions did not include covariates in the model. This was mainly because the statistical assumptions for the logistic model of this type with our relatively small sample size seemed not to be fulfilled when covariates were included. We tested for differences in the general level of scores and for differences in the slope or trend, tests that are analogous to those used for the ANOVA model (see Wei and Johnson). In all statistical hypothesis testing we used two-sided tests with a significance level of five percent.
RESULTS

The main characteristics of the two study groups and of all employees who answered the questionnaire in the early spring of 1995 (two months before this study) are shown in Table II. During this period, no interventions whatsoever were done in the two schools. The test groups are fairly representative of the total populations of the personnel at the two schools. The teachers at the target school complained more about the quality of the indoor air than those at the control school, and also reported a higher prevalence of mucosal membrane symptoms.

The results of the provocation test are shown in Table III, and graphically in Fig. 1. The mucosal congestion has been calculated as the deviation from the baseline. The ANOVA model showed significantly different growth curves in the two study groups (p < 0.01) with respect to the provoked side of the nose. This can be interpreted as different slopes in the regression of mucosal swelling on provocation concentrations, with a more marked gradient for group A. On the control side, only slight mucosal congestion was recorded around the baseline.

The prevalence of local reactions (nasal blockage, secretion, and sneezing) during the provocation is shown in Table IV. We found a borderline significance in the two groups with respect to the general degree of nasal blockage, p = 0.06, with higher scores in group A. With respect to secretion we noted no difference in level, but instead a steady increase in the scores and a different trend in group A than in group B, p = 0.03. Thus, as shown by the table, at low provocation concentrations the scores are higher in group B, but at the highest provocation concentration, 4 mg/mL, the scores are higher in group A. For sneezing no statistically significant results were found.

Eighteen percent of the employees of the target school and seventeen percent of the employees in the control school had a positive skin-prick test (Table II). Allergy to birch pollen was commonest (13%). No allergy to mites or molds was detected in the two schools.

DISCUSSION

The analyses showed significantly more nasal mucosal reactivity to histamine among teachers working in the school with moisture problems than among those in the control school, the differences being more marked at higher provocation concentrations. The control school had had severe ventilation problems before renovation. However, the growth curves of the teachers in the two schools differed significantly, which might indicate that specific pollutants in the water-damaged school had affected the mucous membranes of teachers in the target school.

The investigations performed by professional consultants before the renovation showed a substantial increase in mold in the ventilation system and severe moisture problems caused by water leakage for many years in the target school. However, the levels of the parameters measured, including relative air humidity and concentrations of molds and volatile organic compounds in indoor air, were no higher than those usually found in Swedish schools without indoor climate problems. The prevalence of severe mold growth in the construction, but "normal" levels of molds in the indoor air, has been described before, especially in Nordic countries. Despite this, some studies have shown a correlation

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**TABLE II**

<table>
<thead>
<tr>
<th></th>
<th>Target School</th>
<th>Control School</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Total*</td>
</tr>
<tr>
<td></td>
<td>(n = 28)</td>
<td>(n = 129)</td>
</tr>
<tr>
<td>Sex (% men)</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>51</td>
<td>48</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Allergic disease (%)#</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Employment (years)</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Poor indoor air quality$</td>
<td>57</td>
<td>66</td>
</tr>
<tr>
<td>General symptoms (%)</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Mucous membrane irritation (%)</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Positive prick test (%)</td>
<td>18</td>
<td>—</td>
</tr>
</tbody>
</table>

*Total means all members of personnel who answered the questionnaire in January, 1995.
#Reported current or previous hay fever or asthmatic symptoms.
$The prevalence of teachers often troubled by perception of dry air, stuffy bad air, or unpleasant smell.

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### TABLE III

Mean Swelling (±SD; mm) of the Mucous Membrane in the Nose

<table>
<thead>
<tr>
<th>Histamine Concentration</th>
<th>Provoked Side</th>
<th>Control Side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A ($n = 28$)</td>
<td>Group B ($n = 18$)</td>
</tr>
<tr>
<td>1 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.50 (0.73)</td>
<td>0.49 (0.54)</td>
</tr>
<tr>
<td>10 min</td>
<td>0.52 (0.75)</td>
<td>0.42 (0.50)</td>
</tr>
<tr>
<td>2 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>0.98 (0.81)</td>
<td>0.82 (0.76)</td>
</tr>
<tr>
<td>10 min</td>
<td>0.88 (0.84)</td>
<td>0.65 (0.67)</td>
</tr>
<tr>
<td>4 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>1.42 (0.83)</td>
<td>0.86 (0.73)</td>
</tr>
<tr>
<td>10 min</td>
<td>1.10 (0.85)</td>
<td>0.59 (0.61)</td>
</tr>
</tbody>
</table>

Measurements of swelling were made from the baseline after provocation with various concentrations of histamine in groups A and B.

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**Mucosal Swelling**

![Mucosal Swelling Graph](image)

*Figure 1. Histamine provocation curves (the mean net change in mucosal swelling from the baseline on the provoked and unprovoked sides) in group A (target school) and group B (control school). The significance of the difference in slope between the two provocation curves is shown ($p < 0.01$).*

between such environments and effects on health, especially respiratory symptoms, among the occupants.23,24

In cold climates, such as that prevailing during winter in the Nordic countries, the relative humidity indoors is usually low (mostly below 35%), even in very mold-contaminated buildings.22,23 The prevalence of dry air is the most prevalent complaint about the indoor climate in Nordic non-industrial buildings.25 The perception of dry air is affected by several indoor air factors, e.g., room temperatures, ventilation flows, and air pollutants.26,27 The complaints about dry air decreased considerably in the control school after renovation of the ventilation system, but increased in the target school. However, no differences between measurements of room temperatures, ventilation flows, concen-

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<table>
<thead>
<tr>
<th>Group A (n = 28)</th>
<th>Group B (n = 18)</th>
<th>Tests for Group Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Blockage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>64</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

|                    | 2 mg/ml          | 36                        | 50                        |
|                    | 29               | 33                        | 11                        |
|                    | 4                | 6                         |
|                    | 1 mg/ml          | 48                        | 56                        |
|                    | 19               | 17                        | 17                        |
|                    | 4                | 12                        |
|                    | 0                | 0                         |
| Secretion         |                  |                           |
| 1 mg/ml          | 78               | 72                        | p for level = 0.38 |
|                 | 19               | 22                        | p for trend = 0.03 |
|                 | 4                | 0                         |
|                 | 0                | 6                         |
|                 | 2 mg/ml          | 52                        | 56                        |
|                 | 26               | 17                        | 17                        |
|                 | 22               | 22                        |
|                 | 0                | 6                         |
|                 | 4 mg/ml          | 48                        | 56                        |
|                 | 15               | 17                        | 17                        |
|                 | 22               | 22                        |
|                 | 15               | 6                         |
| Sneeze           |                  |                           |
| 1 mg/ml          | 71               | 72                        | p for level = 0.40 |
|                 | 11               | 11                        | p for trend = 0.26 |
|                 | 14               | 17                        |
|                 | 4                | 0                         |
|                 | 0                | 6                         |
|                 | 2 mg/ml          | 82                        | 72                        |
|                 | 11               | 6                         | 16                        |
|                 | 4                | 16                        |
|                 | 4                | 0                         |
|                 | 0                | 6                         |
|                 | 4 mg/ml          | 82                        | 67                        |
|                 | 7                | 17                        | 17                        |
|                 | 11               | 11                        |
|                 | 0                | 6                         |
| The tests use the ordinal character of the score measurements and they are based on all repeated measurements in the subjects. “p for level” tests if the overall levels of the scores are equal in the two groups, p < 0.05 indicates difference in overall level. “p for trend” tests if there is a uniform trend in the two groups, i.e., an interaction between the group and the provocation concentration with respect to the scores. p < 0.05 indicates different trends in the two groups.

The reactivities of airborne particles, and volatile organic compounds were detected between the two schools (data not presented here). The high prevalence of complaints about dry air in the target school cannot be explained by the technical measurements. However, the lack of persistent signs of water damage in the building construction and the reduction in complaints about air stuffiness, unpleasant smell, and perceived indoor dust indicates an improvement in the indoor climate after the renovation.

In many cases, no satisfactory explanation can be found for the mucous membrane irritations reported by inhabitants of buildings with indoor climate problems. It is now being discussed whether the symptoms are due to not fully described reactive pollutants that form in these environments.\(^7\)

The increased mucosal reactivity among teachers in the target school more than one year after renovation could mean that the remedial measures had not sufficiently improved the indoor climate. However, it seems more likely that although they were adequate, the increased reactivity persisted. This agrees with the findings of another study, where persons who had lived in a residential area with severe indoor climate problems continued to have increased reactivity more than two years after they moved away.\(^10\) It is also noteworthy that the nasal histamine reactivity of the teachers in our control school was almost the same as that of people living in a residential area without any indoor air problems, but the histamine provocation procedure in that study differed somewhat from ours.\(^15\)

The mucosal swelling, the perception of nasal blockage, and nasal secretion increased with increasing histamine concentrations in the provocation test. This agrees with a study by Hallén and Juto, who found a significant correlation between the symptom score and mucosal swelling.\(^29\) The amount of secretion followed the same trend as the provocation curves for mucosal swelling in the two groups, while the trend for perceived nasal blockage did not quite follow the same pattern. This may be because histamine provocation mainly caused ipsilateral mucosal swelling, and the perception of nasal blockage depends on the degree of obstruction of both nasal cavities. The onset of sneezing does not follow this pattern. An explanation may be that unprotected sensory nerve endings in the nasal mucosa react more to the first histamine concentration, but then develop tolerance with repeated challenges.

The sample size was relatively small. Data in other stud-
ies using the same technique, however, indicate that differences between groups can be detected if a dozen people are included. The study groups were fairly representative of all employees in both schools and there were no signs of biased selection. The prevalence of atopy, defined as a positive skin-prick test, was similar among the teachers in the target and control schools, indicating that the increased reactivity of the nasal mucosa in the target group might be of a non-specific type. This accords with the finding that no allergy to mites or molds was found in the study group.

Rhinostereometry permits standardization of the nasal mucosal swelling reaction, without interference from secretion or stenosis. Measurements of small changes in mucosal congestion can be made because of its high sensitivity, which is probably necessary when studying groups of people with moderate symptoms of mucosal disease.

The nasal mucosa is very sensitive and easily affected by internal and external disturbances. These effects are reduced by using histamine, a potent substance with a broad action on the nasal mucosa. Histamine directly affects sensory c-fibers of the nasal mucosa and receptors in vessels. The nasal mucosal swelling seems to be induced mainly by dilatation of mucosal vessels and, to some extent, leakage from the microcirculation. The combination of rhinostereometry and laser Doppler flowmetry may improve understanding of the mechanisms of intranasal challenge reactions.

In conclusion, this study indicates that after one spends a long time in a building with moisture problems, increased mucosal sensitivity tends to develop in the upper airways. Moreover, such hyperreactivity may persist for at least one year after remedial measures have been taken.

ACKNOWLEDGMENT

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REFERENCES

Slowly decreasing mucosal hyperreactivity years after working in a school with moisture problems

Abstract In our first study in 1995, teachers, who had worked in a water-damaged school for more than 5 years, were tested for nasal histamine reactivity by rhinostereometry. They were found to have significantly increased reactivity compared with teachers in a school without these indoor-climate problems. This finding could not be explained by differences in atopy or other personal characteristics. In this 2-year follow-up study (1995–97), 26 of 28 teachers in the target school and all 18 teachers in the control school, who participated in the initial study, accepted to take part. They were tested with the same histamine provocation procedure and answered the same questionnaire as 2 years earlier. Technical measurements of temperature, relative humidity, dust, carbon dioxide, formaldehyde and total volatile organic compounds (TVOC) were carried out in both schools during the time period between the two test occasions. In this provocation test, the teachers from the repaired water-damaged school still demonstrated an increased reactivity to histamine compared with the teachers in the control school, but the difference between the growth curves of the provocation tests was less than in 1995. Teachers in the target school still complained about the indoor air quality more than their colleagues, although the complaints were less common. No major differences were observed in the technical investigation between the two schools and the measurements were all within values usually seen in schools in northern countries. Our conclusion is that the observed nasal mucosal hyperreactivity among the teachers in the renovated water-damaged school seems to persist over years and only slowly decrease even after successful remedial measures have been taken.

Practical Implications Long-time exposure to building dampness may increase the risk for hyperreactivity of the upper airways. This acquired hyperreactivity may last for years and only slowly decrease even after the indoor climate has been properly improved. A possible explanation to this slowly decreasing reactivity might be a slow but ongoing restoring process in the mucosa of the upper airways.

Introduction

The health impact of the indoor environment has been increasingly recognized over the past 30 years. Poor indoor air quality has been discussed – but also rejected by some authors – as one of the factors related to the increased prevalence of allergies and asthma in the western world (Björkstén, 1997; Heinrich et al. 1998; Strannegård and Strannegård 2001). Mucous-membrane irritations and general symptoms that the occupants attribute to the indoor climate are frequent in many non-industrial buildings, including schools (World Health Organization, 1983; Kreiss, 1989). It has been shown in a number of studies that moisture, dampness, and consequent microbial growth is associated with respiratory symptoms and infections, increased risk of asthma and general symptoms among both adults and children (Ruotsalainen et al., 1995; Chih-Shan et al., 1997; Peat et al. 1998). Frequently reported symptoms are, e.g. sore throat, cough, wheeze, rhinitis and eye symptoms.

It has been difficult to objectively verify the effects on the mucous membranes of the upper airways reported by occupants in buildings with moisture problems. During the last decade, however, some new techniques have been used, among these acoustic
rhinometry, nasal peak flowmetry, and rhinostereometry (Hallén and Juto, 1993; Hilberg et al., 1995; Hellgren et al., 1997). Acoustic rhinometry has been used in studies related to school environments. Increased nasal congestion was demonstrated for personnel in a school with a low air-exchange rate and increased relative humidity compared with personnel in a control school, although weekly reports of nasal congestion were similar in the two schools (Wålinder et al., 1997). Both low air-exchange rates and mechanical ventilation systems based on mixing were shown by the same research group to be associated with increased nasal obstruction (Wålinder et al., 1998). Increased nasal reactivity to histamine, measured with rhinostereometry, was demonstrated for tenants living in a domestic area plagued by severe indoor climate problems (Ohm et al., 1997). This increased mucosal reactivity may persist for years after having moved from the problem buildings (Ohm et al., 1995).

In 1995, a random sample of teachers (n = 28), who had been working in a water-damaged school for at least 5 years, was tested by nasal histamine provocation and skin-prick tests and compared with a random sample of teachers (n = 18) from a control school with ventilation problems but without moisture problems (Rudblad et al., 2001). Technical investigations of the target school before renovation, which was carried out in 1993–94, showed severe dampness because of inadequate drainage and numerous water leaks from the roof causing growth of moulds in the building construction, and decomposition and discoloring of building materials. Although no systematic measurement programme was used, measurements of temperature, relative air humidity, airborne microorganisms and chemicals [total volatile organic compounds (TVOC)] showed no deviations from what is usually seen in school environments in northern countries (Smedje et al., 1997).

The renovation in the target school was directed toward the water-damages and included change of damaged building materials, reconstruction of the drainage system and removal of wall-to-wall carpets. The mechanical ventilation system in the target school was adequately functioning before the renovation and was only slightly adjusted. The cleaning routines were not changed. In the control school, however, substantial improvements were made in the ventilation system.

A significantly increased swelling of the nasal mucous membrane was demonstrated for the teachers from the school with moisture problems, although the environment had been improved 1 year earlier. This difference in nasal mucosal reactivity could not be explained by atopy or different personal characteristics. In the target school, 18% of the teachers had a positive skin-prick test compared with 17% among the teachers in the control school.

The aim of this follow-up study was to determine whether the increased nasal reactivity to histamine, measured in 1995, still persisted 2 years later, i.e. 3 years after remedial measures had been taken.

Methods
Methodology
All teachers from the study in 1995 – except one from the target school who had died – were invited to participate. The provocation tests in 1995 were performed at the hospital with a single-blind design and the investigator was not given details about the work place of the teachers. In 1997, the provocations were carried out in a room in direct connection with the respective schools.

The study groups
Twenty-six teachers from the target school (one woman declined participation because she was breastfeeding her baby) and all 18 teachers from the control school accepted to be investigated.

Questionnaire
During the period of acclimatization, before the histamine provocation, the same questionnaire as in 1995 was answered (MM 040 NA) (Andersson, 1998). The inquiry contains questions concerning perceived indoor climate during the last 3 months, experienced symptoms and their possible relationship to the work environment.

The provocation test
A standardized nasal provocation procedure, as described by Hallén and Juto was used with a histamine solution in three concentrations (1, 2 and 4 mg/ml) (Hallén and Juto, 1993). The histamine solution was applied with a syringe on the medial side of the right inferior conchae. The left inferior conchae was unchallenged and served as a control. Nasal mucosal swelling was measured with rhinostereometry.

Rhinostereometry is an optical measuring method for studying changes in congestion of the nasal mucosa. It is not invasive and allows direct determination of changes in mucosal congestion with high accuracy (±0.18 mm) (Juto and Lundberg, 1982; Rudblad et al., 2001). The method permits standardization of the nasal mucosal reaction, without interference from secretion or stenosis. After at least 15 min of acclimatization and an additional 15 min to check decreasing mucosal reactivity
the stability of the mucosal position, the provocation started with the lowest histamine concentration (1 mg/ml). Recordings of the mucosal surface, on both sides, were made after 5 and 10 min with increasing histamine concentrations every 10 min.

Exposure measurements

After renovation of the target school in 1993–94, exposure measurements were performed in both schools on three occasions. To reflect possible influence of outdoor climate on the indoor conditions, technical measurements were performed at different times of the year. The first series of measurements were performed in spring 1996, the second in autumn 1996, and the third in winter 1997. In both schools six rooms were chosen including representative classrooms, rooms for group work, and teachers offices. The measurements performed were identical on the three occasions. The following factors were studied: total concentration of airborne dust (sampling time 7 a.m. to 5 p.m. during three consecutive workdays), particle size distribution of airborne dust, indoor air temperature, relative humidity in indoor air, concentration of carbon dioxide (continuously during at least 3 days), concentration of formaldehyde (24 h), and concentration of volatile organic compounds (VOC) (14 days).

The total concentration of airborne dust was determined gravimetrically after sampling during 3 workdays, from 07.30 a.m. until 4.30 p.m. A microbalance (Cahn C-31) was used for the weighing, and the particulate matter was sampled on cellulose acetate filters (Waters) with a porosity of 0.8 μm. For one classroom the concentration was also followed continuously with a personal monitor (DataRam pDR1000) for a couple of days. Particle size distribution of airborne dust, indoor air temperature, relative humidity in indoor air, concentration of carbon dioxide (continuously during at least 3 days), concentration of formaldehyde (24 h), and concentration of volatile organic compounds (VOC) (14 days).

Sampling of volatile organic compounds was carried out by diffuse samplers (Tenax TA). Laboratory analysis was performed on a gas chromatograph with mass selective detector (Hewlett Packard HP 6890) equipped with a thermal desorber (Perkin Elmer ATD 400). Formaldehyde was sampled on a diffusive sampler based on chemisorption on a filter impregnated with 2.4 - di-nitro-phenyl-hydrazine (GMP). The analysis was performed on a high-performance liquid chromatograph (LDC Analytical System 5000).

Meteorological outdoor conditions were obtained from continuous measurements performed by the Swedish Meteorological and Hydrological Institute.

Statistics

An analysis of variance model (ANOVA) for repeated measurements was used to analyze differences in the mucosal congestion between the study groups. The analysis was aimed at modelling the mucosal swelling as a function of increased provocation levels corresponding to 1, 2, and 4 mg/ml of the histamine solution (growth curves for the swelling) including a number of additional explanatory variables. In its final form the ANOVA model included one ‘between subjects’ factor, i.e., the study group factor with different individuals in the two groups. Two ‘within subjects’ factors were included, i.e. dose and year of investigation, where repeated measurements were made on every individual for each dose and year. A third ‘within subjects’ factor, time with readings at 5 and 10 min, was considered for inclusion but in the end the estimations were performed with the 5-min readings only because this substantially reduced the number of parameters in the model. A separate analysis of the 10-min readings was carried out as a validation of the model. Age, gender, smoking habits and status of allergy were included as covariate factors. Differences in the mucosal swelling between the groups were evaluated primarily with respect to the shape, in particular the slope of the growth curves. Interactions between group, year and dose were tested sequentially, starting with those of the higher order, i.e. the third order interaction group × dose × year. Of particular interest is the interaction between group and dose as it is the ANOVA parameter of the slope of the growth curve. With a slight reformulation of the model we also estimated the average increase in swelling per 2\log per mg/ml histamine (actual doses 1, 2, and 4 which give the logarithmic values 0, 1 and 2) for each school in 1995 and in 1997. This is equivalent to an estimate of the slope in the linear regression of mucosal swelling on 2\log provocation level, restricted to the interval 1–4 mg/ml histamine solution. The estimates were supplemented with 95% confidence intervals (95% CI). In the ANOVA model two-sided tests were used with a significance level of 5%.

Differences regarding the perceived indoor climate and symptoms between the two groups were tested by application of Fisher’s exact test for bivariate and trivariate data. As the groups are fairly small we used a version of Fisher’s exact test adopted for small samples and implemented in the program StatXact (StatXact 1998).

Results

Perceived indoor climate and symptoms

The perceived indoor climate and symptoms reported by the two test groups are presented in Table 1 for the
observation years 1995 and 1997. Differences in the outcome for the two schools analyzed separately for each year resulted in significance only for dry air in 1995 where the target school had significantly higher prevalence (Fisher's exact test, \( P = 0.0002 \)). Regarding differences in improvement between 1995 and 1997 we defined three different individual outcomes as worse, equal or improvement. For dry air the target school had 1 worse, 20 equal, and 7 improvements, whereas the control school had, respectively, 2, 16 and 0. There is a significant improvement for the target school compared with the control school (Fisher’s exact test = \( P = 0.03 \)), although 31% of the teachers still complained about perceived dryness of the air. No other symptoms showed significant improvement and most individuals reported the same outcome in 1995 and 1997.

**Provocation test**

The mean congestion figures of the nasal mucosa for the two provocation tests (1995 and 1997) and the two groups are presented graphically in Figure 1. Only the outcome of the provocation side is shown because nothing but slight congestion oscillating around the baseline were registered for the control side. The three-way interaction group \( \times \) dose \( \times \) year was not significant (\( P = 0.085 \)) indicating that this factor could possibly be eliminated from the model and the two-way interactions were more important to consider. The interactions group \( \times \) year and year \( \times \) dose were not significant (\( P = 0.91 \) and \( P = 0.58 \), respectively) whereas the interaction group \( \times \) dose was highly significant (\( P = 0.0011 \)) indicating different slopes for the two groups. As the three-way interaction was not significant, we could not reject the hypothesis that the difference in slopes between the two schools was constant over the 2 years. However, as the three-way interaction on the other hand might be considered as a borderline significance (0.05 < \( P < 0.10 \)), we estimated the slope in the regression of mucosal swelling on the provocation level under two different hypotheses: the first assuming that the difference in slopes was constant between 1995 and 1997, and a second hypothesis that a change has occurred.

Under the first hypothesis we obtained for the target school a slope of 0.35 (95% CI 0.29–0.41) and for the

---

**Table 1** Perceived indoor climate and symptoms in the two study groups in 1995 and 1997

<table>
<thead>
<tr>
<th></th>
<th>Target school ( (n = 28/28) )</th>
<th>Control school ( (n = 18/18) )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stuffy ‘bad’ air</strong></td>
<td>1995: 6 (21.4) ( \times ) 2 (11.1)</td>
<td>1997: 3 (11.1) ( \times ) 0</td>
</tr>
<tr>
<td>Dry air</td>
<td>1995: 14 (50.0) ( \times ) 0</td>
<td>1997: 8 (30.8) ( \times ) 2 (11.1)</td>
</tr>
<tr>
<td>Unpleasant smell</td>
<td>1995: 3 (10.7) ( \times ) 0</td>
<td>1997: 0 ( \times ) 0</td>
</tr>
<tr>
<td>General symptoms*</td>
<td>1995: 12 (42.9) ( \times ) 7 (38.9)</td>
<td>1997: 12 (38.5) ( \times ) 8 (44.4)</td>
</tr>
<tr>
<td>Mucous-membrane irritations*</td>
<td>1995: 7 (25.0) ( \times ) 3 (16.7)</td>
<td>1997: 8 (30.8) ( \times ) 1 (5.6)</td>
</tr>
<tr>
<td>Skin symptoms</td>
<td>1995: 7 (25.0) ( \times ) 3 (16.7)</td>
<td>1997: 3 (11.5) ( \times ) 3 (16.7)</td>
</tr>
</tbody>
</table>

* The frequency of teachers often troubled by tiredness, headache, nausea, vertigo or concentration problems.

* The frequency of teachers often troubled by irritation from eyes, nose, throat or cough the preceding 3-month period.

---

**Fig. 1** The histamine provocation curves (mean values) for the two groups in 1995 and 1997. Readings for 5 as well as 10 min after provocation are shown (Table 1)
control school 0.20 (95% CI 0.13–0.28), that is, non-overlapping confidence intervals. The second hypothesis gives four different slopes: Target school 1995, 0.46 (95% CI 0.36–0.57), and 1997, 0.27 (95% CI 0.19–0.36). For the control school the slopes are for 1995, 0.18 (95% CI 0.05–0.32) and for 1997, 0.21 (95% CI 0.11–0.31). The results for the 10 min readings confirmed the results of the 5 min readings, the basic difference was that the P-value for the three-way interaction was substantially higher, $P = 0.41$. We conclude, based on the first hypothesis, that there still is a difference between the two schools, but as shown by the analysis based on the second hypothesis, the difference appears to be decreasing.

Technical measurements

The results from the exposure measurements are presented in Table 2. Repeated measurements of physical and chemical factors are normally presented as calculated mean values with standard deviations. Because of varying number of persons in the classrooms, as well as different activities during the three measuring periods, it was chosen not to calculate the mean values but present minimum, maximum and in some cases the median values. The total concentration of airborne dust was found to be low in all rooms and at the same level as found in dwellings. Continuous measurements during a couple of days showed that the concentration could vary considerably during the day. When the classroom was unoccupied the concentration was very low, less than 5 μg/m$^3$, whereas the concentration momentarily could reach values of approx. 100 μg/m$^3$ when persons entered or left the classroom.

Table 2. The results from the exposure measurements. Figures given in the table represents minimum and maximum values over the measuring periods (three occasions). The values in brackets represent the median values and the number of measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Target school</th>
<th>Control school</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total concentration of airborne dust (μg/m$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rooms for group work or teachers offices</td>
<td>2–14 [8, $n = 5$]</td>
<td>11–18 [16, $n = 4$]</td>
</tr>
<tr>
<td>Room temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classrooms</td>
<td>18–23</td>
<td>17–25</td>
</tr>
<tr>
<td>Rooms for group work or teachers offices</td>
<td>18–23</td>
<td>20–23</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classrooms</td>
<td>30–55</td>
<td>25–45</td>
</tr>
<tr>
<td>Rooms for group work or teachers offices</td>
<td>30–55</td>
<td>35–45</td>
</tr>
<tr>
<td>Carbon dioxide concentration (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classrooms$^a$</td>
<td>7/32</td>
<td>9/25</td>
</tr>
<tr>
<td>Total concentration of volatile organic compounds (μg/m$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classrooms</td>
<td>35–85 [50, $n = 12$]</td>
<td>45–210$^b$ [90, $n = 7$]</td>
</tr>
<tr>
<td>Rooms for group work or teachers offices</td>
<td>45–60 [50, $n = 6$]</td>
<td>50–110 [95, $n = 3$]</td>
</tr>
<tr>
<td>Formaldehyde (μg/m$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classrooms$^c$</td>
<td>8–14 [8, $n = 12$]</td>
<td>8–11 [8, $n = 7$]</td>
</tr>
<tr>
<td>Rooms for group work or teachers offices</td>
<td>8–13 [8, $n = 6$]</td>
<td>17–22 [22, $n = 3$]</td>
</tr>
</tbody>
</table>

$^a$ Number of classrooms with concentrations of carbon dioxide at steady-state exceeding 1000 ppm and the total number of days of measurements.

$^b$ The highest value recorded in a room where chemicals were used occasionally.

$^c$ Lowest quantification levels is considered to be 8 μg/m$^3$ at the sampling procedure used. Values below the quantification level are given the value 8.

The concentration of compounds in the air generally associated with water-damages in the construction ($n$-butanol and 2-ethyl-1-hexanol) were below the detection level (1 μg/m$^3$).

The ventilation systems were not equipped with humidifiers and therefore the relative humidity indoors was mainly a function of temperature and relative humidity outdoors. The highest values (55%) were recorded in the autumn and the lowest (30%) in the winter. When a classroom was occupied the steady-state concentration of carbon dioxide during a lesson exceeded the recommended highest level, 1000 ppm (AFS, 1993) in 25% of the classrooms in the target school and in 33% of the classrooms in the control school. The concentrations of VOCs, TVOC and formaldehyde were low and well within expected levels. Also individual organic compounds were those expected considering the type of construction and surface materials used.

Discussion

The provocation tests in 1995 and 1997 showed a statistically significant different nasal mucosal reactivity to histamine between the teachers from the two schools, although the difference in 1997 was less pronounced than in 1995.

There is a possibility that the persistent mucosal hyperreactivity among teachers in the target school more than 3 years after renovation could mean that the renovation was not successful.

According to the technical measurements, performed after the renovation, the physical environments in both schools were essentially of the same quality. Exposure
levels of most chemical factors measured were low. Occasionally the carbon dioxide concentration reached levels indicating too many persons in the classroom or improperly functioning ventilation system. Besides this the levels of measured parameters were all within limits usually seen in schools without obvious indoor-climate problems (Smedje et al., 1997).

The teachers in the target school experienced the indoor-air quality as being slightly better in 1997 than in 1995. The complaints about ‘dry air’ had decreased, although still more frequent in comparison with those of their colleagues from the control school and the perception of unpleasant smell had disappeared. The indoor climate in the target school therefore seems to have been properly improved. The perception of dry air is probably more associated with other indoor-climate factors, including temperature and chemical emissions than the physical air humidity (Sundell and Lindvall, 1993; Andersson et al., 1995).

The renovation in the target school was directed toward the water-damages and in the control school toward the ventilation problems. In fact, the difference between the schools before the renovations was the moisture problems in the target school and the ventilation problems in the control school. Therefore it seems likely that the moisture problems in the target school may have caused the observed mucosal hyperreactivity.

The provocation tests in 1995 were performed at the hospital and in the respective school in 1997. The reason for this change was partly practical because the teachers did not have to leave the school and the acclimatization before the provocations was simplified. The practical procedure was identical at the two schools. We have no reason to believe that this performance did influence the results of the provocation tests.

Our conclusion is that long-time exposure to building dampness may increase the risk for nasal mucosal hyperreactivity. Although building reconstruction measures eliminating the building dampness are performed, this hyper-reactivity may persist and only slowly decrease for years. A possible explanation to this slowly decreasing reactivity among the teachers at the target school might be an ongoing physiological changing process in the nasal mucosa.

Acknowledgments
The Research Committee of the Örebro County Council financed the primary study in 1995. This study was supported by grants from the Swedish Working Life Research Fund. The Ethical Committee of the Örebro County Council approved the study. The assistance at the provocation tests and computer handling of the data were made by research assistant Inger Fagerlund at the Department of Occupational and Environmental Medicine, Örebro Medical Center Hospital, Örebro, Sweden.

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Nasal mucosal histamine reactivity among young students and teachers, having no or prolonged exposure to a deteriorated indoor climate

**Background:** In a study performed in the spring of 1995, we found a significantly greater nasal mucosal histamine reactivity among teachers, who had worked for several years in a recently renovated moisture-damaged school, than in those in a control school. In the present study we investigated the students who begun their high-school studies at both schools in the autumn of 1995 and compared them with the teachers as regards mucosal reactivity, atopy and symptoms.

**Methods:** Twenty-eight teachers in the target school, 18 teachers in the control school and 45 students from each school underwent a nasal histamine provocation test and a skin-prick test. They also answered a standardized questionnaire.

**Results:** The teachers in both schools had more marked nasal mucosal histamine reactivity at the lowest provocation concentrations than the students. The histamine provocation curve of the target school teachers had consistently higher values than that of the students ($P = 0.0001$), but its slope and shape were similar ($P = 0.15$), while the slope of the provocation curve of the control school teachers was flatter. However, there was only a borderline significance in this respect compared to the students ($P = 0.07$). Teachers with a dry and crusty appearance of the nasal mucosa on anterior rhinoscopy reacted more strongly to histamine provocation than those without this finding ($P = 0.0004$). There was a significantly higher frequency of skin-prick test positivity (SPT+) among the students ($P = 0.03$). There were no significant differences in nasal mucosal histamine reactivity between atopic and non-atopic subjects out of pollen season.

**Conclusions:** Teachers had a significantly greater mucosal histamine reactivity than the students, whereas the latter had a significantly higher frequency of atopy. These results are compatible with an age-related pattern of mucosal reactivity. A crusty appearance of the nasal mucosa seems to predispose to an increase in histamine reactivity. There were no significant differences according to histamine reactivity between atopic and non-atopic subjects.

Office workers (1, 2), school personnel (3, 4) and the general population (5, 6) commonly complain about poor indoor air quality. Many studies have shown a relationship between staying in damp buildings and irritative symptoms from the mucous membranes of the airways (7, 8).

The indoor environment is also one of many factors discussed as contributing to an increase in the prevalence of atopy in western industrialized countries, mainly in young people (9). However, it is probably much less important than a lower exposure to bacterial infections and changes in eating habits early in life connected with the western lifestyle (10–12).

Many houses built during the last few decades are airtight and poorly ventilated. Irritating volatile organic and inorganic compounds in the indoor air may, under these conditions, emanate from building materials and act as adjuvant factors for allergic sensitization (13, 14). A high level of indoor humidity may also provide optimal conditions for the growth of mites and allergy to them (15, 16).

IgE-mediated diseases of the airways (asthma and rhinitis) are usually accompanied by a contemporary hyperresponsiveness to non-allergic stimuli, such as cold air, dust and various irritants (17, 18).

It seems likely that an allergic inflammation affects the mucosal sensory nerves, which become hyperreactive (19). However, some studies also indicate a mucosal hyperresponsiveness out of season in patients suffering from seasonal allergy to pollen (20, 21).
Among persons who develop symptoms of chronic rhinitis, the proportion with a non-allergic aetiology increases steadily with age and exceeds 60% after the fifth decade of life (22). This suggests that age-related changes in mucosal function, at least partly, are responsible for the increase in mucosal reactivity.

Histamine, a non-allergic stimulant, routinely used for nasal provocation induces sneezing, rhinorrhea and nasal congestion in people with allergic and/or non-allergic rhinitis as well as in healthy persons (23, 24). The nasal congestion can be assessed by various methods i.e. acoustic rhinometry, rhinomanometry or nasal peak flowmetry (25, 26). All of these methods are indirect and the values depend on a combination of mucosal swelling and accumulated secretions in the nasal cavity.

Rhinostereometry is an optical high-precision method which allows direct inspection of the nasal mucosa with the subject held firmly to an individually formed tooth-splint (27). By this method, one could confirm the presence of an increased reactivity to histamine, measured as mucosal swelling, in non-atopic tenants living in a residential area with severe indoor climate problems compared to people living in a control area without these problems (28).

We have previously shown that teachers who had been working for a long time in a water-damaged school with moisture problems had increased mucosal sensitivity of the upper airways subjectively (as shown in questionnaires) and objectively (using nasal histamine provocation) vs. teachers in a control school (29). This was found although the target school had been renovated more than one year before the study. We therefore considered it necessary to do a follow-up study of the students beginning their high-school education at the two schools. This also gave us an opportunity to compare the students and the adult teachers.

In this study we aimed to determine how students in these two schools, beginning their first grade of senior education at the two schools, beginning their first grade of senior education, were asked to participate in the study. Forty-five of them from each school accepted and formed the study groups from the target and the control school.

To judge the presence of a selection bias the students in the first group of randomly selected students (50 at each school), who had declined to participate, were offered a simplified test procedure including a skin-prick test and answering the questionnaire, but without the nasal histamine challenge. Fifty-four (27 from each school) of these students had already undergone the complete investigation and now a further 38 students (19 from each school) agreed to take part in the skin prick test. This means that a total of only eight students gave no information at all in the first selected group. The studies were done in the autumn of 1995 about 2 months after the start of the term.

Questionnaires
A validated standardized questionnaire (MM 040 NA) was used for the teachers and a somewhat modified one (MM 060 NA) for the students (31). They both contained the same questions about the perceived indoor climate, symptoms often referred to in indoor climate research and allergic manifestations specified in Table 2 and some background factors (age, gender, smoking habits, etc.). For the questions about the indoor air quality and symptoms a recall period of 3 months was used and three alternatives were given, ‘yes, often’ (every week), ‘yes sometimes’ and ‘no, never’. Only the first alternative was interpreted as a positive outcome.

Skin-prick test
All teachers and students underwent a standardized skin-prick test (32). The test panel consisted of allergens against pollen (birch, grass and mugwort), mites (D. pteronyssinus and D. farinae), furred animals (cat, dog and horse) and moulds (Cladosporium, Alternaria and Aspergillus fumigatus) besides negative and positive histamine controls. Skin-prick test positivity (SPT+) was defined as the formation of a wheal of a mean diameter of at least 3 mm in response to any of the allergens used and atopy as the combination of SPT+ and reported allergic manifestations.

Provocation test
The nasal histamine provocation test in the teachers was done in a single-blind manner at the hospital outside the two schools (29). To simplify transport and avoid unnecessary exposure to cold air before the provocation, the students were investigated in the respective schools.

A standardized provocation test described by Hallén and Juto (33) involves the use of three concentrations of a histamine solution (1, 2 and 4 ng/ml). After 15 min of acclimatization and a further 15 min to assess the stability of the nasal mucosal position, the provocation started with the lowest histamine concentration (1 mg/ml). Recordings of the mucosal position on both sides of the nose were made with rhinostereometry (27) after 5 and 10 min with an increase in the histamine concentration each 10 min.

Anterior rhinoscopy was done before provocation. The findings were divided by the examiner, a physician with considerable experience, into two groups i.e., with or without a dry and crusty appearance of the nasal mucosa.

Material and methods
Study populations
A random sample of teachers who had worked for at least 5 years in the renovated water-damaged school (39 persons) and all teachers in the control school who fulfilled the same inclusion criteria (30 persons) were invited to take part in the study in the early spring of 1995. Twenty-eight and 18 teachers, respectively, accepted and formed two study groups.

A random sample of 180 students (90 from each school), beginning their high-school studies, were asked to participate in the study. Forty-five of them from each school accepted and formed the study groups from the target and the control school.

To judge the presence of a selection bias the students in the first group of randomly selected students (50 at each school), who had declined to participate, were offered a simplified test procedure including a skin-prick test and answering the questionnaire, but without the nasal histamine challenge. Fifty-four (27 from each school) of these students had already undergone the complete investigation and now a further 38 students (19 from each school) agreed to take part in the skin prick test. This means that a total of only eight students gave no information at all in the first selected group. The studies were done in the autumn of 1995 about 2 months after the start of the term.
Statistical analysis

The two study groups of teachers and the two study groups of students, concerning nominally scaled variables, were compared with Fisher’s exact test specially adapted for use in small samples (34). An analysis of variance model (ANOVA) for repeated measurements was used to analyse differences in mucosal congestion. It aimed at modelling the growth curves for mucosal swelling as a function of the provocation concentrations of 1, 2 and 4 mg/ml of the histamine solution. The main group factor or ‘between subjects’ factor was divided into four categories. In the sequel, the factor is denoted simply by the group and it is composed of the fourfold combination of two factors, i.e., school (target or control) and person category (teacher or student). Two ‘within subjects’ factors were included, i.e., dose (with levels 1, 2 and 4 mg/ml, as defined above) and time (5 and 10 min after each provocation). The ‘between subjects’ and ‘within subjects’ factors and interactions between these were the main explanatory variables in the model, but a set of additional variables was also included in the analysis to control for confounding, i.e., as covariate factors (gender, smoking habits and allergic manifestations). We also analysed the effect of a dry crusty appearance of the nasal mucosa. These analyses were done only in the teachers. We introduced crusty appearance as an additional covariate in the analysis of both the baseline values and the whole provocation curve. In all statistical tests, two-sided tests were used with a significance level of five percent. The ANOVA was first done with all interactions included and followed by a sequential search for parsimonious models where interactions of no statistical significance were excluded. Evaluations of the goodness-of-fit of the models were done with Akaike’s information index (35).

When a final ANOVA model had been found we tried to describe the basic shape of the provocation curves by slightly reformulating the model. In this model we estimated the linear regression of an increase in swelling per ²log mg/ml histamine (doses 1, 2 and 4 mg/ml which give the logarithmic values 0, 1 and 2) for each school and category. We also determined the 95% confidence intervals (95% CI) for the estimates of the intercept and slope in these regressions.

In addition we analysed differences in the baseline values (at provocation level 1 mg/ml) with the ANOVA model. Only the ‘within subjects’ factor time (5 and 10 min) was included in this model, but the school and person category were included as ‘between subjects’ factors.

In a supplementary ANOVA model, atopy was introduced as an additional factor.

Results

Study population

Table 1 shows a few characteristics of the students and teachers in the two schools. The proportion of boys was higher in the target school than in the control school, but it only reflects the gender ratio in the two schools (47.9% vs. 33.4% boys in the target and control schools, respectively). A description of the teachers with an analysis of the completion rate, has been reported in more detail elsewhere (29).

Some data on the primary randomly selected 100 students are given in Table 2. Fifty-four students took part in the histamine provocation study and 46 declined to participate, mostly because they had no time or did not feel like it.

<table>
<thead>
<tr>
<th>Provocation group</th>
<th>Target school</th>
<th>Control school</th>
<th>Target school</th>
<th>Control school</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n = 45</td>
<td>n = 45</td>
<td>n = 28</td>
<td>n = 18</td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>46.7</td>
<td>28.9</td>
<td>46.4</td>
<td>66.7</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>16.1</td>
<td>16.1</td>
<td>51.4</td>
<td>50.6</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>15.6</td>
<td>13.3</td>
<td>7.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 2. Comparison between students who took part in the histamine provocation test (provocation group) and those who declined to participate (non-provocation group) among the first randomly selected 100 students. Q = questionnaire, P = prick test, Hi = histamine provocation

Thirty-eight of these 46 students who declined to participate in the provocation test agreed to have the skin-prick test and answered the questionnaire. There was no selection of students to participate in the histamine provocation study according to reported allergic manifestations (asthma/hay fever), mucosal symptoms or positive skin-prick test (SPT+). The participation rate of girls was higher.

Reported allergy and symptoms

The reported prevalence of allergic manifestations was similar in students and teachers in the target school, but the teachers in the control school reported a somewhat lower prevalence. However, the overall difference between students and teachers was not significant, $P = 0.52$.

(3 Table). Teachers had a higher frequency of mucosal symptoms (21.9%) than students (10.0%), but the difference was only of borderline significance, $P = 0.07$.

Skin-prick test

The frequency of SPT+ and the prevalence of mucosal symptoms are shown in Table 3. The total frequency of SPT+ among the students was much higher than in the

Nasal mucosal histamine reactivity

Table 1. Some personal characteristics of the students and the teachers at the two schools
adult teachers (35.6% vs. 17.4%, $P = 0.03$). The frequencies of SPT+ teachers and students were similar in the two schools. 75% (6/8) of the SPT+ teachers and 69% (22/32) of the SPT+ students reacted to pollen allergens.

The provocation test
The results of the histamine provocation test in the students and teachers on the provoked side of the nasal cavity are given in Table 4 and Fig. 1. Only small oscillations around the baseline were seen on the control side. The ANOVA analysis of the baseline values (provocation concentration 1 mg/ml) showed a borderline statistical significance for the interaction factor person category and time after provocation, $P = 0.07$, primarily due to an increase in the value at 10 min in teachers at the target school. Of greater relevance and more statistical significance is the difference in the main factor teachers vs. students, $P = 0.0001$, with students having much lower values than teachers (on average, about half the teacher's value).

Since we had previously determined separated provocation curves in the teachers in both schools, from 1 mg/ml to 4 mg/ml (29), it was only necessary to compare the students at the schools with the teachers. The first ANOVA model with all main factors and interactions showed that the interaction group × concentration level was highly significant, $P = 0.008$, which indicated that the four groups had different provocation curves. Figure 1 shows that the statistical significance is due to two features, i.e., the curves are different in the two groups of teachers and those of the students are much lower than those of the teachers. We therefore did stratified subgroup analyses of the person category (teachers and students separately) and school (target school and control school separately).

The separate analysis of the teachers is mentioned above. Since we found no difference between students in both schools in any respect, they can be viewed as a homogeneous group. Our analysis of each school showed that the provocation curve of the target school teachers had consistently higher values than that of the students, $P = 0.0001$, but its slope and shape were similar, $P = 0.15$. In the control school, however, the slope of the curve was of borderline significance, the interaction category × provocation concentration had $P = 0.07$.

Table 5 summarizes the essential features of the analysis of the provocation curves. We expressed mucosal swelling as a linear function in the logarithms of the provocation concentration separately for 5 and 10 min. The differences and similarities in intercepts and slopes in the four groups accord with our results from the ANOVA model. Students had less mucosal swelling than teachers, as shown by the intercepts, but there were few, if any, differences between the students in the two schools, as shown by both the intercepts and the slopes. The reactions of the teachers at the control school to the provocation concentration differed considerably from their colleagues at the target school, and those of both groups of students were intermediate...
compared to the groups of teachers, as assessed by their slopes.

A separate analysis of differences between atopic and non-atopic subjects showed no significant differences in the provocation curves (Fig. 2).

The rhinoscopic examination of the teachers showed that 15 had a dry and crusty mucosa and 31 did not. No significant difference was found between teachers in the target school and those in the control school (10/28 and 5/18, respectively, *P* > 0.50). Among the students less variation was noted in the rhinoscopic findings and only one had such a finding on rhinoscopy, although seven students had a slightly dry mucosa.

When a crusty appearance was included as a covariate in the analysis of the baseline values and of the whole provocation curve (target school vs. control school, teachers only), we found a definite statistical significance for the crusty appearance, *P* = 0.002, in the analysis of baseline values and *P* = 0.0004 in the analysis of the provocation curves. However, more importantly, the significant difference between the target and control schools in the latter analysis did not disappear with the introduction of a crusty appearance, *P* = 0.002 for the different slopes.

**Discussion**

The nasal histamine provocation tests differed markedly between teachers and students as regards nasal mucosal histamine reactivity. The fact that even the teachers from the control school showed more marked mucosal swelling in response to the lowest histamine provocation concentrations than the students suggests that age and/or various types of environmental exposures are of importance. Such a mucosal reaction pattern may partly be due to a lowered number of goblet cells (36) and a reduced secretory ability with age (37), which might lead to a lack of protective mucus layer and make the mucosal sensory nerve endings more sensitive to nonspecific stimulation. Among the teachers the curves for mucosal swelling, based on the rhinoscopic findings, rose sharply in those with a dry and crusty nasal mucosa. The development of such a dry mucosa may be constitutional, but it may also be age dependent, and predispose to an increase in mucosal reactivity to histamine.

The difference in provocation curves between teachers from the target and control schools could not be explained by differences in appearance of the nasal mucosa. It is also noteworthy that in another study, using a somewhat modified histamine provocation procedure, the shape and level of the curve for mucosal swelling in the teachers in the control school resembled that of a group of people living in a residential area with no indoor air problems (28).

The teachers in the control school had only a moderate increase in mucosal swelling when exposed to the highest histamine concentration compared to the students. The marked mucosal reaction to the lowest histamine concentration may induce tachyphylaxis of the neural response during repeated stimulation. This is unlike the teachers in the target school who reacted most to the highest histamine concentration. Their curve for mucosal swelling closely resembled that of the students, but at higher levels.

As expected, the students reported mucosal symptoms less frequently than the teachers in the renovated moisture-damaged school. The difference was of only a borderline significance, but this may have been because of the small number of teachers in the study group.

The frequency of SPT+ was at least twice as high among the students as in the teachers (36% vs. 17%) and comparable to other studies of school children (38). This accords with an increasing frequency of atopy in the western world (7–9) and indicates that the increase in nasal histamine reactivity among the teachers at the
renovated damp school is not related to an increased frequency of manifestations of atopy. Indeed, the relationship between working and/or residing in these buildings and increased mucosal reactivity seems to be due to emissions in the air from the moisture-damaged foundation by microbial growth or chemical degradation of the material (39–41).

No differences in nasal histamine reactivity were found between the students at the two schools. More girls participated in the provocation test, but the frequency of symptoms from the mucus membranes and reported allergic manifestations as well as the frequency of SPT + did not differ between those who participated in the provocation test and those who declined to do so.

The study was done outside the pollen season and no significant differences in mucosal swelling curves due to histamine provocation were seen between atopic and non-atopic subjects. Although some studies show an increase in nasal mucosal reactivity to histamine among young atopic subjects outside the pollen season (20, 21), it seems that the ongoing mucosal inflammation among young atopic during pollen season may disappear almost entirely outside the pollen season. Nor could we find a significant difference in this matter between atopic and non-atopic teachers but the group of atopic teachers (five subjects) is obviously too small to draw any further conclusions.

A limitation of this study was the relatively small number of teachers in both groups. Data in other studies using the same method, however, indicate that differences between groups can be detected in as few as a dozen subjects (28).

In conclusion we found a significantly greater mucosal histamine reactivity among the teachers than in the students, although the students had a significantly higher frequency of manifestations of atopy. The teachers in the control school showed a higher reactivity to histamine with the lowest provocation concentration and the curve for mucosal swelling was flatter compared to that of the students. In teachers with a dry and crusty nasal mucosa, based on the rhinoscopic findings, the curves for mucosal swelling were markedly increased. The findings accord with an age-related pattern of mucosal reactivity. No significant differences in nasal histamine reactivity was found between atopic and non-atopic subjects out of pollen season.

Acknowledgments

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References

Nasal mucosal histamine reactivity


Nasal histamine reactivity among adolescents in a remediated moisture-damaged school – a longitudinal study

Abstract In a previous study, in the spring of 1995, we found that teachers, who had been working for several years in a moister-damaged school, 1 year after the renovation still reported a higher frequency of complaints and symptoms and showed significantly higher mucosal histamine reactivity compared with teachers in a control school, although the school seemed to be properly renovated. A longitudinal study of 90 randomly selected senior high school students entering the two schools was initiated to exclude or verify if the indoor air still exerted an irritant effect on the upper airways of an earlier unexposed group. The students went through a nasal histamine provocation test and answered a questionnaire on three occasions, in 1995, 1996 and 1997. No significant differences in the nasal histamine provocation curves for the students at the target school and those at the control school could be shown from start to endpoint of the study period. Neither was there any differences concerning perceived indoor air or mucosal symptoms between the target and the control group and technical measurements showed no noteworthy differences between the two schools. In conclusion, this study indicates that based on both technical and objective medical measures, the current indoor air in the remediated moisture-damaged school does not exert an irritant effect on the upper airway mucosa of the students. A 2-year follow-up of the teachers showed a decreased reactivity to histamine, giving further support to this statement. The increased mucosal reactivity observed among the teachers is probably a result of the previous long-term exposure to building dampness. No differences were seen between atopic and non-atopic students.

Practical Implications Persistent symptoms and increased nasal mucosal reactivity among personnel in a remediated damp building does not necessary imply an inadequate renovation. A longitudinal study with registration of subjective (questionnaires) and objective (nasal histamine reactivity) data of an earlier unexposed group residing in the same building further contributes to the evaluation whether the renovation was successful or not.

Introduction

Concern about possible health effects resulting from indoor air pollution is increasing. Disorders that have been associated with indoor air pollution include asthma, allergies and non-specific eye, upper airway and facial skin symptoms (Kreiss, 1989; Norbäck, 1997). During recent years there has been growing concern about possible health effects due to indoor air pollution in schools with respect to building-related asthma (Smedje et al., 1997a; Taskinen et al., 1997) allergies (Taskinen et al., 1997) and irritative upper respiratory tract symptoms (Rudblad et al., 2001; Wälinder et al., 1998).

Students’ perceptions of indoor air quality (IAQ) are currently the focus of increasing attention. Among 16–19-year-old students, poor IAQ in school was reported more frequently by those with atopy compared with those without, which could be an expression of greater sensitivity to the IAQ in students with atopy (Lundin, 1999). Allergen contact can cause not only immediate symptoms of asthma, but also a prolonged period of bronchial hyperreactivity (Cockerod, 1983). In Scandinavian schools the main allergen exposure problem seems to be that of pet allergens (Dotterud et al., 1997; Munir et al., 1993).

Mold exposure at schools and its relation to respiratory symptoms has been investigated. Students
attending a moisture-damaged school were found to have more asthmatic symptoms (Rylander et al., 1998; Taskinen et al., 1997). Exposure to moldy building environments has been associated with upper (nasal) and lower respiratory symptoms (Dahlqvist and Alexandersson, 1993; Hirvonen et al., 1999; Rudblad et al., 2001).

Nasal symptoms are common even among healthy individuals (International Rhinitis Management Working Group (IRMWG), 1994), and in buildings with indoor air problems there has been a high prevalence of nasal symptoms among reported work-related symptoms (Hedge et al., 1989; Jaakkola et al., 1991). Environmental factors that may cause nasal reactions include environmental tobacco smoke (Bascom et al., 1996), formaldehyde (Pazdruk et al., 1993), mites (Garrelds et al., 1995), volatile organic compounds (Möhlave et al., 1993) and wood-dust (Ahman et al., 1995).

Several studies have had problems verifying a relationship between measured low-level indoor air pollution and reported symptoms (Andersson et al., 1997; Peat et al., 1998). In an investigation of school environments, it was difficult to find a correlation between subjective IAQ and ventilation flow or carbon dioxide concentration (Smedje et al., 1997b). Psychosocial factors and exposure to low concentrations of several pollutants may contribute to this complexity (Oliver and Shackleton, 1998; Stenberg et al., 1994).

There has been a growing demand for sensitive and reproducible tests that can objectively register the effects of a bad indoor climate. Acoustic rhinometry, a method that has become increasingly common in recent years, measures the minimal cross-sectional area of the nasal cavity through changes in acoustic impedance. With this method it was possible to find a relationship between dust accumulation in classrooms and nasal obstruction among school personnel (Wählinder et al., 1999). Rhinostereometry is a direct optical method with high accuracy (Juto and Lundberg, 1982) that has been used in a nasal histamine provocation test to record an increased mucosal swelling reaction in a group of people exposed to severe indoor climate problems as compared with a control group (Ohm et al., 1997).

In a previous study, in the spring of 1995, we found that teachers who had been working for several years in a moisture-damaged school still reported a high frequency of perceived poor IAQ (especially the experience of ’dry and stuffy bad air’) and symptoms of mucosal irritation, although the school in 1993–94 seemed to have been properly renovated. The remedial measures in the target school had been directed toward the water damages and included change of damaged building materials, reconstruction of the drainage system and removal of wall-to-wall carpets (Rudblad et al., 2001). The teachers were exposed to a nasal histamine provocation test (Hallén and Juto, 1993).

Mucosal swelling was registered with rhinostereometry (Juto and Lundberg, 1982). The provocation test showed significantly higher mucosal histamine reactivity among teachers at the remediated moisture-damaged school as compared with teachers at a control school. The persistent complaints of poor indoor air climate, mucosal irritative symptoms and an increased nasal histamine reactivity among the teachers who were exposed over a long period to building dampness raised the question of whether these symptoms and findings were an expression of previous exposure or if the current indoor climate still exerted an irritant effect on the airways. We therefore decided to initiate a longitudinal study of ‘non-exposed’ high school students attending the remediated moisture-damaged school and the control school in the fall of 1995. The question at issue was whether the symptoms and/or the nasal mucosal reactivity among the students at the target school would change during their 3 years of high school studies as compared with the students at a control school. We also wanted to see if atopic students differed in this respect during the study period as compared with non-atopic students.

**Methods**

**Methodology**

Students attending the target school and the control school in 1995 were followed with nasal histamine provocation tests on three occasions during their 3 years of high school studies.

The tests were carried out at approximately the same time each year, i.e. the late fall (about 2 months after the start of the term) of 1995, 1996 and 1997.

The students also had a skin-prick test (SPT) in 1995, and they answered the same questionnaire on all three occasions. As we expected a relatively heterogeneous background of exposure among the students, we also performed technical investigations and measurements of their home environments.

**Study population**

In the first wave (Table 1), on the basis of class-lists of all students, a random sample of 100 students (50/50 from the remediated moisture-damaged school and the control school respectively) who began their senior high school studies in 1995 were invited to participate in the study. Fifty-four (27/27) accepted to participate. In the second wave, in order to increase the statistical power of the study, another 80 students (40/40) were randomly selected from the same class-lists and invited to participate. Thirty-six students (18/18) accepted this time to participate, which means that a total of 90 students (45/45) took part in the study. In order to estimate the possibility of a selection bias, those
students among the initially randomly selected first wave (100 students) who refused participation (23/23) were offered a simplified test procedure including questionnaire and an SPT, but without the nasal histamine challenge. Thirty-eight students (19/19) accepted this reduced test procedure. This means that only eight students left no information at all in this initially selected group.

The number of subjects in the two study groups is identical in all phases but that is simply what happened by chance. The proportion of boys who participated in the study was lower in the control school than in the target school, but this reflected the actual proportion of students attending the two schools (48% vs. 33% boys in the target and control schools respectively).

The first investigation was performed in the fall of 1995. The next two investigations were performed in the fall of 1996 (38/38 participated) and the fall of 1997 (38/35 participated). In 13 cases the reason for non-participation in 1996 and/or 1997 was that the students no longer attended the school in question. In no case health reasons were reported as the reason for leaving school or changing schools. One student in the target school who had participated in 1995 but not in 1996 took part also in 1997. Thus, 37 students from the target school (37/45, 82%) and 35 students from the control school (35/45, 78%) participated in all investigations during the study period, including nasal histamine provocation tests.

Exposure measurements
Exposure measurements were performed in both schools on three occasions (May 1996, October 1996 and February 1997) to cover different seasons. Airborne dust was determined gravimetrically on a microbalance, particle size distribution by a light scattering instrument, temperature and relative humidity by a hygro thermograph, concentrations of carbon dioxide (CO₂) by a photo-acoustic instrument, concentration of volatile organic compounds (VOC) by adsorption on Tenax followed by gas chromatography–mass spectrometry analysis and formaldehyde by adsorption on 2,4-di-nitro-phenyl-hydralazine-impregnated glass fiber filters followed by high-performance liquid chromatography. All samplings and direct recordings were carried out continuously for at least 3–14 days. Finally meteorological outdoor conditions were obtained from continuous measurements performed by Swedish Meteorological and Hydrological Institute.

The results of these measurements were presented in another paper concerning the teachers at the two schools (Rudblad et al., 2002). To summarize, it was shown that the physical environments in the two schools were of essentially the same quality. Exposure levels of most chemical factors measured were low. The concentration of the compounds n-butanol and 2-ethyl-1-hexanol, generally associated with the influence of too high moisture on adhesive bonded PVC and linoleum flooring, were below the detection level (1 µg/m³). The carbon dioxide concentration in both schools occasionally reached levels indicating too many persons in the classroom or an improperly functioning ventilation system. Other than that the levels of measured parameters were all within limits usually seen in schools without obvious indoor-climate problems (Smedje et al., 1997b).

Technical measurements of the home environments
During March–May 1996, 81 of 90 home environments were examined (39/42, respectively, of the target group and control group environments). Dropouts were due mainly to a recent move to a new home, difficulty in finding a suitable time during the limited time of the investigation or, in a few cases, unwillingness to participate. Moisture and mold damage in different rooms and spaces were noted, and a question was posed as to whether there was condensation on the inside of bedroom windows in the wintertime, which is an indication of insufficient ventilation. A dichotomous moisture-damage index was constructed where a value of 1 meant the presence of moisture damage in the basement, the apartment or in bathrooms, or a perceived smell of mold at the time of the examination. Experienced building engineers estimated the index assigned.

Room temperatures and relative humidity were measured in 76 of the homes (36/40 respectively). The additional dropout here was because of technical
problems. The average indoor temperature in the students’ homes was measured during the heating season with an integrated electronic device over a period of approximately 30 days. Relative humidity in the indoor air was determined in parallel with the temperature using a diffusive sampler containing lithium chloride monohydrate as absorption compound. Changes in the mass of the absorption medium, determined by weighing, vary linearly with the relative humidity. The accuracy of measurements of room temperature was ±0.3°C and for relative humidity it was ±1%. The internal moisture supply (difference between the absolute water content in indoor and outdoor air), as an indirect measure of ventilation function, was calculated from measured indoor and official outdoor data.

Questionnaires

A validated, standardized questionnaire (MM060NA) was answered by the students (Andersson, 1998). It contained questions about perceived indoor climate and about symptoms and their presumed relationship to the school environment. There were also questions about some background factors including age, gender, smoking habits and cumulative prevalence of allergic manifestations (Have you ever suffered from hay fever? Have you ever had asthmatic problems?). The same questionnaire was used on all three occasions (1995, 1996 and 1997).

In 1996, two additional questions were asked in order to determine the current prevalence of allergic symptoms [Have you had allergic eye or nose symptoms (itching, sneezing, runny eyes/nose) during the past 12 months? Have you had asthmatic symptoms during the past 12 months?).

Skin-prick test

All students underwent a standardized SPT in 1995, at the beginning of the study (Dreborg, 1987). The test panel consisted of allergens for pollen (birch, grass and mug worth), mites (d. pteronyssinus and d. farinae), furred animals (cat, dog and horse) and molds (cladosporium, alternaria and aspergillus fumigatum), in addition to negative and positive histamine control. SPT positivity (SPT+) was defined as a weal with a mean diameter of at least 3 mm in response to any of the included allergens. Atopy was defined as SPT+ and a reported cumulative prevalence of allergic manifestations in 1995 (Johansson et al., 2001).

Provocation test

To avoid transportation problems and unnecessary exposure to cold air prior to provocation, tests carried out on the students were performed at the respective schools. A standardized provocation procedure described by Hallén and Juto (1993) was used, comprising the use of three concentrations of histamine solution (1, 2 and 4 mg/ml). Histamine dihydrochloride (0.14 ml) was applied with a syringe to the medial part of the right inferior turbinate, one of the most reactive parts of the nasal mucosa. The left side served as a control. After 15 min of acclimatization and an additional period of approximately 15 min to check the stability of the nasal mucosal position, the provocation was started with the lowest histamine concentration (1 mg/ml). Recordings of both sides of the mucosal position were made after 5 and 10 min using rhinostereometry, with increasing histamine concentrations every 10 min.

Statistical methods

Analysis of variance models (ANOVA) for repeated measurements were used to analyze differences in mucosal congestion between the groups. The analyses aimed at modeling the mucosal swelling as a function of increased provocation levels corresponding to 1, 2 and 4 mg/ml of the histamine solution (growth curves for the swelling), and including a number of additional explanatory variables. In its most expanded form the first ANOVA model included one between-subjects factor, i.e. the study group factor with different individuals in the two groups (target school and control school). Two within-subjects factors were included, i.e. dose and year of investigation, where repeated measurements were made on every individual for each dose (three levels, i.e. 1, 2 and 4 mg/ml) and year (three levels, i.e. 1995, 1996 and 1997). A third within-subjects factor, time with readings at 5 and 10 min, was also considered. Gender, smoking habits and atopy were included as co-variate factors. Differences in the mucosal swelling between the groups were evaluated primarily with respect to the shape, in particular the slope of the growth curves. Interactions between group, year, time and dose were tested sequentially, starting with those of the highest order, i.e. the fourth order interaction school × dose × year × time. The interaction between school and dose is of particular interest, as it is the ANOVA parameter of the slope of the growth curve. The interaction of the third order, school × dose × year, shows if the provocation curves for schools and years are parallel or not. Starting from the full model with all factors and interactions included, we tried to formulate a more parsimonious model where the essential features of the data were still present but fewer factors and interactions had to be included and estimated.

The second ANOVA analysis was therefore specifically designed for analyzing the differences at the endpoint, 1997, taking into consideration the baseline
measurements in 1995. Since the students had not been randomly allocated to the two groups, differences at baseline may have existed, although we previously reported that in comparison with the teachers, the students constituted a fairly homogenous group (Rudblad et al., 2002). In the analysis, the outcome was the measurements in 1997, and the factors being studied were school, dose, and time, and the covariates were the baseline values for 1995 as well as gender, smoking and atopy. In addition to the introduction of atopy as a covariate in our models, we also performed separate analyses with atopy as a main between-subjects factor. These analyses were performed both according to the first, enlarged ANOVA model and the second model based on the 1997 measurements.

Different specifications of the correlation structure of the repeated measurements were also tested, and in our search for the best models we used Akaike's (1973) information criterion for evaluating the goodness-of-fit of the models.

To analyze differences in self-reported symptoms between the two schools, we used logistic regression analysis and chi-square tests. The main analysis was performed using the symptoms reported in 1997. They were analyzed by logistic regression analysis, and the factors included in the logistic model were school, gender, smoking, atopy and the symptoms reported in 1995, i.e. the baseline values. Interaction factors were introduced and tested with likelihood ratio tests. We performed a secondary analysis of the baseline values from 1995, and used a chi-square test for 2 by 2 tables with continuity correction.

Results

Evaluation of selection bias

Of the initially randomly selected 100 students in 1995 (Table 1), 54 took part in the histamine provocation study and 46 declined to participate, mostly with the explanation that they had no time or did not feel like it. Data (SPT and questionnaire) were obtained from 38 of these 46 students who refused the provocation test. There was no selection of students who participated in the histamine provocation study according to reported allergic manifestations [25% (13/53) and 29% (11/38) respectively], mucosal symptoms [11% (6/54) and 8% (3/38) respectively] or positive SPT [33% (18/54) and 29% (11/38) respectively]. The participation rate was higher for girls than for boys [61% (33/54) and 29% (11/38) respectively].

Technical investigation of home environments

The results of the technical investigations and measurements of the home environments are shown in Table 2. Students at the target school more often came from the suburbs and lived more often in multi-family houses. Their homes less often had natural ventilation, condensation on the bedroom windows in wintertime or a high internal moisture supply, all of which are indicators of better ventilation than in the homes of students at the control school. There were, however, no important differences in the homes of the students at the target and control schools with respect to room temperature (mean values 22.2/22.5°C respectively) or relative humidity (mean values 43/42% respectively).

Questionnaires

The results of the questionnaire surveys in 1995, 1996 and 1997 are shown in Table 3. Complaints about variable and low room temperatures were more frequent at the target school during the years of the study, while the perception of air quality was similar at the two schools. The prevalence of mucosal and dermal symptoms was low and fairly similar in the two schools and did not change during the study period. General symptoms decreased in the target school and increased in the control school. However, the differences in prevalence in 1997 did not reach statistical significance, \( P > 0.05 \).

Symptoms and perception of the indoor climate among atopic and non-atopic students are shown in Table 4. No significant differences regarding the perceived indoor air climate or mucus membrane irritation were seen between atopic and non-atopic students, and we did not observe any statistical interaction between school and atopy. However, numbers in this more detailed comparison were small.

Table 2. Results from the technical investigations and measurements of the students' home environments

<table>
<thead>
<tr>
<th>Factor</th>
<th>Target school</th>
<th>Control school</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of the home (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City center</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Suburban area</td>
<td>39</td>
<td>10</td>
</tr>
<tr>
<td>Residential area</td>
<td>44</td>
<td>69</td>
</tr>
<tr>
<td>Thinly populated area</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Ventilation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>33</td>
<td>71</td>
</tr>
<tr>
<td>Mechanical</td>
<td>64</td>
<td>29</td>
</tr>
<tr>
<td>Mixed</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Condensation on bedroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>windows in wintertime</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Moisture damage (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In basement/apartment level</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>In bathrooms</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Smell of mold</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Moisture index(^a)</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>Internal moisture supply (^b)</td>
<td>Homes with &gt;2 g/m²</td>
<td>11% (4/36) 18% (7/40)</td>
</tr>
</tbody>
</table>

\(^a\) At least one moisture-damage sign (see text)

\(^b\) Difference between the absolute water content in indoor and outdoor air
The frequency of SPT positivity among the students in 1995 was 36% at each school (16/45), which is in good accord with results from other studies of young people (Bråbkäck et al., 1994). In 1996, 70% (19/27) of the SPT+ students reported having had symptoms of nasal allergy and/or asthma during the past year. During the same time, 18% (9/49) of the SPT− students reported having had symptoms of nasal allergy, but there were no reports of asthma. Analyses of differences between SPT+ students and SPT− students, analogous to that in Table 4, showed differences for general and mucosal symptoms, with the SPT− students having a higher prevalence. This difference was only seen at baseline in 1995, and at the end of the study period the prevalences were similar.

Provocation test

The results of the provocation tests are shown in Table 5 and Figure 1. In the figure, the swellings for 5 and 10 min after provocation are averaged so that the figure is easier to interpret, and therefore the time factor is not shown. It is evident that the target school had a somewhat different provocation curve in 1996, both in within-group comparisons as well as between-group comparisons. The ANOVA with outcome values for 1995, 1996 and 1997 gave a borderline significance for school, and for dose and time (P < 0.001 for both). For all factors where school was included as a covariate baseline (1995), gender, smoking and atopy were also covariates in addition to the atopy factor. A chi-square test was performed (with continuity correction) on the baseline values in 1995. No tests were carried out on 1996 values.

Table 3 Symptoms and perception of indoor climate among the students at the two schools for the period 1995–97

<table>
<thead>
<tr>
<th>Factor</th>
<th>Year</th>
<th>Target school (%)</th>
<th>Control school (%)</th>
<th>PValues for group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varying room temperature</td>
<td>1995</td>
<td>9</td>
<td>2</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>26</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>42</td>
<td>17</td>
<td>0.007</td>
</tr>
<tr>
<td>Low room temperature</td>
<td>1995</td>
<td>42</td>
<td>9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>47</td>
<td>26</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>55</td>
<td>35</td>
<td>0.08</td>
</tr>
<tr>
<td>Bad indoor air quality</td>
<td>1995</td>
<td>11</td>
<td>27</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>18</td>
<td>26</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>26</td>
<td>20</td>
<td>0.05</td>
</tr>
<tr>
<td>General symptoms</td>
<td>1995</td>
<td>38</td>
<td>44</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>42</td>
<td>47</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>28</td>
<td>51</td>
<td>0.17</td>
</tr>
<tr>
<td>Mucus membrane irritation</td>
<td>1995</td>
<td>9</td>
<td>11</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>11</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>13</td>
<td>11</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td>Skin symptoms</td>
<td>1995</td>
<td>11</td>
<td>16</td>
<td>&gt;0.50</td>
</tr>
<tr>
<td></td>
<td>1996</td>
<td>11</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>13</td>
<td>11</td>
<td>&gt;0.50</td>
</tr>
</tbody>
</table>

The analyses with atopy as a main between-subjects factor did not show any statistical significances neither for atopy itself or any of the interaction factors where atopy was included. Thus we could find no evidence for differences between atopic and non-atopic students during these 3 years of study.
Discussion

The statistical analysis showed no significant difference regarding nasal histamine reactivity between students at the target school and the control school from start to endpoint of the time of observation. The explanation for the different appearance of the provocation curve in 1996 in the target school is not clear, but a contributing factor to the more marked mucosal swelling in response to the first and lowest histamine concentration might be known problems with indoor temperature control in the building in combination with the cold weather that particular autumn. The rhinostereometric method is very sensitive, and small changes in nasal mucosal reactivity are easily recognized. For the final investigation during the last year of senior high school, the provocation curves are fairly equal, indicating that the indoor air conditions in the target school are satisfactory. This finding is also strengthened by the fact that no differences in nasal histamine reactivity were seen between the usually more sensitive atopic and non-atopic subjects during the years of study.

The technical investigations and measurements of the homes showed no important selection of students to the respective schools. The observed differences reflected that a greater proportion of the students at the control school lived in single-family houses. The students at the target school complained to a greater extent than students at the control school about low and varying room temperatures. In a single-story building such as the target school, which has a widespread area, it is difficult to maintain a uniform temperature in all areas at the same time. Some studies have shown a positive relationship between complaints about above all high room temperatures and building-related symptoms (Jaakkola et al., 1991; Reinikainen and Jaakkola, 1993). However, the complaints by students at the target school about low and varying room temperatures do not seem to contribute to building-related symptoms, as there was a similar frequency of perceived bad indoor climate and mucosal symptoms among the students in the two schools. Furthermore, the perception of bad indoor climate as well as general and mucosal symptoms among the students did not differ particularly from those of the teachers in the control school (Rudblad et al., 2001). It needs mentioning that this study was designed primarily to analyze differences in histamine reactivity and for that purpose the sample size was considered well in line with experiences of earlier investigations (Rudblad et al., 2001). The secondary aim was to analyze the questionnaire data, where the variables were measured on a nominal or ordinal scale with few categories. For that purpose, it would have been better to increase the sample somewhat, which was not possible for practical reasons.

The exposure measurements did not show any deviation from what is usually seen in Swedish schools (Smedje et al., 1997b). However, exposure to indoor air pollutants, including respiratory dust and pet allergens,
in these 'normal' concentrations has been related to airway symptoms, particularly among atopic individuals (Munir et al., 1993; Norbäck et al., 2001). No significant differences were found between SPT+ and SPT− students or between atopic and non-atopic students at the two schools regarding perception of indoor climate or mucosal irritative symptoms, indicating a decent IAQ in both schools.

In 1996, 70% of the SPT+ students reported having had allergic symptoms during the preceding year. Consequently, in the majority of cases a positive SPT in individuals in this age group is indicative of active allergic disease. It is noteworthy that none of the non-atopic students reported asthmatic symptoms, indicating that asthma in this age group (16–18 years) is predominately a manifestation of atopy and not an acquired non-allergic manifestation.

The students were followed for a period of almost 3 years in renovated environments, while the teachers had been exposed to a bad indoor climate for at least 5 years before the renovation. In 1997, 3 years after the renovation, the teachers in the target school still complained of irritative mucosal symptoms (31%), but the nasal histamine reactivity had decreased (Rudblad et al., 2002).

The lack of significant differences regarding both nasal histamine reactivity and reported symptoms between the students in the two schools and over the years may either indicate that the current indoor air is adequate or that 3 years of exposure is too short for manifestation of objective signs of effects. However, both the technical measurements performed during the study period, the low frequency of irritative mucosal symptoms among the students and the decreased mucosal reactivity between 1995 and 1997 among the target schoolteachers (Rudblad et al., 2002) support the former conclusion.

In conclusion, the nasal mucosal reactivity in the high school students at the remediated moisture-damaged school did not change during the 3 years of follow-up compared with that in students at a control school. Nor were any differences seen between atopic and non-atopic students. The perceptions of IAQ and mucosal irritative symptoms among the students were also similar in the two schools, and no significant differences were seen between atopic and non-atopic students. These facts indicate that the current indoor air in the remediated moisture-damaged school no longer exert an irritant effect on the upper airway mucosa of the students, and they also indicate that the increased mucosal reactivity among the teachers in the target school is mostly a result of the previous long-term exposure to building dampness.

This is also in line with the results of the follow-up study of the teachers, which showed a decreased nasal reactivity with time after restorative measures were carried out.

Acknowledgements

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References


Nasal histamine reactivity among adolescents


Nasal mucosal histamine reactivity among teachers six years after working in a moisture damaged school.

by Stig Rudblad, MD,1 Kjell Andersson, MD,2 Lennart Bodin, PhD,3 Göran Stridh, PhD,2 and Jan-Erik Juto, MD,4

Running head: Nasal histamine reactivity after working in a moisture damaged school

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Abstract

Objectives The objective of this study was to determine whether an earlier verified increased nasal histamine reactivity among teachers, who had been working in a school with severe moisture problems, still persistenced six years after remedial measures had been taken.

Methods Twenty-four teachers in the target school and sixteen teachers in the control school, who participated in all investigations (1995, 1997 and 2000), answered a standardized questionnaire and underwent a nasal histamine provocation test. Besides registration of mucosal swelling the microcircular reaction to histamine provocation was measured with Laser-Doppler flowmetry.

Results The nasal histamine reactivity among the teachers, measured as mucosal swelling, was no longer increased. However, Laser-Doppler flowmetry showed that the teachers in the target school had significantly divergent microcircular perfusion (p=0.0022) and CMBC (concentration of moving bloodcells) (p=0.0009) on histamine provocation compared to the teachers in the control school, indicating a more pronounced plasma leakage and oedema from the nasal mucosa.

Conclusions A restored nasal histamine reactivity measured as mucosal swelling reaction was observed among the teachers six years after an obviously successful renovation of the school environment. However, changes in the microcirculation indicate a remaining effect on the nasal mucosa.

Key terms: histamine provocation, laser-Doppler flowmetry, longitudinal study, microcircular perfusion, mucosal swelling, rhinostereometry.
Introduction

Epidemiological studies of nasal hyperreactive symptoms have mainly been focused on allergic rhinitis (1-3). Most of these studies are based on questionnaires or interviews. The assets of epidemiological data on occupational rhinitis are limited (4). Studies of exposed individuals in a specific office or industrial environment are the predominant sources of information concerning work-related nasal symptoms.

Environmental or occupational factors that may cause nasal reactions include formaldehyde (5), amines (6), flour in the baking industry (7) and wood-dust (8). During recent years there has been a growing concern about indoor air quality in schools, i.e. with respect to irritative respiratory tract symptoms (9-11). A study of Swedish schools has shown that failing and polluted ventilation systems, water leakage and building dampness are quite common (12). It has been shown that building dampness and mold exposure are associated with an increase in respiratory symptoms (13-15).

Sensitive methods have been developed for the study of nasal mucosal reactivity, i.e. acoustic rhinometry and rhinostereometry (16, 17). These methods are not invasive and are well tolerated by the subjects investigated. By using histamine, a non-allergic stimulant, for nasal provocation, an increase in mucosal reactivity has been found among a group of subjects exposed to a deteriorated indoor climate as compared to a control group (18, 19). Nasal mucosal swelling, as a measure of histamine reactivity, was recorded with rhinostereometry (20). There were also indications that long term exposure to a bad indoor climate might be related to increased nasal mucosal reactivity that persists for years, even after removal of the afflicted environment (21, 22).

With the combination of rhinostereometry and laser Doppler flowmetry it has been possible to simultaneously measure congestion and microcirculatory patterns of the nasal mucosa (23, 24). In one study using these two methods of measurements, nasal histamine provocation seemed to increase the congestion and the vascular permeability, producing an interstitial oedema, in patients with allergic rhinitis as compared to healthy subjects (25).

In 1995, a random sample of teachers (n=28), who had been working in a water-damaged school for at least five years were exposed, one year after remedial measures had been taken, to a nasal histamine provocation test and compared to teachers (n=18) from a control school with previous ventilation problems but without moisture problems (19). Technical investigations of the target school before renovation showed severe dampness because of inadequate drainage, and numerous water leaks from the roof causing growth of molds in the building construction and decomposition and discolouring of building materials. Renovation in the target school was directed towards the water-damages and included replacement of damaged building materials, reconstruction of the drainage system and removal of wall-to-wall carpets.

A significantly increased nasal mucosal swelling reaction to histamine provocation was demonstrated in the teachers in the school with previous moisture problems. In 1997 a follow-up study showed that there was still a significant difference in nasal histamine reactivity between the teachers in the two schools, although there was a decrease in reactivity among the teachers in the target school (22).

The aim of this study was to determine whether the increased nasal reactivity to histamine among the teachers in the target school, that was measured in 1995, still persisted five years later, i.e., six years after remedial measures had been taken. In addition to mucosal swelling as a measure of mucosal reactivity, the microcircular reaction of the nasal mucosa to histamine provocation with laser-Doppler flowmetry was measured. The purpose was to see if there was a difference in this respect between the two teacher groups and also if there was a correlation
between mucosal swelling and measured microcirculation. The study had been approved by the Ethics Committee of the Örebro County Council.

**Subjects and methods**

**Methodology**

The nasal histamine provocation tests in 1995 were performed at the hospital using a single-blind design and the investigator was not given details about the work place of the teachers. In 1997 and 2000 the provocations were carried out in an identical manner in the respective schools (22). In 2000 the teachers were also exposed to Laser-Doppler flowmetry (LDF) in order to study the microcirculation of the nasal mucosa.

**Study populations**

In 1995 a random sample of teachers, who had worked at least five years in the target school before the renovation (39 persons), and all teachers in the control school who fulfilled the same inclusion criteria (30 persons), were invited to participate in the study. Twenty-eight and 18 teachers, respectively, gave their informed consent, and formed the two study groups (19). In 1997, 26/18 teachers from the target and control schools, respectively, participated (22). All teachers from the study in 1995 (19), except one from the target school and one from the control school who had died, were now, in 2000, invited to participate (27/17 from the target and control schools, respectively). Twenty-four teachers from the target school and sixteen from the control school agreed to participate and formed the two study groups who participated on all three occasions. The reason for non-participation was mainly lack of time or no longer working at the school. All three investigations were performed in early spring (March/April).

**Questionnaires**

During the period of acclimatisation, before the histamine provocation, the same standardized questionnaire as in 1995 and 1997 was answered (MM 040 NA) (26).

**Skin-prick test**

In 1995 all teachers underwent a standardised skin-prick test [19, 27]. Skin-prick test positivity (SPT+) was defined as the formation of a wheal with a diameter of at least 3 millimetres in response to any of the allergens used. SPT+ was found in 18% (5/28)/17% (3/18) of the teachers in the target and the control schools, respectively.

**Provocation test**

A standardized nasal provocation procedure as described by Hallén and Juto was used with a histamine solution in three concentrations (1, 2 and 4 mg/ml) (28). The histamine solution was applied through a self-retaining nasal speculum with a syringe on the medial side of the right inferior conchae. The provocation was started with the lowest histamine concentration (1 mg/ml). Recordings of the mucosal swelling, on the provoked side, were made with rhinostereometry (RSM) before and 2, 5 and 10 minutes after the first and lowest histamine concentration (1 mg/ml) and 5 and 10 minutes after provocation with the two higher histamine concentrations (2 mg/ml and 4 mg/ml) (20).

Laser-Doppler flowmetry (LDF) is a non-invasive method for studying microcirculation and has the advantage of giving continuous and instantaneous measures of the nasal mucosa blood flow (23). LDF was performed using a Periflux 4001 (Perimed, Sweden). We used a specially designed micromanipulator-guided probe for the LDF measurements which was positioned approximately 0.3 mm from the medial surface of the inferior turbinate (29).
equipped with the micromanipulator (Rhinomed, Sweden) was used to combine LDF and RSM. The microcircular parameters, the concentration of moving bloodcells (CMBC), the velocity of blood flow (VU) and the product of these, the perfusion of flow (PU), were recorded. The recordings were done before and 2 and 5 minutes after the lowest histamine concentrations and 5 minutes after the provocation with the two higher histamine concentrations. Because of the lack of calibrations against other methods for measuring blood flow in the specific tissue, the data are expressed in arbitrary units and as deviations from base line. The measurements register the microcirculation to a depth of more than 1 mm in the tissue but above the big venous vessels, the sinusoids. The measurements were recorded on a PC with special software (PeriSoft for Windows, version 1.10).

Statistical analyses

Analyses of complaints, symptoms and mucosal swelling over the three observation occasions as well as perfusion and CMBC were done with methods for repeated measurements. Different models were used depending on the distributional properties of the outcome variables.

The prevalence of complaints and symptoms were analysed with a generalised linear model analogous to a logistic regression model but with allowance for correlated responses for the three occasions. The outcome parameter was the odds ratio for the prevalence of the complaints and symptoms. Explanatory factors in this model were school (two levels: target and control), observation year (three levels: 1995, 1997 and 2000), and the interaction between school and year.

The continuous outcomes of mucosal swelling, perfusion and CMBC were also analysed with a generalised linear model, but in this case the model was analogous to an ANOVA model with repeated measurements. For mucosal swelling there were up to seven explanatory variables included in the model, i.e. school, year and histamine dose level (three levels: 1 mg/ml, 2 mg/ml and 4 mg/ml) and interactions between two of these factors as well as the three-factor interaction. A potential explanatory factor, time after provocation with two levels, 5 and 10 minutes, was not introduced in the model but handled through separate analysis for each time point.

For perfusion and CMBC we had observations only in 2000. The model could therefore be simplified by excluding the factor year. Otherwise the statistical model was similar to the one used for mucosal swelling. In an additional analysis of perfusion and CMBC our interest was not the effect of dose but rather the effect of time after provocation. In this experiment, dose was fixed at 1 mg/ml and readings were done at 2, 5 and 10 minutes after provocation. The statistical model was quite similar to the previous model but instead of the factor dose we used the factor time (with three levels: 2, 5 and 10 minutes) and the interaction between school and time.

The ANOVA was first done with all interactions included, followed by a sequential search for parsimonious models where interactions of no statistical significance were excluded. Evaluations of the goodness-of-fit of the models were done with Akaike’s information index (30).

When a final ANOVA model had been found, we tried to describe the basic shape of the provocation curves (the response variable modelled as a function of dose, or time) by slightly reformulating the model. In this model we estimated the linear regression of an increase in swelling per $\log$ mg/ml histamine (doses 1, 2 and 4 mg/ml which give the logarithmic values 0, 1 and 2) for each school. We also determined the 95% confidence intervals (95% CI) for the estimates of the intercept and slope in these regressions. This linear model was also used in the analysis of perfusion and CMBC on dose. For these outcomes we also analysed the effect of time with a linear regression. Time was then set at 2, 5 and 10, that is, the actual times recorded.
Correlation coefficients for the linear association within and between swelling, perfusion and CMBC (measured over different dose and time levels) were calculated according to Pearson’s as well as Spearman’s correlation coefficients.

Our analytical models were implemented in the statistical packages SAS, version 8.1 (modules GENMOD and MIXED) and BMD, version 7 (procedure 5V).

**Results**

**Reported complaints and symptoms**

As shown in table 1, there were more complaints of varying and low room temperatures among the teachers in the target school as compared to the teachers in the control school. The analysis of varying room temperature and low room temperature showed a significant difference between the two schools, p=0.01 and p=0.04 respectively. The odds ratios for complaints, with the control school teachers as referents, were 5.7 (95% CI 1.2 – 27.7) and 5.4 (95% CI 1.1 – 25.6), respectively, indicating a much higher risk for complaints at the target school.

For poor indoor air quality there was also a significant difference between the two schools, p=0.01, and the odds ratio was 5.3 (95% CI 1.2 – 22.3) for the target school, but no significance for the factor year or interaction school*year.

For the symptoms none of the investigated factors, i.e. difference between schools, difference between years and interaction school*year, showed statistical significance.

(Table 1 in)

**Provocation test**

**RSM/mucosal swelling reaction**

Table 2 shows the mean swelling of the nasal mucosa provoked with increasing histamine concentrations. The ANOVA showed that in 1995 the teachers in the two schools had a statistically significant difference in their response to increased provocation levels, (p=0.001 for the 5-minute readings). In 1997 the difference had decreased compared to 1995 (the difference was still statistically significant). In 2000 there were no statistically significant differences. The linear regression analysis of mucosal swelling in table 3 illustrates the estimates of intercepts, slopes and 95% confidence intervals of the provocation curves of the teachers when they underwent repeated histamine provocation. The response to the provocation levels, and how this response slowly converges from 1995 to 2000 to almost the same values for the schools is clearly seen from the slope estimates. For the target school the slopes are 0.46, 0.28 and 0.23, respectively, and for the control school they are 0.15, 0.21 and 0.18, respectively, using values from the 5-minute readings. A similar development is also seen for the 10-minute reading, although the slope estimates are generally smaller.

The swelling reaction 2 minutes (in 2000) after provocation with the lowest histamine concentration is more pronounced in the two study groups than after 5 or 10 minutes (figure 1c).

(table2, 3 and figure1 a-c in)

**LaserDoppler flowmetry**

Table 4 shows the perfusion and CMBC, expressed as difference from baseline in arbitrary units, and table 5 shows a linear regression analysis of perfusion and CMBC induced by histamine provocation. Part A, in table 5, shows the histamine provocations using 1 mg/ml, 2 mg/ml and 4 mg/ml for 5-minute readings, and part B shows the 2-minute, 5-minute and 10-minute readings for the 1 mg/ml provocation level. An analysis of variance of repeated
measurements of perfusion for various dose levels in Schools, based on 5-minute readings, shows a significant difference for School*Dose (p=0.0022) indicating a difference in reaction patterns between the two teacher groups during the provocation procedure. This is well illustrated in figure 2a where the microcircular perfusion in the nasal mucosa steeply increases from baseline 2 minutes after the first provocation with the lowest histamine concentration in the two teacher groups. The control group then seems to restore the level of perfusion rapidly and reacts more strongly to the following provocations compared to the weaker reaction from the teachers in the target school. There is also a significant difference over time (2, 5 and 10 minutes) (p=0.0064) when considering the lowest histamine concentration (1 mg/ml).

Tables 4 and 5 and figure 2b show the changes in CMBC during the histamine provocation procedure. The teachers in the target school, in contrast to those in the control school, show a decrease in their CMBC after the first histamine provocation and this remains low during the whole provocation procedure. There is a significant difference based on the 5-minute readings for the factor School (p=0.0009), but not for the factor School*Dose (p=0.25), indicating that the provocation curves are well separated with a similar reaction pattern during the provocation procedure, which is clearly illustrated in figure 2b. As with perfusion there was also a significant difference over time (p=0.0048) when considering the lowest histamine concentration.

There was no correlation between measured mucosal swelling and Laser-Doppler flowmetry (perfusion/CMBC values) during the histamine provocation procedure, with correlation coefficient almost all in the order of –0.10 to 0.10. On the other hand correlations within the measured mucosal swelling as well as within flowmetry were much higher, in the order of 0.6 – 0.7, clearly indicating the need for the analysis of these measurements with allowance for correlations between repeated measurements, i.e. our chosen method of analysis.

*(table4, 5 and figure 2a, 2b in)*

**Discussion**

The statistical analyses showed no significant differences regarding nasal histamine reactivity, measured as the mucosal swelling reaction, between the teachers in the target school and those in the control school six years after remedial measures had been taken. We interpret the results, in combination with the outcome of the intermediate study in 1997 (22), as an indication that long-term exposure to building dampness increases the risk for nasal mucosal hyperreactivity, which only slowly decreases during years after the indoor environment has been restored. The cause of this mucosal hyperreactivity is unclear but might depend on exposure to different chemical and/or microbial emissions from moisture-damaged building constructions, not easily determinable using current methods of measurements (31, 32). The reason for this prolonged increased reactivity, even after remedial measures have been taken, is probably an ongoing physiological restoring process in the mucosa of the upper airways.

In conformity with another study (25), we could also see that the maximal swelling reaction upon provocation with the lowest histamine concentration occurred in both groups as early as after 2 minutes. This fact encourage us to consider the 5- and 10-minutes reactions as essentially quite different, indicating that they should be handled separately in the statistical analyses.

The high frequency of experienced poor indoor air among the teachers in the target school in 1995 only marginally decreased during the years of observation. This may have something to do with difficulty in maintaining a uniform temperature in a single-story building, leading to a significantly higher prevalence of complaints of varying and low room temperature among the target school teachers. Uniformity of temperature and well adapted ventilation are important for comfort and seem to be associated with a lower incidence of experiencing air dryness (33),
which was a dominating complaint in the experience of poor indoor air climate among the target school teachers (19, 22).

The frequency of reported mucosal irritative symptoms and skin symptoms decreased among the teachers in the target school during the time of observation. However, there were no statistically significant differences regarding these symptoms over time between the teachers in the target school and the teachers in the control school, probably because the study was not fully dimensioned to evaluate differences in these parameters.

Upon repeated histamine provocation the nasal microcircular flow (perfusion) among the teachers in the target school showed a slower reaction pattern with a flatter provocation curve than that of the teachers in the control group. This slow reaction might correlate to a leakage of plasma from the capillaries in the mucosa, followed by interstitial oedema.

The CMBC is probably affected by changes in the amount of interstitial fluid (34). The flatter perfusion curve and the decreasing CMBC during the histamine provocations among the teachers at the target school strongly indicates an increased tendency for plasma leakage and interstitial oedema. Hence, although there were no significant differences between the two study groups regarding nasal mucosal swelling upon histamine provocation, there still seems to be a difference in the superficial microcircular pattern. This fact indicates that long term exposure to building dampness might do more damage to the superficial subepitelial layer of the nasal mucosa, and is followed by a longer restoring process compared to that of the deeper structures. Accordingly, the absence of correlations between the mucosal congestion and the microcircular pattern upon histamine provocation probably depends on that RSM measures mucosal congestion due to dilatation of the deeper situated sinusoids in the nasal mucosa, while LDF measures the superficial microcircular pattern (23).

In conclusion we found a restored nasal histamine reactivity, measured as mucosal swelling reaction, among the teachers six years after long term exposure to building dampness.

We also found remaining changes in the microcircular pattern in this teacher group compared to the control group.

**Acknowledgements**

The study received financial support from the Research Committee of the Örebro County Council. It was also supported by grants from the Swedish Working Life Research Fund. Assistance with the provocation tests and computer analysis of the data was performed by research assistant Inger Fagerlund at the Department of Occupational and Environmental Medicine, Örebro University Hospital, Örebro, Sweden.
References

Table 1. Prevalence of complaints and symptoms in Target and Control Schools in 1995, 1997 and 2000.

<table>
<thead>
<tr>
<th>Year</th>
<th>Target school (n=24)</th>
<th>Control school (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes / n of answers (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>COMPLAINTS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Varying room-temperature</td>
<td>1995</td>
<td>5/23 (21.7)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>9/22 (40.9)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>5/20 (25.0)</td>
</tr>
<tr>
<td>Low room-temperature</td>
<td>1995</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>7/22 (31.8)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>5/20 (25.0)</td>
</tr>
<tr>
<td>Poor indoor air quality&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1995</td>
<td>13/24 (54.2)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>8/24 (33.3)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>10/24 (41.7)</td>
</tr>
<tr>
<td><strong>SYMPTOMS:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General symptoms&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1995</td>
<td>12/24 (50.0)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>10/24 (41.7)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>8/24 (33.3)</td>
</tr>
<tr>
<td>Mucosal irritative symptoms&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1995</td>
<td>7/24 (29.2)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>8/24 (33.3)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>4/24 (16.7)</td>
</tr>
<tr>
<td>Skin symptoms&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1995</td>
<td>5/24 (20.8)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>3/24 (12.5)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>2/24 (8.3)</td>
</tr>
</tbody>
</table>

<sup>1</sup>The percentage of teachers often troubled (= at least every week) by stuffy bad air, dry air or an unpleasant smell the preceding three month period.

<sup>2</sup>The frequency of teachers often troubled by tiredness, feeling heavy-headed, headache, nausea, vertigo or concentration problems.

<sup>3</sup>The frequency of teachers often troubled by irritated eyes, nose, throat or cough.

<sup>4</sup>The frequency of teachers often troubled by facial dryness- or redness, scaly and itchy hairbottom/ears or dry,itchy skin on the hands (eczema of the hands).
Table 2. Mean Swelling (SD; mm) of the mucosal membrane in the nose for different levels of histamine. Subjects at the two schools who were present at all investigations. Recordings at 5 and 10 minutes after provocation.

<table>
<thead>
<tr>
<th>School</th>
<th>Year</th>
<th>Histamine level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg/ml</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>Target</td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>(n=24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>0.48 (.79)</td>
<td>0.49 (.79)</td>
</tr>
<tr>
<td>1997</td>
<td>0.70 (.66)</td>
<td>0.44 (.68)</td>
</tr>
<tr>
<td>2000</td>
<td>0.60 (.44)</td>
<td>0.56 (.50)</td>
</tr>
<tr>
<td>Control</td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1995</td>
<td>0.53 (.56)</td>
<td>0.44 (.53)</td>
</tr>
<tr>
<td>1997</td>
<td>0.45 (.22)</td>
<td>0.30 (.28)</td>
</tr>
<tr>
<td>2000</td>
<td>0.51 (.36)</td>
<td>0.44 (.37)</td>
</tr>
</tbody>
</table>

Table 3. Linear regression analysis of mucosal swelling induced by histamine provocation (logarithmic values 0, 1 and 2) stratified for time after provocation, year and school. Estimates of intercepts, slopes and 95% confidence intervals (95% CI).

<table>
<thead>
<tr>
<th>Time</th>
<th>Year</th>
<th>School</th>
<th>Intercept Estimate (95% CI)</th>
<th>Slope Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>1995</td>
<td>Target</td>
<td>0.46 (0.19 – 0.73)</td>
<td>0.46 (0.35 – 0.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.43 (0.11 – 0.76)</td>
<td>0.15 (0.01 – 0.30)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>Target</td>
<td>0.68 (0.48 – 0.88)</td>
<td>0.28 (0.19 – 0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.43 (0.18 – 0.68)</td>
<td>0.21 (0.11 – 0.31)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Target</td>
<td>0.61 (0.45 – 0.77)</td>
<td>0.23 (0.17 – 0.30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.54 (0.35 – 0.74)</td>
<td>0.18 (0.11 – 0.26)</td>
</tr>
<tr>
<td>10 min</td>
<td>1995</td>
<td>Target</td>
<td>0.48 (0.21 – 0.75)</td>
<td>0.29 (0.18 – 0.39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.43 (0.09 – 0.76)</td>
<td>0.10 (.003 – 0.23)</td>
</tr>
<tr>
<td></td>
<td>1997</td>
<td>Target</td>
<td>0.44 (0.22 – 0.66)</td>
<td>0.28 (21 – 0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.30 (0.03 – 0.35)</td>
<td>0.17 (0.09 – 0.26)</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>Target</td>
<td>0.63 (0.46 – 0.80)</td>
<td>0.20 (0.13 – 0.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0.53 (0.32 – 0.74)</td>
<td>0.17 (0.08 – 0.25)</td>
</tr>
</tbody>
</table>
Table 4. Perfusion and CMBC, expressed as difference from baseline in arbitrary units. Mean (SD) for different levels of histamine and different time points after provocation in the investigation performed in 2000. Subjects at the two schools who were present at all investigations.

<table>
<thead>
<tr>
<th></th>
<th>Histamine provocation level</th>
<th>1 mg/ml</th>
<th>2 mg/ml</th>
<th>4 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 min</td>
<td>5 min</td>
<td>10 min</td>
</tr>
<tr>
<td><strong>Perfusion</strong></td>
<td>Target school (n = 24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>346 (200)</td>
<td>308 (205)</td>
<td>289 (226)</td>
</tr>
<tr>
<td></td>
<td>Control School (n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>324 (260)</td>
<td>203 (195)</td>
<td>212 (245)</td>
</tr>
<tr>
<td><strong>CMBC</strong></td>
<td>Target school (n = 24)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3.0 (11.0)</td>
<td>-3.8 (12.5)</td>
<td>-7.0 (13.7)</td>
</tr>
<tr>
<td></td>
<td>Control School (n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.7 (17.0)</td>
<td>6.0 (11.6)</td>
<td>1.4 (12.8)</td>
</tr>
</tbody>
</table>
Table 5. Linear regression analysis of Perfusion and CMBC induced by histamine provocation (logarithmic values 0, 1 and 2) stratified for school. Estimates of intercepts, slopes and 95% confidence intervals (95% CI).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Study object</th>
<th>School</th>
<th>Intercept Estimate (95% CI)</th>
<th>Slope Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion Part A¹</td>
<td>5-minute readings</td>
<td>Target</td>
<td>308 (230 – 386)</td>
<td>2.1 (-29.7 – 33.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>206 (110 – 301)</td>
<td>90.8 (51.9 – 129.7)</td>
</tr>
<tr>
<td></td>
<td>Part B¹</td>
<td>1 mg/ml histamine</td>
<td>Target</td>
<td>324 (248 – 400)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>243 (150 – 336)</td>
<td>-0.8 (-7.4 – 5.7)</td>
</tr>
<tr>
<td></td>
<td>Part A¹</td>
<td>5-minute readings</td>
<td>Target</td>
<td>-3.8 (-8.5 – 1.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>6.0 (0.2 – 11.8)</td>
<td>-0.9 (-2.9 – 1.1)</td>
</tr>
<tr>
<td></td>
<td>Part B¹</td>
<td>1 mg/ml histamine</td>
<td>Target</td>
<td>-1.4 (-6.7 – 3.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>9.2 (2.8 – 15.7)</td>
<td>-0.8 (-1.4 - -0.1)</td>
</tr>
</tbody>
</table>

¹ Part A consists of readings after 5 minutes with increasing histamine provocation level, 1, 2 and 4 mg/ml. Part B consists of readings after 2, 5 and 10 minutes with 1 mg/ml histamine provocation level.
Figure 1a

1995

- Target school
- Control school

Swelling in mm

Histamine provocation level mg/ml

Figure 1b

1997

- Target school
- Control school

Swelling in mm

Histamine provocation level mg/ml

Figure 1c

2000

- Target school
- Control school

Swelling in mm

Histamine provocation level mg/ml
**Figure 2a**

![Perfusion, difference from baseline graph](image)

**Figure 2b**

![Histamine provocation level graph](image)
Legends

**Figure 1a-1c.** Histamine provocation curves (the mean net change in mucosal swelling from baseline) for teachers from the target and control schools measured on three occasions, in 1995, 1997 and 2000.

**Figure 2a-2b.** Perfusion and CMBC, difference from baseline. Values read at 2, 5, and 10 minutes after provocation for the 1 mg/ml histamine provocation level, and at 5 minutes for the 2 and 4 mg/ml levels.