



Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl



Acoustic rhinometry, spirometry and nitric oxide in relation to airway allergy and smoking habits in an adolescent cohort

Kristina Irander^{a,*}, Magnus P. Borres^{b,c}, Jörgen P. Palm^d

^a Allergy Centre, ENT Section, University Hospital, 58185 Linköping, Sweden

^b Department of Pediatrics, Sahlgrenska Academy of Göteborg University, Göteborg, Sweden

^c Phadia AB, Uppsala, Sweden

^d Department of Physiology and Pharmacology, Karolinska Institutet, Sweden

ARTICLE INFO

Article history:

Received 20 May 2010

Received in revised form 19 October 2010

Accepted 24 October 2010

Available online xxx

Keywords:

Allergic rhinitis

Smoking habits

Acoustic rhinometry

Spirometry

Nasal nitric oxide

Exhaled nitric oxide

ABSTRACT

Objective: This study aimed to analyze upper and lower airway function and the impact of smoking habits in a cohort of allergic and healthy adolescents. The influence of smoking habits on the outcomes of rhinitis and asthma is well documented, but only few reports are available showing smoke related upper airway impairment by rhinometric measurements, and none with focus on early changes in adolescents. **Methods:** A cohort followed from infancy was re-examined at the age of 18 years concerning allergy development. Acoustic rhinometry (VOL2), spirometry (FEV₁) and measurements of nitric oxide levels from the upper (nNO) and lower airways (eNO) were performed before and after physical exercise, and smoking habits were registered.

Results: Active smoking habits were reported by 4/21 subjects suffering from allergic rhinitis, by 1/4 from probable allergic rhinitis, by 0/3 subjects with atopic dermatitis and by 2/10 healthy controls. Smoking habits were reported as daily by 2 and occasional by 5 of the 7 active smokers. VOL2 did not increase in smokers after exercise as in non-smokers, resulting in a post-exercise group difference (7.3 ± 1.1 cm³ vs. 8.8 ± 1.5 cm³; $p = 0.02$), and FEV₁ values were lower in smokers compared to non-smokers ($89 \pm 7\%$ vs. $98 \pm 8\%$; $p = 0.02$). The nNO and eNO levels were, however, only slightly reduced in smokers. Airway allergy was discerned only in subjects with current allergen exposure by increased eNO levels compared to healthy controls (41 ± 44 ppb vs. 13 ± 5 ppb). The levels of VOL2, nNO and FEV₁ did not differentiate allergic subjects from healthy controls.

Conclusions: Low levels of tobacco smoke exposure resulted in reduced airway functions in this adolescent cohort. Acoustic rhinometry and spirometry were found to be more sensitive methods compared to nitric oxide measurements in early detection of airway impairment related to smoke exposure. A possible difference in airway vulnerability between allergic and healthy subjects due to smoke exposure remains to be evaluated in larger study groups.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Prediction, diagnosis and management of allergic rhinitis and other forms of hypersensitivity in the upper airways is of great importance, as these disorders are common and the impact is considerable on affected individuals [1]. During infancy and childhood symptoms are mainly due to allergy mechanisms. As age advances exposure to air pollutants may increasingly contribute to airway symptoms, many of which are due to non-allergic mechanisms. Tobacco smoke is one of the most common indoor air pollutants, affecting many subjects during childhood by

passive exposure and during adolescence and adult life also by active smoking habits. The harmful effect of tobacco smoke on the outcomes of rhinitis and asthma is well described [2–4]. However, only a few studies have analyzed smoking habits and nasal obstruction by means of acoustic rhinometry or rhinomanometry [5,6], and no reports have been found using these methods with focus on early smoke related nasal impairment in adolescents.

In this report the functions of the upper and lower airways were objectively evaluated and related to current smoking habits in a group of allergic and healthy adolescents. The subjects were all included during infancy in a prospective study with focus on a presumed allergy predictor. The cohort had previously been checked twice [7,8], and a third follow-up was performed at the age of 18 years [9]. At this age some of the participants were presumed to have gained smoking habits but not experience of

* Corresponding author. Tel.: +46 142 363023.

E-mail address: kristina.irander@telia.com (K. Irander).

occupational irritants. Thus, the group was regarded as suitable for a study of early airway impairment due to smoking habits. Measurements by acoustic rhinometry, spirometry and analyses of nasal nitric oxide (nNO) and exhaled nitric oxide from the lower airways (eNO) were performed before and after provocation of the airways by means of physical exercise. Analyses of nitrite and nitrate concentrations in nasal lavages were also included, as these end products in the NO metabolism were supposed to be of interest.

2. Materials and methods

2.1. Study design

The study was performed out of the pollen season. The subjects had to be free from airway infections for at least 10 days prior to the examination. All participants were examined in the morning before noon to avoid influence from the diurnal variations of NO levels [10]. Each participant was acclimatized to the temperature and humidity of the indoor air in a sitting position for at least half an hour prior to examination, while allergy symptoms, use of medications and smoking habits reported in a questionnaire were scrutinized. The participants were also interviewed about the breakfast, as intake of vegetables is found to affect NO measurements [11–13]. However, breakfast was not reported to contain vegetables by any of the participant. The tests were performed in the following order: Acoustic rhinometry, spirometry, nasally exhaled NO and orally exhaled NO after mouthwash. Physical exercise was performed to evaluate the functional status of the airways. The four tests were repeated in the same order immediately following exercise, and spirometry was repeated another two times as described below. These examinations were then followed by a skin prick test (SPT), blood sampling for Phadiatop® analysis, inspection of the upper airways and a nasal lavage for the analysis of nitrite/nitrate concentrations.

2.2. Subjects

2.2.1. Number of participants

A cohort, including 67 infants with high and low risk of allergy development, was followed up at the age of 18 years in order to evaluate the age of allergy debut in relation to a presumed allergy predictor [9]. At this follow-up, the invitation to participate in clinical examinations, allergy tests and tests of airway functions was accepted by forty-nine subjects, but due to technical failures of NO measurements in four individuals and exclusion of results from seven subjects using pharmacological agents with possible effects on airway functions and NO levels [14–16], the evaluations of the upper and lower airways were based on results from 38 individuals. Unfortunately, two of them refused to participate in the exercise test, providing only pre-exercise test values.

2.2.2. Allergy diagnoses and allergy tests

The diagnoses of allergy and the allergy tests in this 18-year follow-up are previously described in detail [9]. In summary, allergy diagnoses of the upper and lower airways were based on the history of relevant symptoms appearing at least twice in relation to exposure to airborne allergens during the previous two years. Diagnosis of asthma was confirmed by pre-defined changes in the spirometry results of the exercise provocation test (see below). Relevant symptoms from the lower airways, but not reaching asthma criteria, were called 'allergic bronchitis'. Atopic dermatitis was evaluated according to the SCORAD index [17]. The term 'probable allergy' was used when symptoms were not quite distinct, had an uncertain relation to allergen exposure or were not repeatable, and thus did not fulfill all of the criteria. According to

these definitions, twenty-one subjects suffered from allergic rhinitis symptoms and some in this group also from lower airway symptoms and atopic dermatitis, three subjects from atopic dermatitis only with no airway symptoms, while reports from four individuals were inconclusive. Ten subjects were symptom free.

The SPT was performed using ALK extracts (ALK, Sverige AB, Sweden) including pollen allergens (birch, timothy, mugwort) and perennial allergens (horse, cat, dog, D pteronyssinus, D farinae, Alternaria, Cladosporium). SPT was defined as positive if the mean wheal diameter (half of the sum of the largest diameter and its perpendicular) was at least 3 mm [7]. Histamine hydrochloride 10 mg/ml served as a positive reference and normal serum albumin as a negative control. Sensitization to inhalant allergens was also examined in all of the subjects using Phadiatop® analysis (Phadia AB, Uppsala, Sweden).

2.2.3. Allergy sub-groups

All participants were allocated into five sub-groups. Subjects with allergic rhinitis were separated into a 'perennial' sub-group ($n = 13$) due to sensitization to perennial allergens, regardless of any concurring positive pollen tests, and a 'pollen' sub-group ($n = 8$) sensitized to pollens only and thus not exposed to any offending allergens for at least three months. The atopic dermatitis group included subjects with eczema only and no airway symptoms ($n = 3$), and the 'probable' group subjects with inconclusive symptoms ($n = 4$). The healthy non-sensitized subjects served as a control group ($n = 10$).

2.2.4. Smoking habits

All subjects were asked to report daily smoke exposure at home or during leisure time. Their own current habits of cigarette consumption were reported as occasional (≤ 1 –2 cigarettes per week; $n = 5$), light (1–9 cigarettes per day; $n = 1$), moderate (10–20 cigarettes per day; $n = 1$) or heavy (> 20 cigarettes per day; none). Thus, altogether seven subjects reported active smoking habits and another two passive smoke exposure.

The numbers of subjects with smoking habits in the allergy sub-groups are shown in Table 1.

2.3. Acoustic rhinometry

Acoustic rhinometry was performed using Rhin 2000 (S.R. Electronics A.S., Lyngby, Denmark). The mean values from three recordings and the sum from both nasal cavities were calculated using the computerized program with pre-determined calculations of minimal nasal cross-sectional areas and volumes [18]. The values of VOL2, corresponding to the volume in the anterior part of the proper nasal cavity (a distance of between 2.20 and 5.40 cm from the nostrils, as calculated in Rhin 2000), were chosen for statistical analyses.

Table 1

Number of subjects in the allergy sub-groups related to smoking habits; perennial=subjects sensitized to perennial ± pollen allergens; pollen=subjects sensitized to pollens only.

Allergy sub-groups	Smoking habits			Totals
	No	Passive	Active	
Allergic rhinitis				
Perennial	10	1	2	13
Pollen	5	1	2	8
Atopic dermatitis	3	0	0	3
Probable allergy	3	0	1	4
Healthy	8	0	2	10
Totals	29	2	7	38

Table 2

Parameter values before and after exercise in the upper and lower airways related to smoking habits.

Parameters	Non-smokers ^a (n = 29)	Passive smokers (n = 2)	Active smokers ^b (n = 7)	Group differences
Upper airways VOL2 (cm ³)				
Before	6.5 ± 1.1	5.5 ± 0.3	7.3 ± 1.7	n.s.
After	8.8 ± 1.5	7.3 ± 1.2	7.3 ± 1.1	a/b p = 0.02
nNO (ppb)				
Before	70 ± 32	90 ± 20	58 ± 18	n.s.
After	67 ± 30	75 ± 17	56 ± 15	n.s.
Nitrites/nitrates (μM)				
After	17 ± 8	14 ± 1	18 ± 10	n.s.
Lower airways				
FEV1 (%)				
Before	98 ± 8	101 ± 7	89 ± 7	a/b p = 0.02
After	97 ± 7	100 ± 11	87 ± 6	a/b p < 0.01
eNO (ppb)				
Before	23 ± 30	18 ± 19	20 ± 28	n.s.
After	16 ± 12	18 ± 20	18 ± 24	n.s.

Differences calculated between the groups of non-smokers (a) and active smokers (b); n.s. = not significant.

2.4. Spirometry and the exercise test

The function of the airways was evaluated before and after a physical exercise test. The individuals had to run on a treadmill for 6 min to achieve a pulse rate of ≥ 160 beats per minute. Spirometry (Microlab) was performed before and after exercise and repeated after a further 15 min. A reversal dose of an inhaled beta-agonist was then given followed by a fourth measurement 15 min later. The best result from three recordings from each of the four repeated measurements was used. The definition of on-going asthma was based on the values of the forced expiratory volume in 1 s (FEV₁): An initial FEV₁ value $\leq 80\%$ of the expected value, a reduction of FEV₁ $\geq 15\%$ after the physical exercise or an increase of FEV₁ $\geq 15\%$ after bronchodilation.

2.5. NO measurements from the upper and lower airways

Measurements of NO followed the ATS/ERS recommendations [14] using NIOX[®] (Aerocrine AB, Sweden) at a flow rate of 3 l/min. The nNO values were calculated according to the 'subtraction method' [10]. Briefly, when measuring nasally exhaled NO, a tight-fitting nasal mask was adapted to the mouthpiece used for oral exhalations, and the mean of three recordings was calculated. In order to avoid the non-airway contribution of NO from saliva in the oropharyngeal tract [11–13] a mouthwash with 20 ml of sodium bicarbonate solution (10%) for 1 min was performed, and eNO was calculated as the mean of three immediately following oral exhalations. This mean eNO value was subtracted from the mean nasally exhaled NO value in the calculation of nNO. Thus, nNO, as calculated using the 'subtraction method', represents the supra-velar airway contribution to nasally exhaled NO.

2.6. Nasal lavage and nitrite/nitrate analysis

Nasal lavage was performed using saline pre-warmed to 37 °C. The subject held the head bent forward with the face horizontally while the left nasal cavity was filled with saline, using a syringe connected to the nostril via a short tube and a nasal olive. After 5 min the saline was aspirated back and immediately centrifuged. The supernatant was kept frozen (–20 °C) until analysis. Nitrites and nitrates were analysed by the Greiss reaction after reduction of nitrate to nitrite according to the method described by Verdon et al. [19].

2.7. Statistics

In general, statistics were analyzed by non-parametric methods, using the Graph Pad Prism software program. Mann–Whitney U-Test (MW) was used in calculations of differences between two groups. Wilcoxon Signed Rank Test (W) was used in calculations of differences between separate occasions in the same group of individuals. Results are presented as mean values ± 1 standard deviation. Spearman Rank Correlation was used in evaluating correlations. $p < 0.05$ was regarded as significant.

2.8. Ethics

The study was approved by the Ethical Committee at the University Hospital in Linköping, Sweden (03694) and performed according to the Declaration of Helsinki. A written informed consent was obtained from each of the participants.

3. Results

3.1. Acoustic rhinometry

3.1.1. Allergy sub-groups

The mean value of VOL2 in the overall group was 6.6 ± 1.2 cm³ before and 8.5 ± 1.6 cm³ after exercise. No statistical differences were found between any of the allergy sub-groups and the healthy group in the pre- or post-exercise values. The increase in VOL2 after exercise was significant ($p < 0.0001$) in all of these groups.

3.1.2. Smoking habits

The values of VOL2 in relation to smoking habits are shown in Table 2. VOL2 was slightly lower in the group of non-smokers compared to the group of active smokers before exercise, but increased after exercise only in non-smokers ($p < 0.0001$) but not in smokers, resulting in a post-exercise significant difference ($p = 0.02$) between the two groups (Fig. 1a).

3.2. Spirometry

3.2.1. Allergy sub-groups

In the overall group the pre-exercise value of FEV₁ was $97 \pm 9\%$ of the expected value, with a significant decrease to $95 \pm 8\%$ immediately following exercise ($p < 0.01$), no further decrease 15 min later, and with a significant increase to $97 \pm 8\%$ after the reversal dose ($p = 0.001$). No significant differences were found in

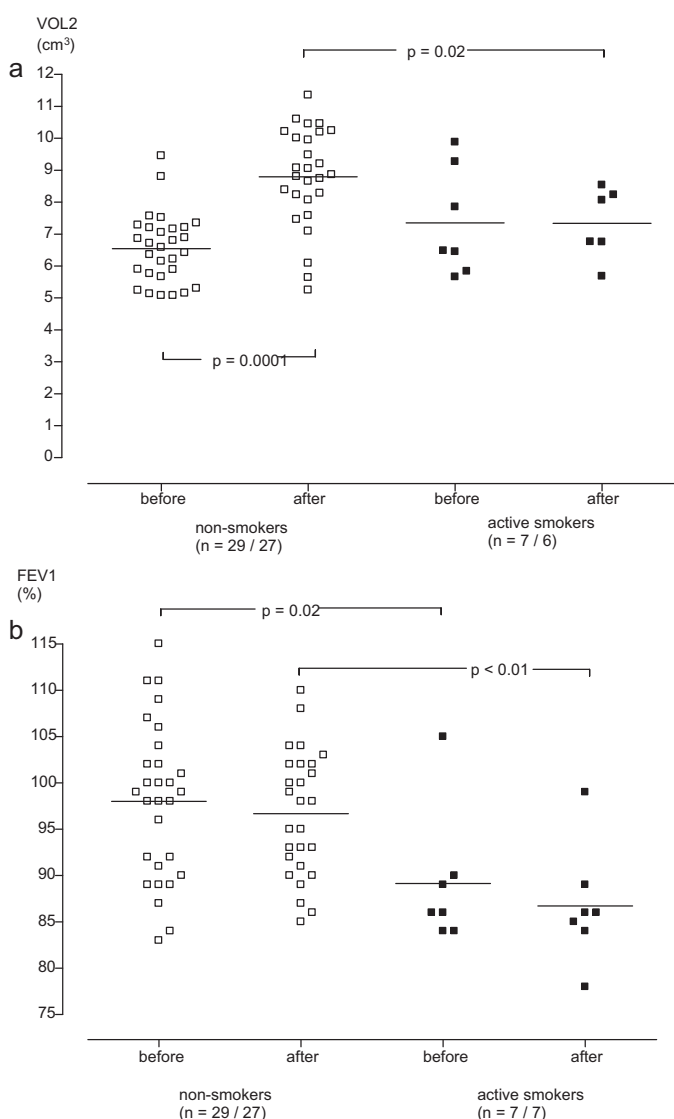


Fig. 1. Levels, with mean values indicated, before and after exercise in non-smokers and smokers: (a) nasal volumes (VOL2); (b) forced expiratory volumes in 1 s (FEV₁) in percent of expected values.

FEV₁ values between any of the allergy sub-groups and healthy controls. None of seven subjects reporting lower airway symptoms fulfilled the criteria of asthma diagnosis, and they were thus regarded to suffer from allergic bronchitis.

3.2.2. Smoking habits

The values of FEV₁ in relation to smoking habits are shown in Table 2. A significant difference was found between the groups of active smokers and non-smokers, as lower levels were found in smokers before exercise as well as after exercise ($p = 0.02$ and $p < 0.01$ respectively; Fig. 1b). The same group differences were found in the further two repeated FEV₁ measurements.

3.3. Nasal NO (nNO)

3.3.1. Allergy sub-groups

The mean value of the nNO level in the overall group was 69 ± 29 ppb before and 65 ± 27 ppb after exercise. No significant differences in the nNO levels were found between any of the allergy sub-groups and healthy controls. A slight reduction in nNO levels after exercise was found in all groups.

3.3.2. Smoking habits

The nNO values in relation to smoking habits are shown in Table 2. The nNO level was lower, but not significantly, in active smokers in comparison to the nNO level in non-smokers.

3.4. Nitrite/nitrate levels in nasal lavage and the relation to nNO levels

The level of nitrites/nitrates in the overall group was 17 ± 8 μ M. No significant level differences were found related neither to allergy nor to smoking habits (Table 2). A positive correlation was found between the levels of nitrite/nitrate and nNO in the overall group ($r^2 = 0.07$; $p = 0.02$).

3.5. Orally exhaled NO (eNO)

3.5.1. Allergy sub-groups

The eNO level was numerically higher in the sub-group sensitized to perennial allergens compared to the healthy group before (41 ± 44 ppb vs. 13 ± 5 ppb) and after exercise (28 ± 22 ppb vs. 13 ± 5 ppb). The levels before and after exercise in the pollen-sensitized sub-group (14 ± 10 ppb and 13 ± 10 ppb, respectively) were not different from the levels in the healthy control group.

3.5.2. Smoking habits

The eNO levels in the overall group were 22 ± 29 ppb before and 17 ± 15 ppb after exercise. No conclusive differences were found between non-smokers and active smokers (Table 2).

4. Discussion

This study of an adolescent cohort showed an obvious influence from early smoking habits on the airway functions. This was not expected, as the number of smokers and the consumption of cigarettes were low. Thus, acoustic rhinometry disclosed disturbed function of the upper airways, as the nasal volume after exercise did not increase in smokers as is normally expected [20]. Signs of smoke-related disturbance were also found by spirometry, as the values of FEV₁ were significantly lower, although within normal limits, in smokers compared to non-smokers. Our results are in accordance with the two previous studies on nasal obstruction analyzed by acoustic rhinometry [5,6] and a recently published study [21] reporting a reduced mucosal decongestion in smokers. Signs of lower airway obstruction were found in adolescents with active smoking habits [22–25], which also is in agreement with our findings. Our study indicates that the very low exposure dose in adolescent smokers with occasional habits may result in a functional disorder with a reduced plasticity of the airways. A possible different vulnerability in allergic rhinitis in comparison to non-allergic subject could not be evaluated, however, due to the low numbers of smokers in the allergy sub-groups and the healthy control group.

In contrast to the results from the functional tests, the inflammatory markers nNO and eNO showed just a slight reduction of the NO levels in the smokers. According to previous studies on smokers the reduction of nasal NO [26] and exhaled NO [27] has been significant. The different outcome in our study might be due to the low cigarette consumption.

Our attempt to identify ongoing airway allergy by means of NO measurements, showing no differences in nNO levels but increased eNO levels, was in accordance with previous studies recently reviewed [28]. None of the techniques used for nNO analysis in recent studies have shown differences between nNO levels in allergic rhinitis subjects and normal controls [28]. Our nNO results, using the method of subtraction of eNO values after mouthwash from the nasally exhaled NO in the calculation of the nNO levels, showed the same results found in the previous study using this

method [29]. Thus, also the 'subtraction method', although repeatable, is inadequate to detect the smaller differences in nNO levels that might be expected in allergic rhinitis. The higher level of eNO, found in the perennial group with continuous allergen exposure in comparison to the pollen group with no current allergen exposure and healthy controls, are in line with results described in previous reports [30,31].

The concentrations of nitrites/nitrates in NAL showed a correlation with the nNO levels, although with a very low correlation coefficient. No advantage of using nitrites/nitrates analyses compared to nNO measurements was found.

The subjects in our study were examined in a condition of optimal health and the allergen exposures were limited to perennial allergens, often in minor doses, and no exposure to pollen allergens. This might explain why an expected nasal obstruction in subjects sensitized to perennial allergens was not identified by acoustic rhinometry. Likewise, this might also explain why none out of the seven subjects suffering from allergic symptoms in the lower airways had the asthma diagnosis verified by FEV₁ results in the physical exercise test. Unfortunately, the sub-group members were low in number, which reduced the possibility to find statistical differences, a negative consequence of using a cohort for comparative studies. Nevertheless, in spite of low subject numbers an obvious influence on the airways due to smoking habits was found.

In conclusion, low levels of tobacco smoke exposure resulted in obvious reductions of airway functions as measured by acoustic rhinometry and spirometry before and after a physical exercise test in our cohort of adolescents. These functional tests were found to be more sensitive compared to nitric oxide measurements in early detection of the smoke related airway impairment. Since our cohort was small and the numbers of smokers were low, our findings have to be confirmed in larger studies, which also are required for an evaluation of a possible difference in vulnerability to tobacco smoke in allergic rhinitis subjects compared to healthy controls.

Conflict of interest statement

MP Borres is affiliated to Phadia AB, Uppsala, Sweden.

Acknowledgements

The authors wish to thank Professor Jon Lundberg, Department of Physiology and Pharmacology, Karolinska Institutet, for guidance and support.

Special thanks also to Lena Lindell, Nina Timelin, Lisbeth Hjälle and Tony Forslund, Linköping University, for excellent assistance.

Financial support was provided by the Swedish Medical Society and the Karl Jeppson Foundation. There was no involvement by the financial sponsors in the performance of this study.

References

- [1] M.S. Dykewicz, S. Fineman, Executive summary of joint task force practice parameters on diagnosis and management of rhinitis, *Ann. Allergy Asthma Immunol.* 81 (1998) 463–468.
- [2] M.S. Benninger, The impact of cigarette smoking and environmental tobacco smoke on nasal and sinus disease: a review of the literature, *Am. J. Rhinol.* 13 (1999) 435–438.
- [3] P.P. Plaschke, C. Janson, E. Norrman, E. Björnsson, S. Ellbjär, B. Järholm, Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensitization and smoking, *Am. J. Respir. Crit. Care Med.* 162 (2000) 920–924.
- [4] C.E. Baena-Cagnani, R.M. Gómez, R. Baena-Cagnani, G.W. Canonica, Impact of environmental tobacco smoke and active tobacco smoking on the development and outcomes of asthma and rhinitis, *Curr. Opin. Allergy Clin. Immunol.* 9 (2009) 136–140.
- [5] J. Numminen, M. Ahtinen 3rd, H. Huhtala, J. Laranne, M. Rautiainen, Correlation between rhinometric measurement methods in healthy young adults, *Am. J. Rhinol.* 16 (2002) 203–208.
- [6] P. Virkkula, M. Hytönen, A. Bachour, H. Malmberg, K. Humerinta, T. Salmi, et al., Smoking and improvement after nasal surgery in snoring men, *Am. J. Rhinol.* 21 (2007) 169–173.
- [7] M.P. Borres, K. Irander, B. Björkstén, Nasal metachromatic cells in infants in relation to allergic disease and family history of atopy, *Pediatr. Allergy Immunol.* 4 (1991) 184–189.
- [8] M.P. Borres, K. Irander, B. Björkstén, Nasal metachromatic cells in infancy in relation to the appearance of atopic disease during the first 6 years of life, *Allergy* 52 (1997) 770–774.
- [9] K. Irander, M.P. Borres, An 18-year follow-up of allergy development related to nasal metachromatic cell findings during infancy, *Allergol. Int.* 59 (2010) 193–200.
- [10] J.P. Palm, P. Graf, J.O.N. Lundberg, K. Alving, Characterization of exhaled nitric oxide: introducing a new reproducible method for nasal nitric oxide measurements, *Eur. Respir. J.* 16 (2000) 236–241.
- [11] W. Zetterquist, C. Pedroletti, J.O.N. Lundberg, K. Alving, Salivary contribution to exhaled nitric oxide, *Eur. Respir. J.* 13 (1999) 327–333.
- [12] H. Marteus, D.C. Törnberg, E. Weitzberg, U. Schedin, K. Alving, Origin of nitrite and nitrate in nasal and exhaled breath condensate and relation to nitric oxide formation, *Thorax* 60 (2005) 219–225.
- [13] A.-C. Olin, A. Aldenbratt, A. Ekman, G. Ljungkvist, L. Jungersten, K. Alving, et al., Increased nitric oxide in exhaled air after intake of a nitrate-rich meal, *Respir. Med.* 95 (2001) 153–158.
- [14] American Thoracic Society, European Respiratory Society, ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, *Am. J. Respir. Crit. Care Med.* 171 (2005) 912–930.
- [15] C. Serrano, A. Valero, J. Bartra, T. Torrego, J. Mullo, C. Picado, Effects on nasal nitric oxide production of 2 mechanisms of vasoconstriction, *J. Invest. Allergol. Clin. Immunol.* 17 (2007) 337–340.
- [16] F.H. Guo, H.R. De Raeve, T.W. Rice, D.J. Stuehr, F.B.J.M. Thunnissen, S.C. Erzurum, Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo, *Proc. Natl. Acad. Sci. U.S.A.* 92 (1995) 7809–7813.
- [17] B. Kunz, A.P. Oranje, L. Labrèze, J.F. Stalder, J. Ring, A. Taïeb, Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis, *Dermatology* 195 (1997) 10–19.
- [18] E. Millqvist, M. Bende, Reference values for acoustic rhinometry in subjects without nasal symptoms, *Am. J. Rhinol.* 12 (1998) 341–343.
- [19] C.P. Verdon, B.A. Burton, R.L. Prior, Sample pretreatment with nitrate reductase and glucose-6-phosphate dehydrogenase quantitatively reduces nitrate while avoiding interference by NADP⁺ when the Greiss reaction is used to assay for nitrite, *Anal. Biochem.* 224 (1995) 502–508.
- [20] A. Valero, C. Serrano, J.L. Valera, A. Barberà, A. Torrego, J. Mullo, et al., Nasal and bronchial response to exercise in patients with asthma and rhinitis: the role of nitric oxide, *Allergy* 60 (2005) 1126–1131.
- [21] T. Kjaergaard, M. Cvancarova, S.K. Steinsvaag, Smoker's nose: structural and functional characteristics, *Laryngoscope* 120 (2010) 1475–1480.
- [22] D.R. Gold, X. Wang, D. Wypij, F.E. Speizer, J.H. Ware, D.W. Dockery, Effects of cigarette smoking on lung function in adolescent boys and girls, *N. Engl. J. Med.* 335 (1996) 931–937.
- [23] I. Urrutia, A. Capelastegui, J.M. Quintana, N. Muñozguren, X. Basanga, J. Sunyer, et al., Smoking habit, respiratory symptoms and lung function in young adults, *Eur. J. Public Health* 15 (2005) 160–165.
- [24] E.O. Vianna, M.R. Gutierrez, M.A. Barbieri, R.D. Caldeira, H. Bettiol, A.A. Da Silva, Respiratory effects of tobacco smoking among young adults, *Am. J. Med. Sci.* 336 (2008) 44–49.
- [25] S. Yoo, H.B. Kim, S.Y. Lee, B.S. Kim, J.H. Kim, J. Yu, et al., Effect of active smoking on asthma symptoms, pulmonary function, and BHR in adolescents, *Pediatr. Pulmonol.* 44 (2009) 954–961.
- [26] A.-C. Olin, J. Hellgren, G. Karlsson, G. Ljungkvist, K. Nolkranz, K. Torén, Nasal nitric oxide and its relationship to nasal symptoms, smoking and nasal nitrate, *Rhinology* 36 (1998) 117–121.
- [27] V. Bessa, E. Tseliou, P. Bakakos, S. Loukides, Noninvasive evaluation of airway inflammation in asthmatic patients who smoke: implications for application in clinical practice, *Ann. Allergy Asthma Immunol.* 101 (2008) 226–232.
- [28] G. Scadding, G.K. Scadding, Update on the use of nitric oxide as a non-invasive measure of airways inflammation, *Rhinology* 47 (2009) 115–120.
- [29] J.P. Palm, K. Alving, J.O. Lundberg, Characterization of airway nitric oxide in allergic rhinitis: the effect of intranasal administration of L-NAME, *Allergy* 58 (2003) 885–892.
- [30] A. Simpson, A. Custovic, S. Pipis, A. Adishes, B. Faragher, A. Woodcock, Exhaled nitric oxide, sensitization, and exposure to allergens in patients with asthma who are not taking inhaled steroids, *Am. J. Respir. Crit. Care Med.* 160 (1999) 45–49.
- [31] A.-C. Olin, K. Alving, K. Torén, Exhaled nitric oxide: relation to sensitization and respiratory symptoms, *Clin. Exp. Allergy* 34 (2004) 221–226.