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Clinical aspects of exhaled nitric oxide in adults:

Associations with atopy, bronchial hyperresponsiveness, smoking and chronic obstruction

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ACADEMIC DISSERTATION

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Knowing is not enough; we must apply.
Willing is not enough; we must do.

Johann Wolfgang von Goethe (1749–1832)

Abstract

Measurement of fractional exhaled nitric oxide (FENO) has proven useful in assessment of patients with respiratory symptoms, especially in predicting steroid response. The objective of these studies was to clarify issues relevant for the clinical use of FENO. The association between allergic sensitization and FENO among healthy subjects with no signs or symptoms of airway diseases was studied in order to assess whether allergic sensitization per se influences FENO. The association between airway inflammation and bronchial hyperresponsiveness (BHR) in steroid-naive subjects with symptoms suggesting asthma was examined, as well as the possible difference in this association between atopic and nonatopic subjects. Influence of smoking on FENO was compared between atopic and nonatopic steroid-naive asthmatics and healthy subjects. The short-term repeatability of FENO in COPD patients was examined in order to assess whether the degree of chronic obstruction influences the repeatability, and to assess the clinically significant change in FENO in COPD patients.

For these purposes, we studied a random sample of 248 citizens of Helsinki, 227 army conscripts with current symptoms suggesting asthma, 19 COPD patients, and 39 healthy subjects. All subjects underwent FENO measurement, spirometry, and histamine and exercise challenges; atopy was assessed by skin prick tests.

Among healthy subjects with no signs of airway diseases, median FENO values were similar in skin prick test-positive (13.2 ppb) and -negative (15.5 ppb) subjects ($p=0.304$), with the non-parametric one-tailed 95% upper limit of FENO respectively 29 and 31 ppb. In atopic and nonatopic subjects with symptoms suggesting asthma, BHR to histamine (HIB) did not significantly differ, but exercise-induced bronchoconstriction (EIB) was more severe among atopic subjects ($p<0.01$). FENO associated with severity of EIB and HIB only in atopic patients. FENO in smokers with steroid-naive asthma was significantly higher than in healthy smokers and nonsmokers ($p=0.001$, both comparisons). Among atopic asthmatics, FENO was significantly lower in smokers than in nonsmokers ($p=0.002$), but with no difference among nonatopic asthmatics ($p=0.89$). However, even among nonatopic asthmatic smokers, FENO was significantly higher than among healthy subjects ($p=0.01$). In COPD patients, the coefficient of variation (CoV) for measurements of FENO repeated after 24 h was 12.4% in COPD patients, and 15.9% in healthy subjects. Among COPD patients with GOLD stage 2 disease, the CoV was 13.7%, and among those with stage 3 or 4 disease, 10.5%.

These findings indicate that allergic sensitization per se does not influence FENO, supporting the view that elevated FENO indicates NO-producing airway inflammation, and that the same reference range can be applied to both skin prick test-positive and -negative subjects. The significant correlation between FENO and the degree of BHR only in atopic steroid-naive subjects with current asthma-like symptoms supports the view that the pathogenesis of BHR in atopic asthma is strongly involved in NO-producing airway inflammation, whereas in the development of BHR in nonatopic asthma other mechanisms may dominate. Attenuation of FENO only in atopic but not in nonatopic smokers with steroid-naive asthma may result from differences in the mechanisms of FENO formation as well as in the sensitivity of these mechanisms to smoking in atopic and nonatopic

asthma. The results suggest, however, that in young adult smokers, FENO measurement may prove useful in assessment of asthmatic airway inflammation. The short-term repeatability of FENO in COPD patients with moderate to very severe disease and in healthy subjects was equally good. A change in FENO exceeding 24% is likely to reflect a significant change in COPD.

Contents

List of original publications

This thesis is based on the following original publications:

I Rouhos A, Kainu A, Karjalainen J, Lindqvist A, Piirilä P, Sarna S, Haahtela T, Sovijärvi ARA. Atopic sensitization to common allergens without symptoms or signs of airway disorders does not increase exhaled nitric oxide. The Clinical Respiratory Journal 2008;2:141-148

II Rouhos A, Ekroos H, Karjalainen J, Sarna S, Sovijärvi ARA. Exhaled nitric oxide and exercise-induced bronchoconstriction in young male conscripts: association only in atopics. Allergy 2005;60:1493-1498*

III Rouhos A, Ekroos H, Karjalainen J, Sarna S, Haahtela T, Sovijärvi ARA. Smoking attenuates increase of exhaled nitric oxide in atopic but not in nonatopic young adults with asthma. International Archives of Allergy and Immunology 2010;152:226-232 (DOI: 10.1159/000283029)

IV Rouhos A, Kainu A, Piirilä P, Sarna S, Lindqvist A, Karjalainen J, Sovijärvi ARA. Repeatability of exhaled nitric oxide measurements in patients with COPD (submitted)

The publications are referred to in the text by their Roman numerals. The original publications I to III are reprinted with the permission of their copyright holders.

* This publication has also appeared in the thesis of Heikki Ekroos 2008, Helsinki, Finland

Abbreviations

AMP	Adenosine monophosphate
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
BMI	Body mass index
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COV	Coefficient of variation
EAACI	European Academy of Allergy and Clinical Immunology
ECP	Eosinophilic cationic protein
EIB	Exercise-induced bronchoconstriction
ECRHS	European Community Respiratory Health Survey
ERS	European Respiratory Society
FENO	Fractional exhaled nitric oxide
FEV1	Forced expiratory flow in one second
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HIB	Histamine-induced bronchoconstriction
ICS	Inhaled corticosteroids
IFN-γ	Interferon γ
IgE	Immunoglobulin E
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LABA	Long-acting β-agonists
NF-κB	Nuclear factor κB
NO	Nitric oxide
NOS	Nitric oxide synthase
OLIN	Obstructive Lung Diseases in Northern Sweden
PD_{15}FEV1	Provocative dose of histamine causing a 15% fall in FEV1
PEF	Peak expiratory flow
SABA	Short-acting β-agonists
SPT	Skin prick test
TNF-α	Tumor necrosis factor α

1 INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common diseases globally and in Finland. Asthma affects approximately 6% of the Finnish population (Pallasaho et al. 2002), and diagnosed COPD about 5%, with undiagnosed disease estimated to affect a further 5% of the population (Laitinen et al. 1999). The prevalence of these diseases is increasing due to an increase in allergic sensitization, continued use of tobacco, and aging of populations.

Treatment of asthma by inhaled corticosteroids (ICS) aims at resolution of symptoms and optimization of lung function. These indices, however, correlate poorly with airway inflammation and bronchial hyperresponsiveness (BHR), the hallmark features of asthma. Treatment strategies aiming at reduction of airway inflammation or BHR by monitoring of sputum eosinophils (Green et al. 2002a) or monitoring responsiveness to methacholine (Sont et al. 1999), in addition to lung function and symptoms, have resulted in better asthma control. Unlike asthma patients, only a subset of COPD patients benefit from ICS. However, differential diagnosis is not always clear-cut; these diseases may often coexist (Marsh et al. 2008), or patients may present with symptoms and findings with features of asthma or COPD but not fulfill the diagnostic criteria of either disease.

Although airway inflammation and bronchial hyperresponsiveness are closely related, they represent different aspects of the airway disease, with their relationship possibly changing over the course of the disease and its treatment. Atopic and nonatopic asthma share these features, but some differences do appear in inflammatory profile and cytokine production (Bettiol et al. 2000, Amin et al. 2000, 2005) as well as in the clinical profile of the disease (Romanet-Manet et al. 2002). Whether these differences are also reflected in the relation between airway inflammation and BHR is unclear.

Since the first report of increased concentrations of nitric oxide (NO) in the exhaled air of asthmatics (Alving et al. 1993), extensive research on fractional exhaled NO (FENO) has provided the rationale for its clinical application. FENO correlates with eosinophilic airway inflammation, and as eosinophilic inflammation is responsive to steroid treatment, FENO may be regarded as a surrogate marker for steroid-responsive airway inflammation irrespective of the exact diagnostic label (Taylor et al. 2006). The use of FENO in the diagnostic work-up of patients with respiratory symptoms could result in better recognition of those with steroid-responsive symptoms, thus helping in focusing appropriate treatment on those who are most likely to gain benefit. FENO is easy to measure, and the repeatability of the measurement is good in healthy subjects, and in subjects with asthma or with respiratory symptoms suggesting asthma (Ekroos et al. 2000, 2002, Kharitonov et al. 2003). Good repeatability has been reported also in COPD patients (Brindicci et al. 2005, Bhowmik et al. 2005, de Laurentiis et al. 2008). However, severe obstruction could impair the repeatability; these studies have included only a small number of such patients. Further studies are needed before assessment of clinically significant changes in FENO in COPD patients during the course of the disease or in response to steroid treatment is possible.

Furthermore, some other aspects of FENO remain unclear or controversial. Reports of elevated FENO in subjects with allergic rhinitis without symptoms from the lower

respiratory tract or atopic subjects even with no current respiratory symptoms (Horváth & Barnes 1999, Franklin et al. 2004, Olin et al. 2006) have inspired criticism of FENO's being merely a feature of atopy rather than of airway inflammation or airway disease. Furthermore, smoking has been shown to reduce FENO in healthy and asthmatic subjects (Kharitonov et al. 1995a, Verleden et al. 1999, Horváth et al. 2004), compromising the clinical value of FENO among smokers.

The present series of investigations were aimed at examining these unclear issues relevant to the clinical use of FENO. Our study of a random sample of citizens of Helsinki was part of the large epidemiological FinEsS study (Meren 2005, Pallasaho 2006a, Kotaniemi 2006, Kainu 2008), and was aimed at assessing the association between allergic sensitization and FENO in healthy asymptomatic nonsmokers with no signs or history of airway diseases, and at comparing this association among those with respiratory symptoms or diagnosed airway diseases. Studies on steroid-naive army conscripts with current symptomatic asthma aimed at establishing the relationship between airway inflammation and BHR to both direct and indirect stimuli, as well as the influence of smoking on FENO levels, and at assessing whether this relationship differs between atopic and nonatopic asthma. In addition, we studied patients with stable COPD to assess whether degree of chronic obstruction influences the repeatability of FENO, and to assess clinically significant change in FENO in COPD patients.

2 REVIEW OF THE LITERATURE

2.1 Exhaled nitric oxide

2.1.1 Overview

Nitric oxide (NO), one of the smallest biologically active substances, plays a key role as a signal molecule in vital physiologic functions such as regulation of vascular tone and modulation of neuronal function, and in host defense. Identification of the endothelium-derived relaxing factor as NO earned the 1998 Nobel Prize in Physiology or Medicine for Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad. In biological tissues, NO is highly reactive, whereas in gaseous form it is fairly stable at low concentrations. It differs from traditional transmitter molecules as it has no cell membrane receptors and is not stored within cells but diffuses freely into the cells. Many of its biological effects are mediated by the activation of guanylate cyclase. In addition, NO can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Its biological effects depend on its concentration. NO is produced by a wide variety of structural and inflammatory cells, including those involved in asthma such as eosinophils, mast cells, epithelial cells, macrophages, and smooth muscle cells (Moncada et al. 1991, Yates 2001a).

NO is formed endogenously from the amino acid L-arginine in a reaction catalyzed by nitric oxide synthase (NOS). Three isoforms of NOS have been identified: endothelial NOS (eNOS or NOS1), neuronal NOS (nNOS or NOS3), and inducible NOS (iNOS or NOS2). The first two, referred to as constitutive NOS (cNOS), are responsible for the physiological production of NO, yielding picomolar concentrations within seconds or minutes in response to small rises in intracellular calcium concentration secondary to cell activation. These low concentrations of NO regulate vascular tone, inhibit platelet aggregation, and act as a transmitter in the non-adrenergic-non-cholinergic nerve endings regulating various respiratory, gastrointestinal, and genitourinary functions (Moncada et al. 1991, Yates 2001a). NO is important for the normal beating of airway epithelial cilia, it also affects mucus secretion and plasma exudation and thus has an influence on mucociliary clearance (Jain et al. 1993, Yates 2001a).

Activation of the inducible isoform of NOS (iNOS) results in production of much larger amounts of NO in nanomolar concentrations for longer periods of time after cell activation. Its activity is independent of calcium concentration and is regulated by transcription factors, most importantly by nuclear factor kappa B (NF-κB), which is activated by several proinflammatory cytokines, especially tumor necrosis factor-alfa (TNF-α) and interferon-gamma (IFN-γ), or microbial products (Yates 2001a, Korhonen et al. 2005). NO derived from epithelial cells and macrophages may be important in the pulmonary host defense mechanism because of its antimicrobial activity (Nathan & Hibbs 1991, Yates 2001a). Increased FENO production may have an amplifying effect on airway

inflammation, as NO can facilitate eosinophil chemotaxis to the airways or can enhance eosinophil viability (Korhonen et al. 2005). NO may also promote the development of Th2 responses, because it reduces IFN-γ production, thus allowing proliferation of Th2 lymphocytes (Yates 2001a). The formation and effects of NO in airway inflammation are presented in Figure 1. The gene for iNOS is located in chromosome 17q11.2-q12 in a region where a linkage with atopy and asthma has been reported, and polymorphisms in the iNOS gene have been reported to associate with severity of asthma and with eosinophilia (Batra et al. 2007).

After its production, NO can be exhaled, metabolized to nitrate and nitrite, or can interact with superoxide to form peroxynitrite. NOS is not the only source of NO, as NO can also be generated nonenzymatically from several chemical sources such as S-nitrosothiols and organic nitrates (Marteus et al. 2005). NO is produced along the entire airway, with hundred-fold higher concentrations derived from upper than lower airways, with most of the NO from the upper airways produced in the nasal cavities (Lundberg et al. 1996). This large NO production in the upper airways has to be taken into account in the measurement technique in order to obtain NO levels representative of NO production in the lower airways.

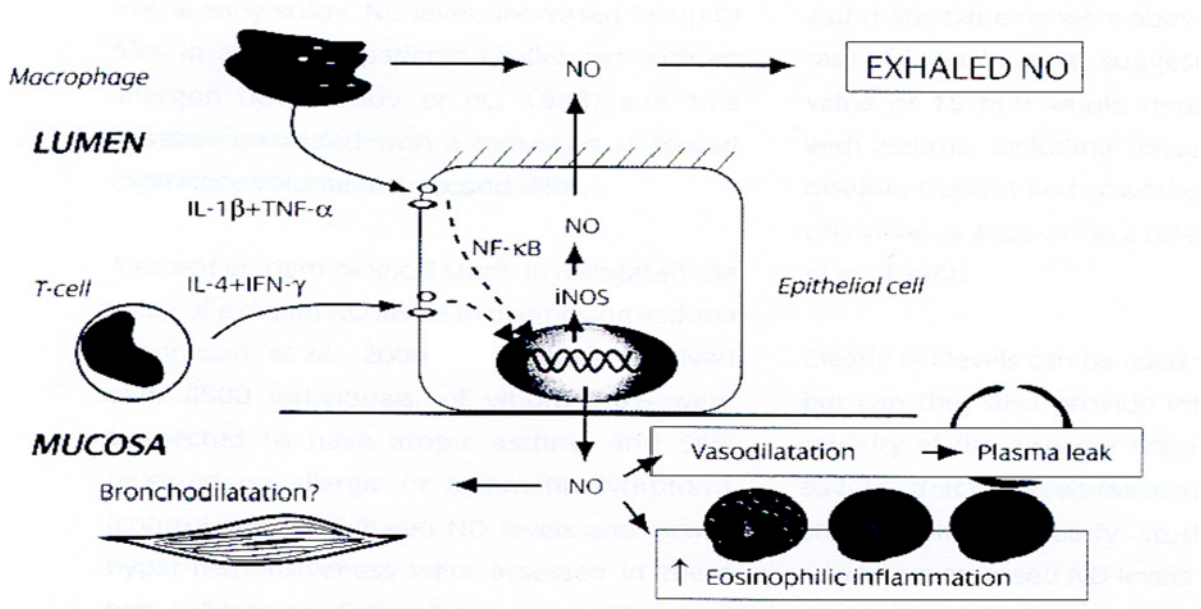

Figure 1 *Formation and effects of NO in airway inflammation (modified from Kharitonov & Barnes 2001)*

2.1.2 FENO and airway inflammation

The beginning of NO research in airway pathology can be attributed to the report by Gustafsson and co-workers in 1991 demonstrating that endogenous nitric oxide can be measured in the exhaled air of experimental animals and humans. A higher amount of exhaled NO in atopic asthma than in healthy subjects was first reported in 1993 (Alving et al. 1993), followed by the finding of iNOS expression in the bronchial biopsies of nonsmoking steroid-naive atopic asthmatics, whereas it was almost absent in healthy subjects (Hamid et al. 1993). Furthermore, normal bronchial epithelial cells were found to express iNOS in response to stimulation with a cytokine, TNF-α (Hamid et al. 1993). An inhaled allergen challenge in asthmatic patients has been shown not to change FENO during the acute bronchoconstriction, but instead, a progressive increase during the late response has been detectable, suggesting increased iNOS expression and consequently increased NO production in response to inflammatory cytokines (Kharitonov et al. 1995b).

As increased levels of FENO have appeared especially in asthmatics, numerous studies have examined the correlation between FENO and airway inflammation assessed directly in induced sputum, or bronchoalveolar lavage (BAL), or in endobronchial biopsies in patients with asthma (Jatakanon et al. 1998, Horvath et al. 1998, Lim et al. 1999, Piacentini et al. 1999, Jatakanon et al. 2000, Berlyne et al. 2000, Lim et al. 2000, van den Toorn et al. 2001, Payne et al. 2001, Grönke et al. 2002, Jones et al. 2002, Warke et al. 2002, Berry et al. 2005, Malmberg et al. 2005). In order to evaluate the use of FENO as a surrogate marker for eosinophilic airway inflammation in clinical practice, the greatest interest has been focused on studies examining the association between sputum eosinophilia and FENO. A significant correlation between FENO and sputum eosinophils has been detectable in several studies (Jatakanon et al. 1998, Horvath et al. 1998, Piacentini et al. 1999, Jatakanon et al. 2000, Berlyne et al. 2000, Jones et al. 2002, Grönke et al. 2002, Berry et al. 2005, Malmberg et al. 2005), the largest study being by Berry and co-workers (2005), in which a significant correlation appeared between FENO and sputum eosinophil count in a study population of 566 stable asthmatics. In general, the strongest associations have appeared among nonsmoking steroid-naive subjects with atopic asthma.

2.1.3 Measurement of FENO

Exhaled nitric oxide can be measured by a chemiluminescence method based on the photochemical reaction between NO and ozone (0_3) generated in the analyzer. The amount of light emitted in the reaction, quantified by a photomultiplier, is proportional to the amount of NO in the exhaled air. The result is expressed as a fractional concentration, parts per billion (ppb). Measurements are usually performed online, meaning FENO testing with a real-time display of NO breath profiles. Off-line measurements, meaning the collection of exhalate into a suitable reservoir for delayed analysis, are also possible but seldom used, because commercial portable hand-held analyzers using a rapid electrochemical method have become available. The repeatability of FENO measurements using a hand-held device has been shown to be good (Takalo et al. 2008). Some differences in FENO results have been reported between different stationary analyzers

(Borrill et al. 2006), whereas agreement has been good between measurements with hand-held and stationary devices (Alving et al. 2006, Khalili et al. 2007). Most chemiluminescence analyzers are sensitive to < 1 ppb of NO.

For the measurement, the subject is instructed to exhale from near-total lung capacity against a flow resistor in order to close the soft palate and to avoid any contamination from the high NO content of the nasal cavities. NO-free air (filtered room air, synthetic air or 100% oxygen) is used for inhalation in order to avoid the influence of variations in ambient air NO. Because breath-holding may elevate FENO, exhalation should then start immediately, and it should last at least 6 seconds in order to allow the airway compartment to be washed out and a reasonable plateau to be achieved. The plateau concentration of FENO is defined as the mean concentration over a 3-second period during the stable end-expiratory plateau. The exhalation procedure is repeated at least twice, reproducible exhalations yielding plateau NO values that agree within 10%. The result of the measurement is the mean of the plateau NO values of these repeated measurements. When standardized techniques have been used, the repeatability of FENO measurements has been good in healthy subjects (Ekroos et al. 2000, Kharitonov et al. 2003), both in nonsmokers and smokers (Bohadana 2008), in asthma (Ekroos et al. 2002, Kharitonov et al. 2003), and in subjects with asthma-like respiratory symptoms (Ekroos et al. 2002). Good repeatability has been reported also in patients with COPD (Brindicci et al. 2005, Bhowmik et al. 2005, de Laurentiis et al. 2008), but these studies have included only a small number of patients with severe disease. Severe obstruction may impair the subject's ability to maintain the required flow or impair the diffusion of NO into the exhaled air due to collapse of the bronchioles during exhalation. These issues must be addressed for clinical use of FENO measurements in assessment of eosinophilic inflammation or steroid response in COPD patients.

2.1.4 Factors influencing FENO

Exhalation flow rate is crucial for obtaining reproducible measurement. Increase in flow rate results in decrease in exhaled NO concentration, as faster air flow through the bronchial tree leaves less time for diffusion of NO from bronchial mucosa to the exhaled air. Following the initial reports on increased FENO in asthma, a wide range of different techniques have been used, and great efforts made to standardize the method. Three sets of guidelines have been published: the first one by European Respiratory Society (ERS) in 1997 (Kharitonov et al. 1997), which recommended a flow rate of 10 to 15 l/min (167-250 ml/s). This was followed by the American Thoracic Society (ATS) guidelines in 1999 (American Thoracic Society 1999) and combined ATS/ERS recommendations in 2005 (ATS/ERS 2005), both of which chose a flow rate of 50 ml/s as a reasonable compromise between measurement sensitivity and patient comfort. For reproducible measurements, the variation in flow rate must remain between 45 and 55 ml/s during the time of the NO plateau generation. Measurements with multiple flow rates can be applied to differentiate between alveolar and bronchial components of FENO (Lehtimäki 2003, Barnes et al. 2006).

Spirometric maneuvers (Deykin et al. 1998, Silkoff et al. 1999, Deykin et al. 2000) and bronchial challenges with direct and indirect stimuli (de Gouw et al. 1998) and sputum induction with hypertonic saline (Beier et al. 2003) may transiently reduce FENO, and it is thus advisable they be performed after FENO measurements (ATS/ERS 2005). The FENO maneuver itself does not influence FENO levels (Silkoff et al. 1999). Non-enzymatic production of NO in the oral cavity may influence FENO. Mouth rinsing with an alkaline solution such as sodium bicarbonate or with an antibacterial mouth wash before the measurement has been reported to rapidly and transiently reduce FENO (Zetterqvist et al. 1999, Marteus et al. 2005), but the duration of this effect is unclear. Current guidelines do not advise on mouth rinsing before FENO measurement (ATS/ERS 2005). The influence on FENO of prior caffeine consumption is controversial (Taylor et al. 2004), alcohol may cause a slight decrease (Yates et al. 1996, Jones et al. 2005), and ingestion of nitrite or nitrate-rich foods a slight increase in FENO (Zetterqvist et al. 1999); thus patients are instructed to refrain from eating and drinking anything for one hour before FENO measurements (ATS/ERS 2005). Influence on FENO of active and passive smoking and smoking cessation is addressed in section 2.6. Respiratory tract infections may raise FENO, with return to baseline level reported after 3 weeks (Kharitonov et al. 1995c, Murphy et al. 1998). Most studies, although not all, have found no circadian rhythm in FENO, making it uncertain whether measurements need to be standardized for time of day (ATS/ERS 2005).

Short-acting β2-agonists (SABA) may cause a slight, transient increase in FENO (Yates et al. 1997, Silkoff et al. 1999), whereas no influence has been detectable with long-acting β2-agonists (LABA) (Yates et al. 1997). Steroid treatment has no inhibitory influence on the physiological NO production by constitutive NOS, whereas the increased FENO in asthma produced by iNOS activity is dose-dependently reduced by steroid treatment (Kharitonov et al. 1996a, Jatakanon et al. 1999a, Jones et al. 2002, Kharitonov et al. 2002). This may be due to inhibition of iNOS expression, or inhibition of synthesis of proinflammatory cytokines as well as reduction in the number of activated cells that release proinflammatory cytokines in the airways (Kharitonov et al. 1996a). The leukotriene receptor-antagonist montelukast (Bisgaard et al. 1999, Sandrini et al. 2003) and the monoclonal anti-IgE antibody omalizumab (Silkoff et al. 2004) reduce FENO in patients with asthma. Neither nedocromil (Carra et al. 2001) nor theophylline (Lim et al. 2001) has been found to reduce FENO.

The effects of gender, height or age on FENO have been conflicting. Several studies have reported lower concentrations in females, even when adjusted for height or weight (Tsang et al. 2001, Olivieri et al. 2006, Taylor et al. 2007, Travers et al. 2007), whereas others have found no difference between genders when other anthropometric factors are taken into account (Olin et al. 2006, 2007). Studies by Travers and by Taylor reported gender, atopy, and smoking status to influence FENO. The largest study, involving 3 376 adults, found FENO levels to be dependent on age and height, independent of gender (Olin et al. 2007), and defined the upper limit of FENO as ranging from 24 to 54 ppb. A summary of some suggested reference values in healthy adults is given in Table 1.

Table 1. *Summary of some suggested reference values for FENO*

Authors	Upper limit of reference value (ppb)	Subjects	Flow rate (ml/s)	Comments
Ekroos et al. 2000*	< 12	adult nonsmokers	90-120	
Olin et al. 2007	24.0-54.0	adult nonsmokers	50	equation: Ln (FENO) = 0.057 + 0.013 x height (cm) + 0.0088 x age (yrs)
Travers et al. 2007	< 41.1	adults	50	

* presented for interpretation of the results in Studies II and III

Not only is eosinophilic inflammation or asthma known to influence FENO. Increased FENO levels have been reported in other inflammatory lung diseases such as allergic alveolitis, fibrosing alveolitis, bronchiectasis, bronchiolitis obliterans syndrome in lung transplants, and decreased levels in cystic fibrosis and in primary ciliary dyskinesia, as well as in pulmonary hypertension; in sarcoidosis results are conflicting (Kharitonov & Barnes 2001). FENO in COPD is addressed in section 2.5.

2.2 Atopy

2.2.1 Overview

The word "atopy," coming from the Greek, meaning "special" or "unusual," was introduced in 1923 (Coca & Cooke 1923). Atopy was considered to be hereditary, limited to a small group of patients, clinically characterized by hay fever and bronchial asthma, and associated with immediate-type (wheal-and-flare) skin reactions. The current definition of atopy as proposed by the Nomenclature Review Committee of the World Allergy Organization reads as follows: "Atopy is a personal and/or familial tendency to become sensitized and produce IgE antibodies in response to ordinary exposures to allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis, or eczema" (Johansson et al. 2004). A 2001 position paper from the European Academy of Allergology and Clinical Immunology (EAACI) recommended that "a healthy asymptomatic person with a positive skin prick test or the presence of specific IgE antibodies should be referred to as "skin prick test-positive" or "IgE sensitized," and the term atopic reserved for a person with this predisposition who is suffering from typical allergic symptoms" (Johansson et al. 2001), whereas the revised nomenclature report concludes that "atopy is a clinical definition of an IgE-antibody high-responder, and allergic symptoms in a person of the atopic constitution may be referred to as atopic, as in atopic rhinitis" (Johansson et al. 2004). According to this recommendation, also the term "atopic dermatitis," which is frequently used to

describe eczema with certain clinical characteristics, should be restricted to those in whom IgE-sensitization can be detected (Johansson et al. 2004).

Atopy is a hereditary trait: If both parents are atopic, the risk of child's developing an IgE-mediated allergy is 40 to 60%. If neither parent is atopic, the risk used to be considered 5 to 10%, but the percentage is increasing (Johansson et al. 2001). Associations have been found between several gene loci and asthma and high IgE levels (Laitinen et al. 2001), but no specific genetic markers for atopy have been identified, suggesting that atopy is a polygenic disorder (Johansson et al. 2001). Recent studies have reported mutations in the filaggrin-protein gene as resulting in impaired skin barrier function predisposing to atopic dermatitis and asthma (Holloway et al. 2010). The wide range in reported prevalence rates of atopic sensitization may depend on the study populations, differences in performing skin prick tests and reading the reactions, differences between allergens and in the potency of allergenic extracts (Baldacci et al. 2001). The prevalence of atopic sensitization in Helsinki, Finland, as based on skin prick test results, has been reported to be high, with 57% of subjects aged 26 to 39 being sensitized to at least one allergen, and 42% of those sensitized responding to at least four allergens (Pallasaho et al. 2006b). However, approximately 40% of the skin prick test-positive subjects in the Finnish adolescent population have been asymptomatic (Haahtela et al. 1980, Kilpeläinen et al. 2001).

Atopic sensitization is not a sign of a disease, but it bears the risk for development of typical allergic diseases such as allergic rhinitis or asthma. Skin prick test-positive subjects have been reported to be about three times as likely to develop asthma, and have more than twice the risk for allergic rhinitis of skin prick test-negative subjects (Settipane et al. 1994). Multiple sensitization carries further increased risk, and it associates strongly with physician-diagnosed asthma, wheezing, and allergic rhinoconjunctivitis (Pallasaho et al. 2006b). The presence of atopy may facilitate the development of exercise-induced bronchoconstriction (EIB). The degree of atopic sensitization indicated by atopy score has been shown to correlate significantly with degree of EIB (Koh et al. 2002). The Koh study used an extensive panel of 53 allergens and defined as a positive response a wheal size of $\geq$ 4 mm.

2.2.2 Assessment of atopy

Atopic sensitization can be assessed either by skin prick test or by measurement of IgE in a blood sample. Allergen-specific IgE is preferable to total IgE, which is significantly influenced by age, sex, smoking habits, and ethnicity (Baldacci et al. 2001). Allergen-specific IgE in a blood sample is considered positive if the level is higher than the lowest detection limit of the assay. In the skin prick tests, an interaction is artificially created between the allergen and the IgE bound to mast cell receptors in the skin. The selection of a reaction size regarded as positive is a compromise between acceptable levels of sensitivity and specificity. Usually a reaction size $\geq$ 3 mm in diameter is considered positive, and subjects with at least one positive reaction regarded as skin prick test-

positive (Dreborg 1989, Dreborg & Frew 1993). A decrease occurs in skin prick test reactivity with aging (Barbee et al. 1981), due to either a real decline in immunologic reactivity because of aging or to a decrease in the ability of the skin to react to immunological challenges (Baldacci et al. 2001). Dermographismus, present in 3 to 5% of the population, prevents interpretation of skin test results.

The number of allergens or specific IgEs required for a panel allowing identification of all atopic subjects in a population is uncertain. The NHANES 2005-2006 study reported that approximately 92% of atopic subjects were identifiable by six specific IgEs, but to increase identification to more than 99% required eleven specific IgEs (Gergen et al. 2009). The ERCHS study on 20- to 44-year-olds concluded that seven allergens in a skin prick test panel would identify almost all the sensitized subjects (Bousquet et al. 2007), but their definition of a positive response as a wheal size > 0 mm differs from the guidelines. This cut-off level of > 0 mm, however, has been assessed in a recent study to be best at identifying those with allergen-specific IgE in epidemiological studies (Bousquet et al. 2008). In addition to the dichotomized results, the quantification of allergic sensitization has also been applied based on atopy score by summing up the wheal diameters of each positive reaction (Miles et al. 1995, Koh et al. 2002) or by summing up specific IgE values (Söderström et al. 2003).

2.2.3 Atopy and FENO

Whether atopic sensitization per se, without the manifestation of any allergic airway disease, may lead to increased FENO has been a matter of debate. Studies based on general population samples have generally found FENO to be higher in atopic than in nonatopic subjects (Salome et al. 1999, Franklin et al. 2004, Olin et al. 2006), and atopic subjects without asthma or asthma-like symptoms but with allergic rhinitis have also shown increased levels of FENO (Henriksen et al. 1999, Gratziou et al. 1999). Study of a heterogeneous population of 115 subjects found higher FENO levels in skin prick test-positive than in skin prick test-negative subjects (Franklin et al. 2004). Although all subjects were asymptomatic at the time of the study, 26% had physician-diagnosed asthma, 17% reported wheezing during the previous year, and 17% had increased bronchial responsiveness; thus they did not represent a healthy population. A large general-population study found atopy as being a significant predictor of FENO even after exclusion of subjects with asthma or current asthma symptoms during the previous month (Olin et al. 2006). However, this study population also included subjects with a history of chronic or recurrent respiratory symptoms, and atopy was defined by measuring total IgE. Horváth and Barnes (1999) found higher levels of FENO in skin prick test-positive than in skin prick test-negative healthy asymptomatic subjects. The majority of the 15 atopic subjects included in the study, however, had mild to moderate bronchial hyperresponsiveness on methacholine challenge.

Salome and co-workers (1999) found higher FENO in skin prick test-positive than in skin prick test-negative young adults without current symptoms (wheeze within 12

months) or bronchial hyperresponsiveness in histamine challenge. In that study, however, the flow rate of FENO measurement was not controlled, thus influencing the results. In contrast, no significant difference in FENO was reported between 28 skin prick test-positive and 22 skin prick test-negative healthy subjects with no symptoms or signs of airway disease, no signs of airway obstruction in spirometry, and no bronchial hyperresponsiveness in methacholine challenge (Berlyne et al. 2000). Equal FENO levels have also been detectable in 20 skin prick test-positive and 80 skin prick test-negative healthy asymptomatic adults with no history of respiratory symptoms suggesting asthma or rhinitis (Gratziou et al. 1999). A similar finding was also reported by Olin and co-workers (2004) in a predominantly male population of 33 IgE-sensitized and 137 non-IgE-sensitized bleachery workers with no reported asthma or rhinitis.

2.3 Bronchial hyperresponsiveness

2.3.1 Overview

Bronchial hyperresponsiveness (BHR) can be defined as “an increase in the ease and degree of airway narrowing to inhaled bronchoconstrictor stimuli of chemical or physical origin, leading to variability in airway obstruction” (Sterk 1993a). Bronchoconstrictor stimuli are classified according to the main mechanism through which they induce airway limitation. Direct stimuli such as histamine and methacholine act directly on bronchial smooth muscle, causing it to contract and the airways to narrow. Indirect airway challenges such as exercise, adenosine monophosphate (AMP), and mannitol induce airflow limitation by acting on intermediary cells, which upon stimulation release mediators that provoke smooth muscle contraction and cause secondary bronchoconstriction (Sterk et al. 1993b).

BHR has been hypothesized to involve two at least semi-independent components: the relatively fixed or persistent BHR and superimposed on this a component of variable or episodic BHR (Cockroft & Davis 2006). The variable component is likely to be closely associated with airway inflammation and to be reflected by indirect bronchial challenges, whereas the underlying persistent component appears related to structural airway changes, collectively referred to as airway remodeling. This includes subendothelial basement membrane deposition of collagen, airway smooth muscle hypertrophy or hyperplasia, or both, increased vascularity, and changes in extracellular matrix composition, leading to increased contractility of the airway smooth muscle and increased airway wall thickness. This persistent component of BHR may be slowly or incompletely responsive to anti-inflammatory therapy, and is likely to be better reflected by direct bronchial challenges (Cockroft & Davis 2006).

Given this background, although BHR to direct stimuli may already show improvement after a few days’ treatment with inhaled steroids (ICS) in mild asthma

(Sovijärvi et al. 2003), BHR continues to improve with prolonged steroid treatment for at least one year (van Schoor et al. 2005), and some degree of BHR may remain even during long-term treatment with ICS (Brannan et al. 2007). In contrast, BHR to indirect challenges has been shown to be more sensitive to ICS, because it appears to be related to concentration of mast cells and eosinophils in the airway (Brannan et al. 2007). In a study on atopic asthma and steroid treatment, multiple regression analysis explained 40% of the variability in BHR: 21% related to reticular basement membrane thickening, 11% related to BAL epithelial cells, and 8% to BAL eosinophils (Ward et al. 2002). The Ward study concluded that airway inflammation and remodeling in asthma are interrelated, and improvement is achieved with ICS, but the changes are not temporally concordant; thus prolonged treatment may be necessary for maximal benefit (Ward et al 2002). To what extent each of these components, persistent and variable, influence BHR may change during the course of the disease, as airway inflammation is suggested to precede structural changes, which may represent a consequence of a chronic airway inflammation. A study on 66 nonsmoking steroid-free subjects with mild to moderate atopic asthma found the relationship between airway inflammation and BHR to depend on the asthma duration: a significant correlation emerged between FENO and BHR to methacholine in patients with duration of the disease $\leq$ 16 years, whereas no significant correlation appeared between these parameters in subjects with longer-duration disease (Grönke et al. 2002).

Bronchial hyperresponsiveness is not specific for asthma; mild BHR may be present also in COPD as well as in smokers. Asymptomatic BHR has been demonstrated in epidemiological studies, being more frequently observed in subjects with atopy, in members of families with asthma, in those exposed to tobacco smoke, and in women (Boulet 2003). Its significance is uncertain, as some follow-up studies have found it to be a risk factor for development of asthma (Laprise et al. 1997, Brutsche et al. 2006), whereas some have not (van den Nieuwenhof et al. 2008).

2.3.2 Assessment of bronchial hyperresponsiveness

2.3.2.1 Direct bronchial challenges

Direct airway challenges cause airflow limitation by acting directly on effector cells, predominantly on airway smooth muscle but also on mucus glands and on airway microvasculature without involving intermediate pathways. Direct stimuli include cholinergic agonists (methacholine, acetylcholine, carbachol), histamine, prostaglandin D2 and leukotrienes C4, D4 and E4, with histamine and methacholine as the agents most widely used (Sterk et al. 1993b). Response to the challenge is usually presented as a provocative dose (PD) or concentration (PC) that produces a 15 or 20% fall in FEV1, for example PD_{15}FEV1 signifying the provocative dose required to induce a reduction of 15% in FEV1. Responsiveness to histamine and to methacholine has correlated significantly with each other (Juniper et al. 1978, Juusela et al. 2008). The direct challenges have high sensitivity for the diagnosis of asthma and will consequently identify most cases of

asthma, even the mild cases. Direct challenges are more useful in excluding a diagnosis of asthma than in establishing one because their negative predictive power is greater than their positive predictive power; thus a negative response in general can be used to rule out asthma (Crapo et al. 2000). However, the sensitivity and the specificity of the tests naturally depend on the defined cut-off levels.

2.3.2.2 Indirect bronchial challenges

Indirect airway challenges induce airflow limitation by acting on cells other than smooth muscle cells, for instance, inflammatory cells, epithelial cells, and neuronal cells which upon stimulation release mediators, cytokines, or neurotransmitters that provoke smooth muscle contraction and cause secondary bronchoconstriction. A wide array of mediators are involved (histamine, leukotrienes, prostaglandins, acetylcholine, tachykins, neuropeptides). Because the responses to these challenges are modified or even completely inhibited by ICS, the airway response to these challenges may more closely reflect active airway inflammation (Joos et al. 2003). Indirect stimuli include physical stimuli such as exercise, nonisotonic aerosols (hyper- and hypotonic saline, distilled water, mannitol), eucapnic voluntary hyperventilation of dry air, and pharmacological stimuli such as adenosine monophosphate (AMP), tachykins, bradykinin, metabisulphite/SO2, and propranolol (Joos et al. 2003). In clinical practice, exercise is the most widely applied indirect stimulus, with use of mannitol probably increasing, now that mannitol capsules and a dry powder inhaler have become commercially available. Eucapnic voluntary hyperventilation is also useful in clinical practice. AMP has been used widely in clinical studies.

Several mechanisms are involved in bronchoconstriction induced by indirect stimuli (Figure 2). Exercise causes loss of water via evaporation from the mucosal lining of the airway surfaces, which results in osmotic (increase in airway osmolarity) effects, which in turn stimulate the release of bronchoconstrictor mediators mainly from mast cells and epithelial cells (Joos et al. 2003). Increased urinary levels of mast cell markers have been detectable in association with exercise-induced bronchoconstriction (EIB) in asthmatics (O'Sullivan et al. 1998). Vascular mechanisms caused by thermal effects (cooling and rewarming) may contribute to bronchoconstriction independent of the release of mediators (McFadden 1994). In addition, changes in airway osmolarity and temperature, as well as the mediators released may also directly activate neural pathways, resulting in reflex bronchoconstriction and increased microvascular permeability and edema (van Schoor et al. 2000). The same mechanisms act in eucapnic voluntary hyperventilation of dry air (Brannan et al. 2007). As for nonisotonic aerosols, their osmolarity appears to be the most important determinant of the airway response (van Schoor 2000). AMP enhances the release of a variety of inflammatory mediators from mast cells, exerting its action through interaction with specific adenosine receptors, one subtype being present on the surface of mast cells (van Schoor et al. 2005).

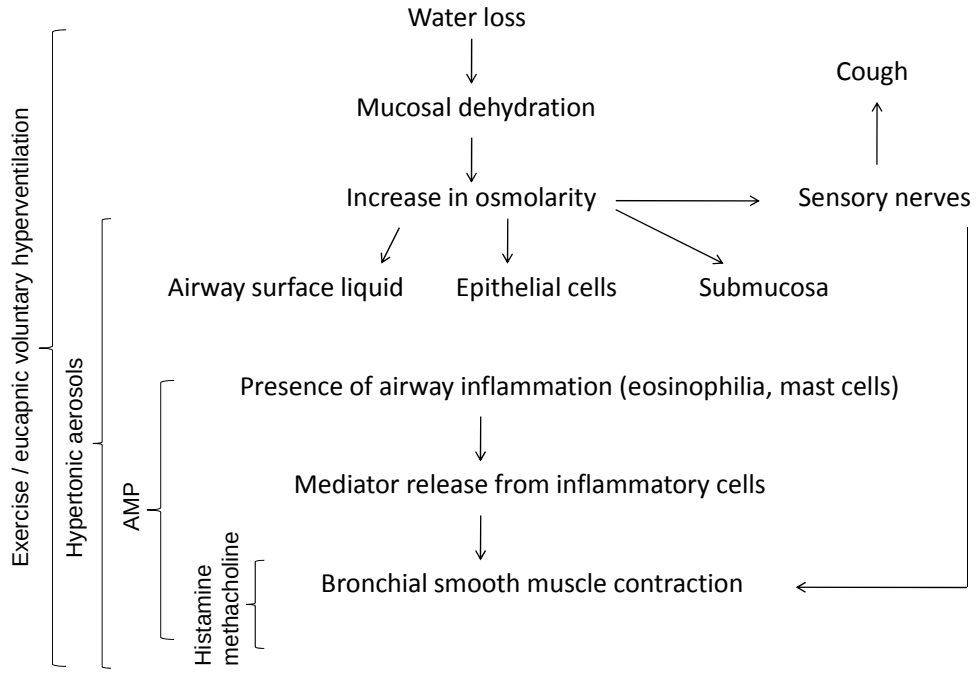

Figure 2 *Mechanisms of indirect and direct bronchial challenges (modified from Brannan et al. 2007)*

Exercise is considered to be the most physiological of all stimuli used for challenge testing. The major factors determining the occurrence and severity of exercise-induced bronchoconstriction (EIB) are the level of the ventilation, the water content and the temperature of the inspired air during the exercise, and the interval since exercise last induced an attack of asthma. Usually the most sensitive way of provoking exercise-induced bronchoconstriction in adult subjects is for them to exercise for 8 minutes at an intensity that raises the minute ventilation to 40 to 60% of the predicted maximum voluntary ventilation (Crapo et al. 2000, Joos et al. 2003). In the absence of a measure of ventilation, as often is the case in a clinical setting, one substitute is a heart-rate target for which 80 to 90% of the predicted maximum should be reached and maintained for the last 4 minutes of the exercise (Crapo et al. 2000, Joos et al. 2003). The most effective exercise is hard and brief, and the target exercise intensity should be reached within 4 minutes, because the prolonged warm-up period may induce refractoriness to EIB (Crapo et al. 2000). Appropriate post-exercise testing should include measurements 5, 10, 15, 20, and 30 minutes after cessation of exercise (Crapo et al. 2000). The maximal airflow limitation is usually detected within 3 to 12 minutes after exercise, with spontaneous recovery within 30 minutes (van Schoor et al. 2000). The criterion for a positive response is controversial: a reduction in FEV1 of 10% or more of the pre-exercise value is generally considered abnormal (Crapo et al. 2000, Joos et al. 2003), whereas some authors suggest that a value

of 15% is more diagnostic of asthma, particularly if exercise has been performed in the field. However, a fall in FEV1 of 10% has been considered a reasonable criterion, because healthy subjects generally demonstrate an increase in FEV1 after exercise (Crapo et al. 2000). The preferred modes of exercise are treadmill or cycle ergometer, but free-range running has been considered useful for screening populations (Crapo et al. 2000).

As all the indirect challenges are thought to work by similar mechanisms, the responses to indirect challenges can be expected to show good mutual correlation. Those asthmatics with a positive response to exercise, AMP, or hypertonic saline have been shown to be positive to mannitol, and asthmatics positive to saline and mannitol have been positive to eucapnic voluntary hyperpnoea (Brannan et al. 2007). In mild cases, indirect challenges will often be negative, whereas a positive response to indirect challenge is highly specific to asthma. Indirect challenges may be better suited to assess therapeutic efficacy and short-term changes in asthma control than direct challenges (Joos et al. 2003). Exercise is the bronchial challenge test that most closely resembles the circumstances of everyday life of an asthmatic subject. Although not very sensitive, an exercise test is highly specific for the diagnosis of asthma, and is particularly useful in children, army recruits, and athletes (van Schoor et al. 2000).

Direct and indirect challenges may have an influence on each other. When performed after a methacholine challenge, the response to a mannitol challenge has been attenuated, but when these two challenges were performed in the opposite order, the mannitol challenge had no influence on responsiveness to methacholine (Gade et al. 2009). Methacholine challenge has also been reported to blunt the response to subsequent eucapnic voluntary hyperventilation (Hurwitz et al. 1994). A repeated or continued exercise challenge can induce tachyphylaxis: 50% of patients are refractory to a second exercise challenge performed within 60 minutes. Most lose this refractory state within 2 hours, but this occasionally takes as long as 4 hours (Crapo et al. 2000). Similarly, repeated inhalation of AMP induces airway refractoriness. This refractoriness is likely to be mediated through depletion of the mediators involved in bronchoconstriction (van Schoor et al. 2000). Exercise challenge has had no influence on airway inflammation, as assessed by induced sputum (Tateishi et al. 1996, Gavreau et al. 2000) or by FENO (Scollo et al. 2000, El-Halawani et al. 2003).

2.3.3 Bronchial hyperresponsiveness, airway inflammation and FENO

If BHR is a combination of persistent BHR related to permanent or somewhat permanent structural changes, and of variable BHR, more related to the inflammatory component, it is understandable that the findings of the relationship between BHR and airway inflammation have been inconsistent. Some studies have reported significant correlations between eosinophilic inflammation assessed by sputum eosinophils or by FENO measurement and BHR to direct stimuli (Dupont et al. 1998, Jatakanon et al. 1998, Lim et al. 1999, Salome et al. 1999, Warke et al. 2002, Langley et al. 2003, Zietkowski et al. 2006); but some others none (Crimi et al. 1998, Yoshikawa et al. 1998, van Rensen et al. 1999, Polosa et al. 2000, Ichinose et al. 2000, Leuppi et al. 2001, Prieto et al. 2002a).

When BHR has been assessed by indirect challenges in study populations of steroid-naive subjects, correlations between these parameters have often been significant (Yoshikawa et al. 1998, Polosa et al. 2000, Prieto et al. 2002a, Berkman et al. 2005, Porsbjerg et al. 2007). If a significant correlation has appeared between eosinophilic airway inflammation and BHR in steroid-naive subjects, the correlation is often lost when steroid-treated subjects have been studied (Dupont et al. 1998, Lim et al. 1999, Leuppi et al. 2001). Despite improvement in eosinophilic inflammation and in BHR during steroid treatment, or during decreased exposure to allergens, no significant correlation between these changes has been detected (van Rensen et al. 1999, Prieto et al. 2002b). This reflects the fact that improvements in various aspects of airway pathology are not directly related to each other and cannot be interpreted interchangeably.

A further discrepancy in the correlation studies can be caused by the subjects' atopic status. Most of the studies have involved only atopic subjects (Jatakanon et al. 1998, Crimi et al. 1998, van Rensen et al. 1999, Polosa et al. 2000, Leuppi et al. 2001, Grönke et al. 2002, Prieto et al. 2002a, 2002b) or mixed populations comprising mainly atopic subjects (Yoshikawa et al. 1998, Scollo et al. 2000, Langley et al. 2003), and the atopic status is not always even assessed (Ichinose et al. 2000, Berkman et al. 2005), or the results have not been presented separately for atopic and nonatopic subjects (Dupont et al. 1998, Langley et al. 2003, Porsbjerg et al. 2007). Data on comparative studies assessing the correlation between airway inflammation and BHR separately in atopic and nonatopic subjects are very limited (Lúdvíksdóttir et al. 1999, Zietkowski et al. 2006). Lúdvíksdóttir's study found a significant correlation between FENO and BHR to methacholine in atopic but not in nonatopic subjects, whereas the study by Zietkowski and co-workers found significant correlations among both atopic and nonatopic subjects, the correlation being stronger among atopics. In the latter study, subjects were steroid-naive, whereas in the former study, the majority were on steroid treatment. Most studies have included only nonsmokers, but some studies also ex-smokers (Warke et al. 2002, Langley et al. 2003, Porsbjerg et al. 2007), and not always is the subjects' smoking status defined (Crimi et al. 1998, Ichinose et al. 2000, Berkman et al. 2005). This adds to the number of factors possibly influencing the correlation results, as smoking history (current or former) may influence the cellular composition of the airway inflammation, and the likelihood of permanent changes in the airways increases with increasing length of smoking history.

In asthma patients, bronchial responsiveness to indirect stimuli seems to be more closely associated with airway inflammation than is responsiveness to direct stimuli. A study comprising 120 atopic asthmatics found eosinophilic inflammation to be more closely associated with BHR to AMP than with BHR to methacholine. Percentage of sputum eosinophils was found to explain 25% of the variance in BHR to AMP, but it was not a significant independent predictor of BHR to methacholine (van den Berge et al. 2001). Berkman and co-workers (2005) studied 85 subjects with respiratory symptoms suggesting asthma but with no evidence of bronchial obstruction in spirometry. In this population with atopic and smoking status undefined, FENO correlated significantly with exercise, AMP, and methacholine challenges, AMP giving the best correlation, followed by exercise. Including only those 45 subjects in whom diagnosis of asthma was verified, a significant correlation emerged only between FENO and exercise challenge. Similarly, in

a study comprising 21 asthmatics, most of whom were atopics, sputum eosinophils correlated significantly with EIB but not with BHR to methacholine (Yoshikawa et al. 1998).

2.4 Asthma

2.4.1 Overview

During the last 10 years, the number of patients suffering from asthma has doubled (Kupczyk et al. 2010), the prevalence in Europe being highest in the UK, 10 to 13% (European Lung White Book 2003). In Finland, the FinEsS studies have reported a 6.6% prevalence of physician-diagnosed asthma in the Helsinki area (Pallasaho et al. 1999) and similarly a 6.4% prevalence in northern Finland (Kotaniemi et al. 2001). The genetic predisposition to develop asthma is well recognized and involves especially genes related to allergy and to certain cytokines or inflammatory mediators involved in asthma. Environmental factors, such as changing patterns of microbial exposure, and exposure to allergens and to environmental pollutants, are likely to interact with the genetic predisposing factors. Factors responsible for the increased incidence are not fully understood, but environmental and lifestyle changes play a major role (Kupczyk et al. 2010).

Global Initiative for Asthma (GINA) guidelines define asthma as “a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing. These episodes are usually associated with variable airflow obstruction that is often reversible either spontaneously or with treatment”. Diagnosis of asthma is based on the detection of reversible obstruction either in spirometry or in serial PEF measurements in conjunction with typical symptoms. For establishing an asthma diagnosis, the Finnish national asthma guidelines require improvement of $\geq$12% (and $\geq$ 200 ml) in FEV1 and/or in FVC, or repeated improvement of $\geq$15% ($\geq$ 60 l/min) in PEF in response to bronchodilator, or repeated diurnal PEF variation of $\geq$ 20% (Haahtela et al. 2006). In addition, the guidelines consider $PD_{15}FEV1 < 0.4$ mg of histamine or $PD_{20}FEV1 < 0.6$ mg of methacholine specific for asthma in adults. Diagnosis can also be established by detecting improvement of $\geq$ 15% (and $\geq$ 200 ml) in FEV1 or of $\geq$ 20% in PEF values during steroid treatment (Haahtela et al. 2006). Biopsy studies have shown airway inflammation to be present even at a clinically early stage of asthma (Laitinen et al. 1993, Jeffery 1996), and several studies have demonstrated early intervention with ICS to provide significant long-term benefits such as prevention of irreversible changes in the airway and reduction in occurrence of severe asthma exacerbations and decline in lung function in adult patients (Haahtela et al. 1994, Pauwels et al. 2003, O’Byrne et al. 2009, Haahtela et al. 2009).

2.4.2 Asthma, airway inflammation, and FENO

Although asthmatic airway inflammation is often referred to as eosinophilic inflammation, it is much more complex than simple eosinophilic inflammation alone. It is characterized by activated mast cells, infiltration of eosinophils, and increased number of T-helper 2 (Th2) lymphocytes, accompanied by increased expression of multiple inflammatory genes, regulated by proinflammatory transcription factors (Djukanovic et al. 1990, Laitinen et al. 1993, Bousquet et al. 2000). Eosinophils play a central deleterious role by release of proinflammatory mediators and cytokines resulting in vascular leakage, in hyperproduction of mucus, in smooth muscle constriction, and in epithelial shedding. They are also involved in the regulation of airway inflammation and initiate the remodeling process by release of cytokines and growth factors (Bousquet et al. 2000). Mast cells are critical "trigger cells" in eliciting acute bronchoconstriction, mucus secretion, and edema by the release of histamine and other vasoactive mediators. Mast cells also release proteases, and mast cell products activate fibroblasts, thus potentially playing a role in airway remodeling (Bousquet et al. 2000). Infiltration of airway smooth muscle by mast cells has been detectable in asthma but not in eosinophilic bronchitis, suggesting their role in bronchial hyperresponsiveness (Brightling et al. 2002). In addition to the increased cell numbers in asthmatic airway inflammation, an essential feature is the cell activation which results in production and release of cytokines such as interleukin (IL) 4, IL-5, and IL-13, which induce the immunoglobulin (Ig) E response and promote eosinophil chemotaxis. An increased number of neutrophils is reported in severe asthma (Jatakanon et al. 1999b), but a subgroup with predominantly neutrophilic airway inflammation has been detected also among patients with mild to moderate asthma (Green et al. 2002b). The epithelium in asthma is partially shed, and goblet cells appear in greater numbers than in healthy subjects. Epithelial cells may be important in regulating airway remodeling and fibrosis because they release fibrogenic growth factors (Bousquet et al. 2000).

Asthma has been divided into two subtypes: atopic (or allergic or extrinsic) and nonatopic (or nonallergic or intrinsic) asthma, since 1947, when Rackeman introduced the concepts of extrinsic and intrinsic asthma based on differences in the clinical phenotype of the disease (Rackeman 1947). Nonatopic asthma in adults is characterized by female dominance, later onset, more severe clinical course, and higher prevalence of nasal polyposis than in atopic asthma (Romanet-Manet et al. 2002). Even though atopic and nonatopic asthma share common immunopathological features (Humbert et al. 1999), some differences have been detected in their cellular pattern of inflammation (Amin et al. 2000, 2005) and in their cytokine production (Amin et al. 2000, Bettiol et al. 2000). Airway inflammation with increased numbers of eosinophils and mast cells is a feature of both atopic and nonatopic asthma, but biopsy studies by Amin and co-workers have found infiltration of eosinophils in the airway mucosa to be more prominent in atopic than in nonatopic asthma (Amin et al. 2000), and mast cell numbers and extracellular deposition of mast cell products in smooth muscle compartment to be higher in atopic than in nonatopic asthma (Amin et al. 2005). Furthermore, increased number of neutrophils and higher expression of IL-8 have both been detectable in nonatopic asthma (Amin et al.

2000). In contrast, biopsy studies by Karjalainen and co-workers (2003) found no difference in inflammatory cell profile between newly diagnosed atopic and nonatopic asthma. Both studies, however, found a significantly thicker basement membrane tenascin layer in atopic than in nonatopic asthma (Amin et al. 2000, Karjalainen et al. 2003). Whether this difference is directly related to the atopic constitution or possibly to a secondary factor such as duration of disease is still unclear. However, differences have been detectable in cytokine production between atopic and nonatopic asthma. Higher numbers of IL-4- and IL-5-positive cells have appeared in atopic asthma (Amin et al. 2000), and atopic but not nonatopic asthma has been characterized by increased IL-4 production, which correlates with eosinophilic inflammation (Bettiol et al. 2000). In atopic asthma, spontaneous production of IFN-γ, which suppresses IgE production, has been found to be impaired (Bettiol et al. 2000).

The first observation of increased FENO in atopic asthmatics was in 1993 (Alving et al. 1993). Since then, numerous studies have confirmed that steroid-naive atopic asthma is associated with increased FENO (Taylor et al. 2006), whereas studies on nonatopic asthma have reported conflicting results, with some reporting increased FENO (Zietkowski et al. 2006, Malinovschi et al. 2009), with others reporting no difference from levels in healthy controls (Lúdvíksdóttir et al. 1999, Graziou et al. 1999, Olin et al. 2004). A study by Ekroos and co-workers (2009) found higher FENO in nonatopic steroid-naive asthmatics than in healthy controls. Interestingly, FENO in low-sensitized atopic asthmatics (defined as total skin prick test wheal sum 3-10 mm) was similar to FENO in nonatopic asthmatics, whereas FENO among high-sensitized atopic asthmatics (defined as total skin prick test wheal sum > 10 mm) was significantly higher (Ekroos et al. 2009). When assessing patients with symptoms suggesting asthma, high FENO values have been found useful both in atopic and nonatopic asthma (Malmberg et al. 2005).

Since asthma is a heterogeneous disease, detailed phenotyping of asthma has been suggested instead of the current dichotomy; this might allow the successful targeting of therapies to those patients most likely to gain benefit (Wenzel 2006, Bradding & Green 2010). Significant differences in airway hyperresponsiveness and in FENO levels have occurred between some suggested subtypes (Porsbjerg et al. 2009); thus, BHR and FENO measurements could help in phenotyping these patients.

2.4.3 FENO and asthma diagnosis

Diagnosis of asthma is based on demonstrating variable airflow obstruction, whereas assessment of airway inflammation, the central feature of asthma, is not included in the diagnostic work-up. Presence of airflow obstruction may be intermittent, or particularly in mild asthma, it may not be present at the time of measurement, thus leaving the diagnosis open. Smith and co-workers (2004) compared FENO measurements with conventional tests (PEF measurements, spirometry) for diagnosis of asthma in 47 consecutive patients with symptoms suggesting asthma, and found sensitivities for conventional tests (0-47%) to be lower than for FENO (88%) or sputum eosinophils (86%), when in addition to symptoms, BHR or bronchodilatation response or both were the diagnostic criteria. A

study by Dupont and co-workers (2003) including 240 nonsmoking steroid-naive patients with respiratory symptoms reported FENO to be highly predictive of asthma, with sensitivity of 85% and specificity of 90%. A large population survey concluded that the combination of elevated FENO and increased BHR is a very specific finding for atopic asthma (Henriksen et al. 2000).

Assessment of airway inflammation in clinical practice would prove useful, as patients with asthma-like symptoms and signs of airway inflammation but with normal lung function are reported to benefit from steroid treatment (Rytilä et al. 2000). The rationale for measuring FENO is its ability to predict response to steroid treatment in patients with respiratory symptoms irrespective of exact diagnosis (Taylor et al. 2006). Moreover, importantly, the predictive value of a normal FENO for the absence of eosinophilic airway inflammation has been high, permitting steroid-unresponsive symptoms to be differentiated from those which are steroid responsive (Shaw et al. 2007).

Chronic cough is a common nonspecific symptom often present also in asthma. Frequently in mild asthma, no signs of reversible airway obstruction appear. Several studies have found FENO measurement useful in the assessment of chronic cough in predicting response to ICS. Chatkin and co-workers (1999) reported a positive predictive value of 60% and a negative predictive value of 93% with FENO $\geq$ 30 ppb serving as a cut-off value, and with FENO measured at a flow rate of 45 ml/s. In a retrospective study of 64 patients with chronic cough, 88% of subjects with elevated FENO showed a significant improvement in their cough following ICS treatment, whereas only 9% of subjects with normal FENO responded to ICS (Hahn et al. 2007). A cut-off of 38 ppb (flow rate 50 ml/s) best differentiated between ICS responders and nonresponders among patients with cough (Sato et al. 2008). A recent large study also supported the view that normal FENO predicts low likelihood of response to ICS (Oh et al. 2008). Furthermore, a recent study conducted in primary health care found FENO measurement to improve diagnostic confidence in assessment of nonspecific respiratory symptoms, with both normal and high FENO values having clinical significance (Hewitt et al. 2008).

2.4.4 FENO and asthma management

Asthma control is usually monitored by frequency of symptoms, serial measurements of PEF, or spirometry. Measures of lung function correlate poorly with the degree of airway inflammation, and perception of symptoms varies considerably, making these tools inadequate for assessment of the sufficient dose of ICS for controlling airway inflammation (Taylor et al. 2006). Furthermore, in mild cases, lung function can be normal even before initiation of ICS, leaving no room for improvement. Management strategies which aim at minimizing eosinophilic airway inflammation by monitoring sputum eosinophilia have shown substantial reduction in frequency of severe asthma exacerbations during one- or two-year study periods without increasing the total corticosteroid dose (Green et al. 2002a, Jayaram et al. 2006).

With steroid treatment, FENO levels rapidly decrease (Kharitonov et al. 1996a, 1996b, 2002, Jatakanon et al. 1999a), and they increase again along with deterioration of asthma

control (Jones et al. 2001). The results of the first study applying FENO-based asthma management in comparison with conventional management in a study population of 94 asthmatics appeared in 2005 (Smith et al. 2005). The FENO-based algorithm resulted in reduction in ICS dose without compromising asthma control, and the exacerbation rate was slightly although not significantly lower than in the conventionally managed group. The study design, however, was criticized for favoring reduction in ICS dose in the FENO group. Conflicting results were reported by Szefler and co-workers (2008), who found no benefit from FENO-based management compared to conventional management in terms of ICS dose, exacerbation rate, pulmonary function or symptoms among 12- to 20-year old mild asthmatics despite the fact that the dose of ICS was higher in the FENO group at the end of the study. In only some subgroups, such as in atopic or obese subjects, did FENO-guided management improve asthma control (Szefler et al. 2008). The study applied a FENO threshold of < 20 ppb to identify good control, this level being lower than the suggested reference range (Olin et al. 2007), and the study design allowed only an increase, not a decrease in ICS dose based on FENO levels. A third study with a similar setting including 118 adults (20-81 yrs) with a study period of 12 months, reported a lower final dose of ICS in the group with FENO-based management, but no significant difference in exacerbation frequency (Shaw et al. 2007).

Exacerbation frequency predicts lung function decline. Clinical markers identifying patients at risk for exacerbation could allow modification of asthma medication in order to prevent exacerbations. The decrease in FENO during steroid treatment for asthma precedes the improvement of symptoms, of FEV1, and of sputum eosinophilia (Jatakanon et al. 1999a), and similarly, an increase in FENO may be detectable with deterioration of asthma control before detection of significant changes in PEF variability or spirometry. A study by Jones and co-workers (2001) reported both single measurements and an increase of > 60% over baseline in FENO (measured at a flow rate of 250 ml/s) having positive predictive values ranging from 80 to 90% for the prediction and diagnosis of loss of asthma control. Gelb and co-workers (2006) measured FENO at a flow rate of 100 ml/s and found combined baseline FENO $\geq$ 28 ppb and FEV1 $\leq$ 76% of predicted value to identify asthmatics with 85% probability of future exacerbation. FENO measurement in subjects with difficult-to-treat asthma could help in identifying subjects who may benefit from an increased steroid dose. In a study by Pérez de Llano and co-workers (2009), subjects with suboptimal asthma control with maximal fluticasone-salmeterol combination were treated with a course of oral steroids. A FENO value $\geq$ 30 ppb demonstrated sensitivity of 87.5% and specificity of 90.6% for identification of responders to the course of oral steroids.

How best to interpret individual FENO measurements during treatment of asthma is still unclear. A large study on unselected asthmatics reported that changes in FENO values rather than absolute cut-off points may be meaningful for longitudinal assessment of asthma control (Michils et al. 2008). A 40% reduction in FENO proved to be a reliable predictor of asthma control optimization, and an increase < 30% to be helpful in excluding the occurrence of any significant deterioration. A recent study by Smith and co-workers (2009) demonstrated in those asthmatics whose phenotype is characterized by elevated FENO levels when symptomatic, that “personal best” FENO levels were lower with oral

than with inhaled steroid treatment. FENO levels after a course of oral steroids approximated the predicted values from the reference equations by Olin and co-workers (2007) and to a lesser extent approximated those suggested by Travers and co-workers (2007). However, targeting reference values may not be justified, as optimized doses of ICS have resulted in well-controlled asthma even when FENO levels exceeded those predicted. The study suggested that, rather than predicted values, the absolute values or the magnitude of changes in FENO in relation to personal best obtained when asthma is well controlled, is more likely to be informative (Smith et al. 2009).

A Cochrane Database review in 2009 concluded that tailoring the dose of ICS based on FENO has been beneficial in reducing the final (but not overall) daily ICS doses in adults, but this approach cannot be routinely recommended for clinical practice at this stage (Petsky et al. 2009). It is clear that routine measurements would not be useful, as FENO is not elevated in all asthmatics, elevated FENO does not equal eosinophilic inflammation, and inflammation in asthma is not always eosinophilic. Further studies are needed to identify the subgroups that benefit from FENO-based management of asthma and to develop a correct algorithm for this purpose.

2.5 COPD

2.5.1 Overview

Chronic obstructive lung disease (COPD) causes a major health burden worldwide, currently being the world's fourth most common cause of death (WHO 2008a), and predicted to rise to third place by the year 2020, just after ischemic heart disease and cerebrovascular disease (Murray & Lopez 1997). The pulmonary pathology of COPD can involve changes in the airway mucosa (increased number of goblet cells responsible for increased mucus production, reduced number of ciliated cells), obstruction in the small airways caused by inflammation and fibrosis, and structural changes in lung parenchyma (destruction of alveolar walls, abnormal enlargement of airspaces, loss of lung elasticity) adding to airway obstruction (Rabe et al. 2007). Systemic inflammation may be present, related to an accelerated decline in lung function and systemic manifestations of the disease (Barnes & Celli 2009).

Despite the multicomponent nature of the disease, diagnosis of COPD is usually based on detection of airflow obstruction in spirometry, because the pulmonary components of COPD are usually reflected in airflow indices detected in spirometry. Prevalence estimates differ, depending largely on the diagnostic definition of the disease and the population studied. The prevalence of physician-diagnosed COPD is much lower than suggested by population-based spirometric surveys, indicating that underdiagnosis of COPD is common (Mannino et al. 2000, Bednarek et al. 2008). The most widely used GOLD guidelines recommend a post-bronchodilator FEV1-to-FVC ratio < 0.7 to define airflow limitation,

and the grading of disease severity by FEV1 in relation to the predicted value (Global Initiative for Chronic Obstructive Lund Disease). The global BOLD study, applying these criteria, reported 10% prevalence of GOLD stage II or higher (FEV1 < 80% of predicted) (Buist et al. 2007). Although easy to use, this fixed cut-off ratio has been widely criticized, since it has been found to result in significant overestimation of COPD in older individuals and underestimation in younger adults (Pellegrino et al. 2008, Vollmer et al. 2009, Miller et al. 2009). Use of the lower limit of normal (LNN) both in FEV1-to-FVC ratio and in FEV1 to diagnose COPD, apparently has the least bias (Vollmer et al. 2009), and its use is recommended in the current ATS/ERS guidelines on lung function testing (Pellegrino et al. 2005). For grading of disease severity, the use of FEV1 alone does not adequately reflect all systemic manifestations of COPD. Multidimensional tools such as the BODE index (combination of body mass index, degree of obstruction, dyspnea score, and exercise capacity as measured by a 6-min walking test) may be useful to capture the multidimensional nature of COPD (Celli et al. 2004, Barnes & Celli 2009).

The direct causal relationship between tobacco smoke and COPD is well established (US Public Health Service 1984), and COPD prevalence corresponds directly to the smoking habits of the population with a 20-year latency. The frequent statement that 15 to 20% of smokers develop airway obstruction has proven to be an underestimate, as recent studies have shown that up to 50% of smokers develop COPD (Lundbäck et al. 2003, Rennard & Vestbo 2006, Kotaniemi et al. 2005, Mannino & Buist 2007). The only recognized genetic risk factor, severe α1-antitrypsin deficiency, concerns only 1 to 3% of patients with COPD (Mannino & Buist 2007). Occupational exposure to vapors, gases, fumes, or dusts raises the risk for COPD, but joint exposure to both smoking and occupational factors has a synergistic effect with markedly increased risk compared to exposure to occupational factors alone (Jaakkola 2009). Globally, also exposure to biomass fuels, especially to cooking gases in poorly ventilated homes, has been assessed to be an important risk factor for COPD (Mannino & Buist 2007).

In general, airway pathology in COPD is unresponsive to steroid treatment, but COPD is a heterogeneous disease, with some subgroups of patients likely to benefit from steroid treatment, and some patients finding it even detrimental (Yang et al. 2007, Drummond et al. 2008). Identification of the correct subjects for steroid treatment is not simple: differential diagnosis between asthma and COPD is not always clear, as the clinical course of asthma sometimes leads to fixed airway obstruction, whereas partial reversibility may be present in COPD, and these diseases are also reported often to co-exist (Marsh et al. 2008). Treatment should also aim at reduction of exacerbation frequency, since exacerbations are known to lead to faster FEV1 decline (Donaldson et al. 2002). Currently, regular treatment with ICS is recommended for COPD patients with FEV1 < 50% of predicted and repeated exacerbations (Rabe et al. 2007).

2.5.2 COPD and airway inflammation

Airway inflammation in COPD is characterized by an increase in macrophages, neutrophils, and cytotoxic (CD8+) T-lymphocytes. This pattern is also seen in smokers

with normal lung function, but in COPD the inflammation is amplified. Alveolar macrophages, activated by cigarette smoke and other inhaled irritants, release increased amounts of proteinases, inflammatory mediators, neutrophil chemotactic factors, and reactive oxygen species (Barnes et al. 2006a, Bourdin et al. 2009). Furthermore, alveolar macrophages of COPD patients show a marked reduction in the activity of histone deacetylase (HDAC), an inhibitor of cytokine production. This impaired function at least partly explains the steroid resistance in COPD, as steroids exert their anti-inflammatory properties via activation of HDAC (Barnes et al. 2006a, Barnes 2006b). Increased numbers of neutrophils are present prominently in the airway lumen, and also in the airway smooth muscle (Rutgers et al. 2000, Baraldo et al. 2004). Their numbers correlate with pack years and number of cigarettes smoked per day as well as with the COPD severity (Chalmers et al. 2001); with worsening disease severity, tissue neutrophilia increases (Barnes et al. 2006a). Neutrophils are the major providers of proteinases, which may contribute to the alveolar destruction and act as potent stimulants for mucus production. Levels of IL-8, a potent neutrophil chemoattractant, are higher in COPD than in healthy smokers, and correlate negatively with disease severity, and IL-8 is further increased during exacerbations (Barnes et al. 2006a). An increase in total elastase activity and a decrease in antielastase activity are reported in COPD patients compared to levels in healthy smokers (Barnes et al. 2006a). CD8+ T-lymphocytes are higher in the airways of smokers with COPD than in healthy smokers. They may cause cytolysis and apoptosis of the alveolar epithelial cells and have been found to correlate negatively with degree of airflow limitation (O'Shaughnessy et al. 1997, Saetta et al. 1998). Eosinophils are present in the airways of some COPD patients, and during exacerbations their numbers may increase considerably (Saetta et al. 1994).

Sputum eosinophilia in COPD patients appears to predict a larger bronchodilator response and improvement with corticosteroids (Papi et al. 2000). A course of oral steroids has resulted in an improvement in FEV1 also in those patients with fixed airway obstruction who have increased eosinophil counts in peripheral blood or in sputum (Brightling et al. 2000, Fabbri et al. 2003). Increased sputum eosinophil count in one study was related to a small but significant improvement in FEV1 following 4-week treatment with inhaled mometasone in subjects with fixed airway obstruction, even though no reduction in symptoms or in sputum eosinophil count was detectable (Brightling et al. 2005). Another study with a similar setting found improvement both in FEV1 and in symptoms as well as normalization of sputum eosinophils following inhaled budesonide treatment in COPD patients with increased sputum eosinophilia at baseline (Leigh et al. 2006). A recent study including 82 COPD patients showed that a management strategy aimed at minimizing sputum eosinophilia resulted in a significant reduction in severe COPD exacerbations compared to that in conventional management strategy, with no overall increase in steroid use (Siva et al. 2007).

2.5.3 COPD and FENO

FENO in stable COPD patients has been reported to range from low (Clini et al. 1998) to elevated (Corradi et al. 1999, Ansarin et al. 2001, Zietkowski et al. 2005, Liu et al. 2007, Beg et al. 2009) when compared to FENO in healthy subjects, with several studies reporting no difference between these groups (Kanazawa et al. 1998, Rutgers et al. 1999, Delen et al. 2000, Ziora et al. 2007). Increased FENO has been reported during COPD exacerbations (Agusti et al. 1999). This variability may in part be explained by the heterogeneous nature of the disease, with multiple factors influencing FENO. At the alveolar level, NO rapidly combines with reduced hemoglobin (400 times faster than CO) and is therefore scavenged by pulmonary capillary blood. In the presence of an altered ventilation-perfusion mismatch in COPD, this scavenging will take place less efficiently. Destruction of alveolar epithelial cells in more severe COPD may influence NO-production in the airways, and the development of cor pulmonale in severe COPD may reduce FENO, presumably as a reflection of endothelial injury. Furthermore, smoking (current or former) influences the composition of the airway inflammation and thus FENO formation. Several studies have included patients receiving steroid treatment, which further influences the results. Similarly, studies trying to correlate FENO and the indices of COPD severity have provided conflicting results (Clini et al. 1998, Ansarin et al. 2001, Bhowmik et al. 2005). Various methodologies and various flow rates for measuring FENO have been applied over the years. The conventional technique of FENO measurement reflects NO derived mainly from the large airways. Measurement of FENO at different flow rates enables one to differentiate airway-derived NO and peripheral NO derived from alveoli and probably from the small airways (Brindicci et al. 2005, Barnes et al. 2006a). An increase in peripheral NO is related to COPD severity and as it is insensitive to smoking, it thus may prove a useful noninvasive biomarker of COPD inflammation (Barnes et al. 2006a). Peripheral NO is, however, insensitive to corticosteroid treatment, and thus not useful as a marker predicting steroid response (Brindicci et al. 2005, Lehtimäki et al. 2010).

Corresponding to the positive results regarding the influence of steroid treatment in COPD patients with sputum eosinophilia, several studies have reported similar results when assessing the presence of airway inflammation by measuring FENO. A study by Ferreira and co-workers (2001) was the first to report a significant decrease in FENO in ex-smokers with COPD during a 2-week ICS treatment as well as a significant negative correlation between the change in FENO and the change in FEV1. Zietkowski and co-workers (2005) found a significant decrease in FENO after 2 months on ICS, with the initial level of FENO correlating with the decrease in FENO and with the increase in FEV1 after ICS therapy. Kunisaki and co-workers (2008) found significantly higher baseline FENO in those ex-smokers with severe COPD who showed significant improvement in FEV1 ($\geq$ 200 ml) during 4-week treatment with ICS. A study measuring bronchial and alveolar NO in 40 COPD patients found higher baseline bronchial NO to be associated with improvement in airway obstruction and symptoms in response to 4-week treatment with ICS. This treatment reduced bronchial NO flux but not alveolar NO concentration (Lehtimäki et al. 2010). As the complexity of COPD is not fully reflected by

measuring only FEV1, it is important that studies examining the influence of steroid treatment assess also its impact on symptoms and exercise capacity.

2.6 Smoking

2.6.1 Overview

Smoking is a risk factor for six of the eight leading causes of death, and the single most preventable cause of death in the world. Tobacco kills up to half its regular users, on average 15 years prematurely, causing today 1 in 10 deaths among adults worldwide, 5.4 million deaths per year (WHO 2008b). Despite the decline in the proportion of smokers in many developed countries, the tobacco epidemic is still increasing in the developing world. In developed countries about 35% of men smoke, compared with almost 50% of men in developing countries (Shafey et al. 2003). In 2008, in Finland, 24% of men and 18% of women (aged 15-64) were current smokers, and 21% of men and 16% of women former smokers (Helakorpi et al. 2009).

The adverse effects of smoking on respiratory health start already in prenatal life. Exposure to tobacco smoke especially during pregnancy and in early childhood permanently impairs lung function, causes a greater frequency of respiratory tract infections and raises the risk for asthma (Svanes et al. 2004, Pietinalho et al. 2009). Results from epidemiological and family studies indicate that genes predisposing to asthma interact with environmental tobacco smoke exposure early in life (Hylkema et al. 2007). In infants and young children, the proinflammatory effects of tobacco smoke may be reflected in increased NO levels (Franklin et al. 2006). Exposure to tobacco smoke (active and passive smoking) is associated with slowed lung function growth, and early onset of lung function decline in adolescents, with girls possibly more vulnerable to this effect (Gold et al. 1996). Smoking is associated with increased bronchial hyperresponsiveness (Willemse et al. 2004a), and several studies have found an increased risk for asthma in smokers, although contradictory results have also appeared (Thomson et al. 2004). Smokers with asthma have worse asthma control independent of FEV1, an accelerated decline in lung function, and an increased mortality rate (Thomson et al. 2004, Hylkema et al. 2007, Chaudhuri et al. 2008), and their response to corticosteroids is impaired (Pedersen et al. 1996, Chalmers et al. 2002, Thomson & Spears 2005). In addition, passive exposure to tobacco smoke has been reported to increase asthma risk (Jaakkola et al. 2002) and to worsen asthma control (Pietinalho et al. 2009). COPD has been reported to develop in up to 50% of those who smoke till their later middle age, with the risk increasing the younger the smoking begun (Lundbäck et al. 2003, Kotaniemi et al. 2005). COPD patients who continue to smoke have a faster decline in lung function, more exacerbations, more symptoms, and increased mortality compared to those who quit smoking (Anthonisen et al. 1994, Willemse et al. 2004a, Godtfredsen et al. 2008).

2.6.2 Smoking and airway inflammation

Smoking has the capacity to damage the airways in several ways: direct toxic damage to the epithelium, oxidative damage, recruitment of inflammatory cells, and increased epithelial permeability. Cigarette smoke contains an abundance of oxygen-based free radicals, peroxides, and peroxynitrite, placing a significant oxidant burden on the lungs. Oxidative stress induces chemotaxis, potent leukocyte adhesion, and initiation of inflammation. Furthermore, oxidants change the proteinase-antiproteinase balance, resulting in reduction in the antiproteinase shield and an increase in proteinolysis (Bourdain et al. 2009, Pietinalho et al. 2009).

Chronic and repeated exposure to cigarette smoke stimulates the activation of macrophages and neutrophils. Activated macrophages secrete cytokines such as IL-8, inducing neutrophil chemotaxis. Increased numbers of neutrophils are present in the induced sputum of healthy and asthmatic smokers (Chalmers et al. 2001, Rytilä et al. 2006), with a significant positive correlation between sputum neutrophils and smoking history (Chalmers et al. 2001). Bronchial biopsies from asymptomatic smokers show an excess of several inflammatory cell types (neutrophils, eosinophils, mast cells, and macrophages) in the bronchial mucosa, having a clear relationship with the impaired epithelial integrity (Amin et al. 2003). Increased numbers of mast cells have been found in all compartments, structural changes correlating with mast cell numbers, especially in the smooth muscle (Ekberg-Jansson et al. 2005). Increased thickness of the basement membrane has been reported to relate strongly to the presence of eosinophils and mast cells, suggesting a role for these cells in the remodeling of the airways that occurs in asymptomatic smokers (Amin et al. 2003). Chronic exposure to cigarette smoke can disturb epithelial regeneration, leading to excessive epithelial proliferation (Lee et al. 2001).

Smoking is known to alter the composition of asthmatic airway inflammation. Inflammation in the asthmatic smoker is shifted from an eosinophil-dominated towards a neutrophil-dominated pattern. Increased numbers of eosinophils do occur, but their proportion in smokers is lower (Chalmers et al. 2001, Broekma et al. 2009). This may in part result from increased apoptosis of activated eosinophils caused by exogenous NO in cigarette smoke (Zhang et al. 2003). Induced sputum both in healthy smokers (Rytilä 2006) and in steroid-naive asthmatic smokers (Chalmers et al. 2001) contains higher numbers of neutrophils than in their nonsmoking counterparts. Increased expression of intraepithelial IL-8 has been detectable in bronchial biopsies of asthmatic smokers compared to that in asthmatic nonsmokers (St-Laurent et al. 2008), and its release is augmented by exposure to cigarette smoke (Mio et al. 1997). IL-8 correlates positively with number of neutrophils in sputum, and increases significantly with increasing pack-years (Chalmers et al. 2001). Similarly, the increased epithelial cell proliferation detected in asthmatic smokers has been reported to correlate with higher number of pack-years (Broekma et al. 2009). Higher mast cell numbers also appears in asthmatic smokers than in nonsmokers (Broekma et al. 2009). Whether the influence of smoking on asthmatic airway inflammation differs between atopic and nonatopic asthma, is, however, unknown.

Smoking cessation occurring sufficiently early can lead to reversal of the inflammation, although normalization may take several months (Willemse et al. 2004a). However, once COPD is established, inflammation may continue to persist, although some improvement may take place (Rutgers et al. 2000, Willemse et al. 2004a). Despite the persisting inflammation and irreversible damage to the lung parenchyma, smoking cessation slows the progression of COPD and clearly reduces the accelerated rate of decline in FEV1 (Fletcher & Peto 1977, Anthonisen et al. 1994, 2002). Smokers with airflow obstruction benefit from quitting despite previous heavy smoking, advanced age, poor baseline lung function, or airway hyperresponsiveness (Scanlon et al. 2000).

In asthma, epithelial changes and the increase in neutrophil and mast cell numbers are likely to be reversed with smoking cessation (Broekma et al. 2009), and steroid responsiveness may at least be partially restored (Hylkema et al. 2007). Cigarette smoke may have direct effects on airway smooth muscle responsiveness, and intensity of smoking, assessed as pack years, has been reported to relate to BHR severity (Piccillo et al. 2008). Smoking cessation has been reported to improve BHR to indirect stimulus earlier and to a greater extent than it improves BHR to direct stimuli, but whether the degree of BHR after smoking cessation reverts to normal levels, is controversial (Willemse et al. 2004a, Willemse et al. 2004b, Piccillo et al. 2008). Smoking cessation, however, may prevent future deterioration of BHR (Wise et al. 2003).

2.6.3 Smoking and FENO

In both healthy and steroid-naive asthmatic subjects compared to their nonsmoking counterparts, active smoking is associated with reduced FENO (Persson et al. 1994, Kharitonov et al. 1995a, Verleden et al. 1999, Horváth et al. 2004, McSharry et al. 2005, Rytilä et al. 2006). Exposure to environmental tobacco smoke may also reduce FENO (Yates et al. 2001b, Laoudi et al. 2009), although increased FENO concentrations have been reported in infants (Franklin et al. 2006). A negative correlation between FENO and the amount smoked has appeared in several studies (Persson et al. 1994, Kharitonov et al. 1995a, Corradi et al. 1999), whereas some have found no such correlation (Verleden et al. 1999, Horváth et al. 2004).

Smoking may reduce FENO by several mechanisms. Decreased iNOS activity caused by negative feedback from high concentrations of NO in cigarette smoke has been proposed to account for the decrease in FENO after acute inhalation of cigarette smoke. Kharitonov and co-workers (1995a) showed, however, that inhalation of NO itself did not result in any decrease in FENO levels. This transient decrease in FENO probably results from a reaction between NO and the high concentration of reactive oxygen species in cigarette smoke, with NO from the airways being captured by peroxynitrite formation (Horváth et al. 2004). Chronically reduced FENO levels in smokers may be due to smoke-induced toxic damage to NO-producing epithelial cells. Exposure to cigarette smoke extract has been reported to inhibit cytokine-induced production of NO by epithelial cells and mast cells, and to inhibit the expression of NO synthase in these cells (Hoyt et al. 2003, Wei et al. 2005). Bergeron and co-workers (2007) reported increased arginase

expression in bronchial biopsies from steroid-naive smoking asthmatics. Because NO is synthesized from arginine, increased arginase activity leads to less substrate for NOS and thus to a decrease in NO formation. In asthmatic smokers, inhalation of nebulised L:-arginine can reverse the cigarette-induced reduction of FENO (Bruce et al. 2010). A significant negative correlation has been reported between FENO and the number of neutrophils in induced sputum (Rytilä et al. 2006). Reduced FENO in smokers may be related to their increased susceptibility to respiratory infections in smokers, since NO production is involved in local host defense and is necessary for normal ciliary beating in the airway mucosa (Nathan & Hibbs 1991, Jain et al. 1993).

Although studies show lower FENO levels in steroid-naive asthmatic smokers than in their nonsmoking counterparts and elevated levels compared to healthy smokers, limited data are available comparing FENO in steroid-naive asthmatic smokers to FENO in healthy nonsmokers. As smoking prevalence among asthmatics is at least at the same level as among the general population (Pietinalho et al. 2009) and as reference values are based on levels in nonsmokers (Olin et al. 2007), it would be essential to know whether FENO in steroid-naive asthmatic smokers differs significantly from FENO in healthy nonsmokers in order to evaluate the usefulness of FENO to reflect airway inflammation in smokers with respiratory symptoms. Furthermore, whether the influence of smoking on FENO between atopic and nonatopic asthmatics differs, is unknown. Horvath and co-workers (2004) found higher FENO among 22 steroid-naive atopic asthmatic smokers than among healthy smokers, but did not report any comparison to healthy nonsmokers. A study by Verleden and co-workers (1999) reported higher FENO among 13 steroid-naive asthmatic smokers than among healthy smokers, but no difference when compared to healthy nonsmokers. That study did not assess subjects' atopic status. Malinovschi and co-workers (2009), studying 65 atopic and 33 nonatopic asthmatics, found increased FENO in atopic and nonatopic steroid-naive asthmatic never-smokers, whereas no difference from non-asthmatics was detectable when current and ex-smokers were included. They performed no comparison between atopic and nonatopic smokers, and did not report the numbers of smokers, ex-smokers and never-smokers within the groups.

Although FENO in asthmatic smokers is lower than in asthmatic nonsmokers, this does not necessarily reduce its ability to reflect asthma control in smokers, if the assessment is based on changes in FENO in repeated measurements rather than on cut-off values in single measurements. Michils and co-workers (2009) reported that a FENO increase < 50% in smokers with controlled asthma would indicate that asthma remains controlled, whereas a FENO reduction of < 20% in smokers with uncontrolled asthma would indicate that it remains uncontrolled (Michils et al. 2009).

After smoking cessation, FENO has been reported to rise gradually within weeks, approaching normal values after 4 to 8 weeks (Robbins et al. 1997, Högman et al. 2002). Whether normal levels are reached may depend on the status of the airways, because a smoking-related decline in FENO may be associated with permanent epithelial damage. Similarly, if eosinophilic airway inflammation is present, FENO levels may be elevated after smoking cessation, when the FENO-reducing effect of smoking is abolished.

3 AIMS OF THE STUDY

1. To examine the association between FENO and allergic sensitization in healthy nonsmoking adults without symptoms or signs of airway disorders and among those with respiratory symptoms or diagnosed airway diseases.

2. To evaluate the association of bronchial responsiveness to direct and indirect stimuli with nitric oxide-producing airway inflammation, and to compare this relationship among atopic and nonatopic patients with various degrees of bronchial hyperresponsiveness and airway inflammation.

3. To compare the influence of smoking on FENO in atopic and nonatopic steroid-naive young adults with current symptomatic asthma, and to compare their FENO levels to those in smoking and nonsmoking healthy subjects in order to evaluate the usefulness of FENO in assessing airway inflammation in asthmatic smokers.

4. To evaluate the influence of chronic obstruction on the repeatability of FENO by assessing short-term variation in FENO in COPD patients and healthy subjects, and to assess the clinically significant change in FENO in COPD patients.

4 MATERIAL AND METHODS

In total, 533 subjects participated in the studies. Their clinical characteristics are described in Table 2. The local ethics committees approved the studies, and all subjects gave their written informed consent.

4.1 Subjects

4.1.1 FinEsS study participants (Study I)

The study population of 248 subjects, aged 26 to 61, originated from among 6 062 responders to a postal survey performed in 1996 in Helsinki as part of the FinEsS study, a large epidemiological investigation on obstructive lung diseases, respiratory symptoms, and type I allergy carried out in Finland (Fin), Estonia (Es), and Sweden (S). The original study sample of 8 000 20- to 69-year-old inhabitants of Helsinki was obtained from the Population Register Centre, randomized by 10-year age cohorts and by gender. Of the 6 062 responders, 1 200 were randomly selected and invited to clinical studies (spirometry, bronchodilatation test, skin prick tests (for those under age 61), and structured interview). Half (600) of them were at the same time further randomized also to undergo FENO measurement and histamine challenge. Of the 600 invited, 310 (51.7%) participated. Technically acceptable FENO measurements were obtainable from 295 of 310 (95%), with skin prick test data available from 248 of them (84%), forming the actual study population.

From this study population of 248 subjects, a subgroup of healthy asymptomatic nonsmoking adults was formed for the main analyses. Subjects were included in this group provided they had 1) no significant smoking history (were nonsmokers or ex-smokers with a history of < 5 pack-years and smoking cessation > 5 years previously), 2) no signs of airway obstruction (FEV1/FVC ≥ 88% of predicted (Viljanen et al. 1982), and bronchodilator response < 12% increase in FEV1), 3) no significant bronchial hyperresponsiveness (PD_{15}FEV1 > 0.4 mg histamine; Sovijärvi et al. 1993), 4) no previously diagnosed asthma, chronic bronchitis, or chronic obstructive pulmonary disease, chronic or recurrent symptoms of the upper or lower respiratory tracts (cough, sputum production, wheezing, shortness of breath, nasal symptoms related to specific allergens), and 5) no symptoms of cardiovascular, gastrointestinal, or neurological diseases. Furthermore, all subjects had to be free from acute respiratory infections for 3 weeks prior to the study. The exclusion process is presented in Figure 3. After this process, the remaining 73 of the 248 subjects formed the study sample comprising healthy asymptomatic nonsmoking subjects.

Table 2. *Characteristics of study populations in Studies I-IV*

Study	group	n	age mean (range)	gender m/f	smoking curr/ex/non	atopy* yes/no	FEV1 % of pred mean (range)	PD_{15}FEV1, mg median (25-75 quartiles)	ΔPEF% mean (range)
I	healthy	73	44 (27-61)	27 / 46	0 / 0 / 73	32 / 41	97.3 (72-129)	†	ND
I	SDS	175	45 (26-61)	79 / 96	48 / 38 / 89	84 / 91	91.3 (41-129)	0.64 (0.38-1.07)‡	ND
II	asthmatic symptoms	181**	20 (18-26)	181 / 0	0 / 0 / 0	128 / 53	94.8 (74-120)	0.55 (0.27-1.29)‡‡	10.2 (-2.1 - 54.0)
III	asthmatics	116**	20 (18-30)	104 / 12	46 / 0 / 70	84 / 32	91.2 (60-119)	0.53 (0.22-1.38)	14.8 (-5.3 - 57.5)
III	healthy	19	19 (18-20)	19 / 0	10 / 0 / 9	ND	99.7 (83-122)	††	ND
IV	COPD	19	65 (54-72)	13 / 6	0 / 19 / 0	ND	53.0 (23.1-79.7)	ND	ND
IV	healthy	20	41 (23-58)	5 / 15	0 / 0 / 20	ND	98.6 (78.4-124.7)	ND	ND

SDS: subjects with symptoms or signs of respiratory or other significant diseases and/or significant smoking history

* atopy = skin prick test-positive

** 70 subjects included in both studies

PD_{15}FEV1: provocative dose of histamine causing a 15% fall in FEV1

ΔPEF%: maximum percentage fall in PEF after exercise

† 94% and †† 89% of subjects with PD15FEV1 > 1.6 mg

‡ result shown for those 48 subjects with any degree of bronchial hyperesponsiveness (PD15FEV1 < 3.2 mg)

‡‡ result shown for those 130 subjects with any degree of bronchial hyperresponsiveness (PD15FEV1 < 3.2 mg)

ND: not done

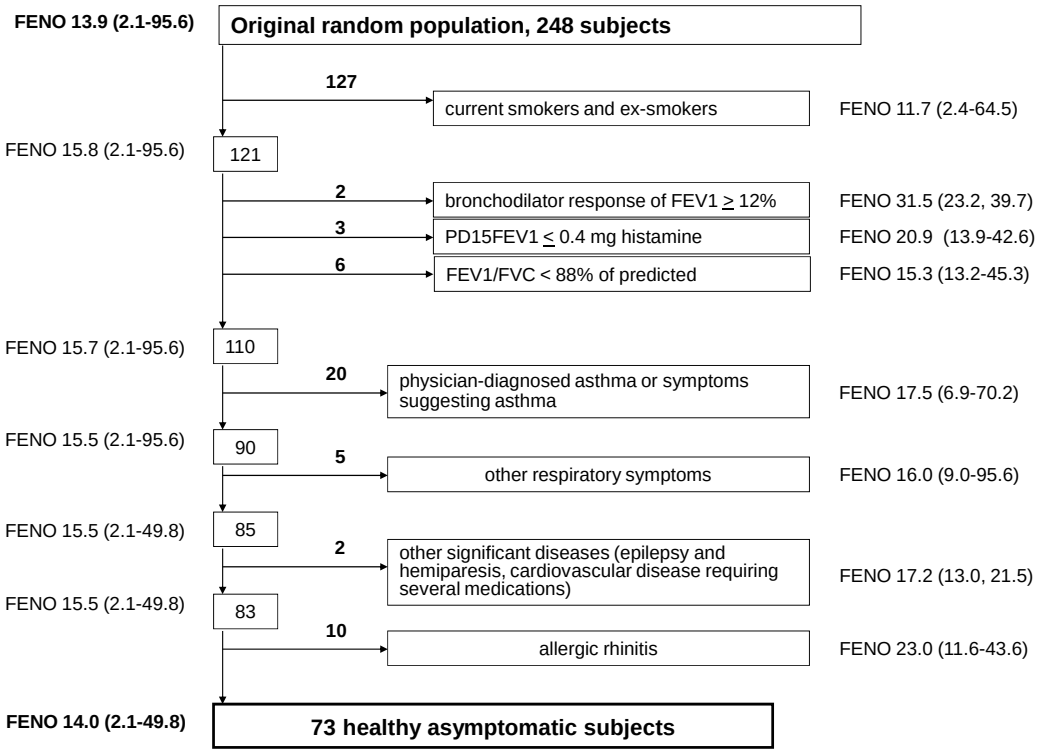

Figure 3 *Exclusion process to select healthy asymptomatic nonsmoking subjects, with median and range of FENO (flow rate 50 ml/s) shown for each subgroup. (Reprinted with permission from: Rouhos et al. 2008, Clin Respir J, 2:141-148. ©Wiley-Blackwell)*

4.1.2 Army conscripts (Studies II and III)

Subjects for Studies II and III were enrolled from among army conscripts (age 18-30) referred to the Central Military Hospital in Helsinki between 1998 and 2002 because of respiratory symptoms (dyspnoea, wheezing, cough, sputum production at rest or related to exercise) suggesting asthma. They were consecutively and prospectively enrolled in the studies, provided that they had used no inhaled or oral steroids, chromones, or leukotriene receptor antagonists during the preceding 2 months, and had no evidence of respiratory infections within the preceding 4 weeks. Asthma was the diagnosis when at least one of the following criteria were applied: 1) $\geq$ 15% reversibility of PEF or of FEV1 in bronchodilatation test with 0.4 mg of inhaled salbutamol, 2) or $\geq$ 20% spontaneous daily variation in PEF compared to the mean value for the day, 3) or $\geq$ 10% fall in PEF after standardized exercise challenge, 4) or $PD_{15}FEV1 \leq 0.4$ mg in the histamine challenge (Sovijärvi et al. 1993). Study III included only subjects diagnosed with current symptomatic asthma by these criteria, whereas Study II included also symptomatic subjects who did not fulfill the criteria of asthma. Study II included only nonsmokers, Study III also current smokers. Information on subjects' smoking history came from an

interview with a nurse upon admission to the military hospital. Study III also included nine nonsmoking and ten smoking healthy conscripts with no signs, symptoms, or history of chronic or recurrent respiratory diseases as a control group.

4.1.3 Repeatability-study participants (Study IV)

At Helsinki University Hospital, 20 subjects with previously diagnosed COPD were recruited from the outpatient department of the Division of Respiratory Diseases and from the Research Unit for Pulmonary Diseases. All had FEV1 < 80% of predicted (Viljanen et al. 1982), and a ratio of FEV1 to FVC under 0.7, and all were ex-smokers with a smoking history of ≥ 20 pack years who had stopped smoking at least one year previously. They were clinically stable, with no changes in their medication during the preceding 4 weeks with the exception of SABA, which were withheld for 12 hours prior to the measurements. From among hospital staff and their relatives we recruited 20 healthy subjects with no history or symptoms of respiratory diseases. These were either life-time nonsmokers or had smoked a maximum of 5 pack-years, but had stopped smoking at least 5 years previously. All subjects had been free of respiratory infections for the preceding 4 weeks.

4.2 Study design

4.2.1 Association between allergic sensitization and FENO (Study I)

On the first study day, subjects underwent a structured interview, flow-volume spirometry, a bronchodilatation test, and skin prick tests. FENO measurement and a histamine challenge, in that order, were performed on the second study day, which was 1 to 14 days after the first session. The measurements were carried out in the Laboratory of Clinical Physiology (FENO and histamine challenge) and in the Research Unit for Pulmonary Diseases of Helsinki University Central Hospital (all other tests) throughout the year from May 2001 to March 2003, with the majority of tests performed outside the pollen season.

4.2.2 Association between FENO and BHR and between FENO, smoking, and atopy in subjects with steroid-naive asthma (Studies II and III)

Flow-volume spirometry, a bronchodilatation test, and skin prick tests were carried out on the first study day, and an exercise challenge in the morning of the second study day. FENO was measured between 7 and 8 a.m. on the third study day, followed by a histamine challenge. The healthy subjects in Study III underwent in the same order the same measurements except for the bronchodilatation test, skin prick testing, and the exercise challenge. The measurements were performed at the Laboratory of Clinical Physiology of

Helsinki University Central Hospital (FENO) and at the Central Military Hospital (all other tests) between 1998 and 2002.

4.2.3 Repeatability of FENO in COPD patients (Study IV)

FENO was determined three times: at baseline, and at 10 minutes and 24 hours after the baseline. Spirometry was carried out on the first study day after the FENO measurements. The measurements were performed in the Laboratory of Clinical Physiology (FENO) and at the Research Unit for Pulmonary Diseases of Helsinki University Central Hospital (spirometry) from April 2006 to November 2008.

4.3 Clinical methods

4.3.1 Exhaled nitric oxide measurement

FENO was measured with a chemiluminescence analyzer (Sievers 270B, Boulder, CO, USA). Two-point calibration of the analyzer was performed daily before FENO measurements. Expiratory airflow and exhaled volume were measured with pneumotachograph (Baby Pneumotachograph, Erich Jaeger GmbH, Wurzburg, Germany) simultaneously with FENO in real time. Before the measurement, subjects rinsed their mouths with sodium bicarbonate solution (Hartwall Novelle®, Oy Hartwall Ab, Helsinki, Finland) to eliminate any nitric oxide produced in the mouth. After inhalation of NO-free gas (synthetic NO-free air in Studies I and IV, 100% oxygen in Studies II and III), subjects exhaled from total lung capacity with a constant flow rate against a flow resistor (Hans Rudolph, Model #7100R, 200 cmH20 l-1s-1, flow range 0-0.1 l/s in Studies I and IV, Hans Rudolph, Model #7100R, 100 cmH20/L/s, flow range 0-0.5 L/s in Studies II and III) to close the soft palate, thus avoiding any nasal NO contamination. No nose clips were used. The subjects maintained the required flow rate with the aid of visual feedback from a computer screen. The exhalation procedure was performed according to guidelines valid at the time of each study (ATS guidelines 1999 in Studies I and IV, ERS recommendations 1997 in Studies II and III). In Studies I and IV, the target flow rate was 50 ml/s, and the mean flow rate during the time of the NO plateau generation for an acceptable measurement was between 45 and 55 ml/s. In Studies II and III, the mean individual exhalation flow ranged between 90 and 120 ml/s. In all studies, exhalation lasted for at least 10 seconds, and the mean value recorded for analysis was taken from a 3-second period from the end-exhaled NO plateau. At least three successive FENO measurements were performed, and their mean value recorded for analysis. The acceptable coefficient of variation (CoV) of the successive FENO determinations was < 0.15 in Studies I to III and ≤ 0.1 in Study IV.

4.3.2 Skin prick tests

The skin prick tests were performed with 15 (Study I) or 13 (Studies II and III) common aeroallergens. Allergens tested in all three studies included cat, dog, cow, horse, birch, timothy, mugwort, the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae*, and the outdoor moulds *Cladosporium* and *Alternaria*. In addition, the storage mites *Acarus siro* and *Lepidoglyphus destructor*, cockroach, and latex were tested in Study I, and cocksfoot, alder, and sheep's wool in Studies II and III. Histamine dihydrochloride (10 mg/ml) served as the positive, and the solvent (glycerol) as the negative control. All extracts were provided by ALK, Denmark, except latex, which was provided by Stallergenes, France. The tests were carried out by experienced nurses on the volar side of the forearm. Reactions were inspected after 15 minutes, and the wheal size was measured in millimeters in two directions perpendicular to each other including the longest diameter, with their mean recorded as the response. A response of $\geq$ 3 mm in the presence of the expected reactions to the control solutions was regarded as positive (Dreborg 1989). Subjects with at least one positive reaction were regarded as skin prick test-positive and defined as atopics. Those subjects who did not react to the positive control or reacted to the negative control were excluded. Study I also assessed the prick wheal sum ("atopy score"), calculated by adding up the mean diameters of each positive reaction (Miles et al. 1995, Koh et al. 2002).

4.3.3 Spirometry

Spirometry was performed with a flow-volume spirometer (SensorMedix Vmax 20C, Yorba Linda, CA, USA, in Studies I and IV, and Medikro M904, Kuopio, Finland, in Studies II and III). The FVC and FEV1 were determined according to guidelines valid at the time of the studies (ATS 1995 for Study I, Quanjer et al. 1993 for Study II and III, and Miller et al. 2005 for Study IV). For the bronchodilatation test, 0.4 mg of inhaled salbutamol aerosol (Ventoline®, GlaxoSmithKline, Brentford, UK) was administered via a spacer (Volumatic®, GlaxoSmithKline). FVC and FEV1 measurements were repeated 15 minutes after salbutamol administration. The results are expressed as absolute values and as percentage of predicted value using published Finnish reference values (Viljanen et al. 1982). Prior to the spirometry, SABA were withheld for 4 hours (Study I) or for 12 hours (Studies II to IV) and LABA for 12 hours (Study I).

4.3.4 Histamine challenge

The histamine challenge for assessment of bronchial responsiveness was performed by a dosimetric method with controlled tidal breathing (Sovijärvi et al. 1993). Buffered histamine diphosphate was administered by an automatic, inhalation-synchronized jet nebulizer (Spira Elektro 2, Respiratory Care Center, Hämeenlinna, Finland) in a four-step non-cumulative dosage scheme (0.025 mg, 0.1 mg, 0.4 mg, and 1.6 mg). A provocative

dose of histamine inducing a 15% decrease in FEV1 (PD_{15}FEV1) was calculated from the logarithmically transformed histamine doses by use of linear interpolation or extrapolation. If FEV1 did not decrease at least 10% after the maximum dose of 1.6 mg of histamine, a PD_{15}FEV1 of 3.2 mg served as the final result. After the final histamine dose, patients received 0.2 mg of inhaled salbutamol aerosol (Ventoline®, GlaxoSmithKline, Brentford, UK) to relieve bronchoconstriction.

4.3.5 Exercise challenge

A standardized 8-minute running test (Karjalainen et al. 1991) was performed outdoors on a 150-meter circular track between 9 and 11 AM. Running speed was adjusted by monitoring the subjects' heart rate with a Sport Tester™ PE 3000 heart rate meter (Polar Electro Ky, Kempele, Finland). Subjects raised their heart rate to 85% of their predicted maximal rate during a 2-minute warm-up and maintained this rate for the remaining 6 minutes of the exercise. PEF was measured just before the exercise and immediately after, and then 5, 10, 20, and 30 minutes after the exercise. Of the three successive PEF measurements obtained on each occasion, the highest was recorded for analysis. The response to exercise challenge was the maximum percentage fall in PEF (ΔPEF%) after exercise: % fall in PEF = [(PEF (baseline) – PEF (after)) / (PEF (baseline)] x 100).

The exercise challenge was conducted outdoors in temperatures ranging from – 17.5 to + 21.4ºC (mean 6.5ºC). Therefore, for all exercise challenges, ΔPEF% was adjusted to a temperature of 6.5ºC by use of the regression coefficient (–0.327) between air temperature (ºC) and ΔPEF%. This coefficient was obtained by linear regression from similar outdoor exercise tests in 1 809 conscripts with similar symptoms (Latvala et al. 2000). Those exercise tests took place during the years 1985 to 1998 at temperatures ranging from -25ºC to +26ºC.

4.3.6 Structured interview

The structured interview was conducted by one of the five trained physicians involved in the study, using a questionnaire developed for the FinEsS studies. The questionnaire contains 162 questions on general health, recent or past respiratory symptoms, diagnosed asthma or other chronic respiratory diseases, provocative factors, and risk factors for respiratory diseases, history of cardiovascular diseases or other major diseases, medications, and smoking history. This questionnaire is based on one developed and validated in the Swedish Obstructive Lung Diseases in Northern Sweden (OLIN) studies (Lundbäck et al. 1991, 1993, Torén, et al. 1993). The FinEsS questionnaire has been described in detail earlier (Kainu 2008).

4.4 Statistical methods

All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows version 11.0 (Studies I and II) or 15.0 (Studies III and IV); SPSS, Chicago, IL, USA). All tests were 2-sided, except the nonparametric ranking for the assessment of a 1-tailed 95% upper limit of FENO in Study I, and a p-value of < 0.05 was considered significant. Since FENO and $PD_{15}FEV1$ were not normally distributed, the results are expressed as median values and 25 to 75% quartile ranges, and for analyses log10 transformation was performed to achieve a near normal distribution. Because each study included several statistical aspects to take into consideration, the details are described separately by study.

4.4.1 Study I

Comparisons of median FENO values between groups were analyzed with the Mann-Whitney U-test. Comparisons of anthropometric and spirometric variables between atopic and nonatopic healthy subjects were performed with an independent samples t-test. Mutual correlations between FENO and number of positive skin prick tests, and between FENO and total sum of wheal diameters, as well as between FENO and anthropometric variables were analyzed with Spearman's rank correlation coefficients. Since FENO was not normally distributed, and normal distribution was not achieved by log-transformation, a nonparametric ranking served for assessing the 1-tailed 95% upper limit in the groups.

4.4.2 Study II

Differences between anthropometric data, FENO, $PD_{15}FEV1$, and exercise-induced ΔPEF% in atopic and nonatopic groups were analyzed with the Mann-Whitney U-test. Mutual correlations between FENO, $PD_{15}FEV1$, and ΔPEF% were analyzed separately in the atopic and nonatopic groups with Pearson's correlation, with 95% confidence intervals calculated. Multiple linear regression analyses were undertaken using log (FENO) values as outcome variables. The first model evaluated the effect of atopy, asthma, ΔPEF%, and log ($PD_{15}FEV1$). In the second model ΔPEF%, and log ($PD_{15}FEV1$) were analyzed separately for atopic and nonatopic patients.

4.4.3 Study III

Multiple comparisons between groups were performed by one-way analysis of variance (ANOVA), and p-values corrected for multiple comparisons by the least significant difference (LSD) method. If significant differences in baseline characteristics (age, weight, height, body mass index) appeared between compared groups, their effect on FENO was tested by the analysis of covariance (ANCOVA) method. Possible interactions

were tested by two-way ANOVA. Correlations between FENO and spirometric variables as well as FENO and hyperreactivity tests were analyzed with Spearman's rank correlation coefficients.

4.4.4 Study IV

Bland-Altman plots served to illustrate the repeatability and intra-subject correlation of FENO measurements. Intraclass correlation coefficients (ICC) with 95% confidence intervals were calculated. Coefficients of variation (CoV) were calculated for intrasession and between-session repeatability by dividing the standard deviation (SD) of the individual measurements by their mean, and were expressed as percentages. Wilcoxon's pairwise test was used for paired observations.

5 RESULTS

5.1 Association between allergic sensitization and FENO

Among healthy subjects, median FENO was similar in our 32 skin prick test-positive (13.2 ppb) and our 41 skin prick test-negative (15.5 ppb) subjects (Figure 4). The 1-tailed nonparametric 95% upper limit for FENO in the whole group of healthy asymptomatic nonsmoking subjects was 30 ppb, among the skin prick test-positive healthy subjects 31 ppb, and among skin prick test-negative healthy subjects 29 ppb.

Among healthy subjects no significant correlation appeared between FENO and degree of atopy defined as number of positive reactions ($r = -0.138$, $p = 0.244$) or as total sum of wheal diameters (calculated by adding up the diameters of each positive reaction (≥ 3 mm) ($r = -0.135$, $p = 0.254$) (Figure 5). In contrast, among those subjects (n = 175), who were excluded because of history, symptoms or signs of respiratory disease, or smoking, a statistically significant correlation appeared between FENO and number of positive reactions ($r = 0.160$, $p = 0.034$) or total sum of wheal diameters ($r = 0.178$, $p = 0.019$), as well as in the subpopulation of excluded nonsmoking subjects (n = 48) ($r = 0.345$, $p = 0.016$) and ($r = 0.343$, $p = 0.017$), respectively (Figure 6). The numbers of positive reactions among healthy subjects and excluded subjects were similar ($p = 0.25$), but mean wheal size and total sum of wheal diameters were significantly higher in excluded subjects than in healthy subjects ($p = 0.005$ and 0.047). Of the healthy skin prick test-positive subjects, 75% were sensitized only to perennial or seasonal allergens and 25% to both groups of allergens, whereas among excluded skin prick test-positive subjects these percentages were 57% and 45%.

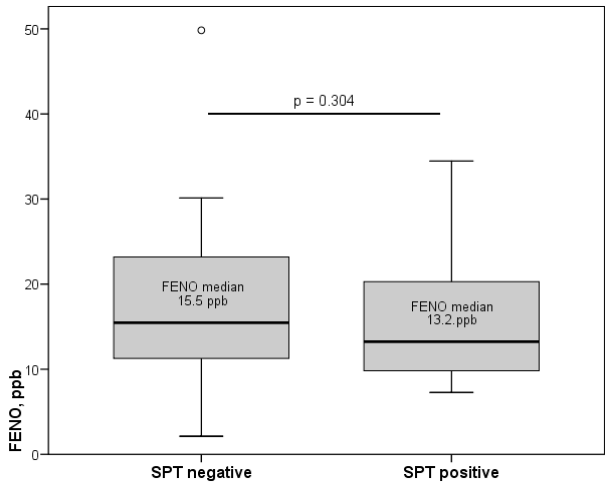

Figure 4 *FENO (flow rate 50 ml/s) in nonsmoking healthy asymptomatic skin prick test (SPT)-positive (n=32) and -negative (n=41) subjects. Data expressed as medians with interquartile range (box) and range (whiskers) excluding an outlier (circle). (Reprinted with permission from: Rouhos et al. 2008, Clin Respir J, 2:141-148. ©Wiley-Blackwell)*

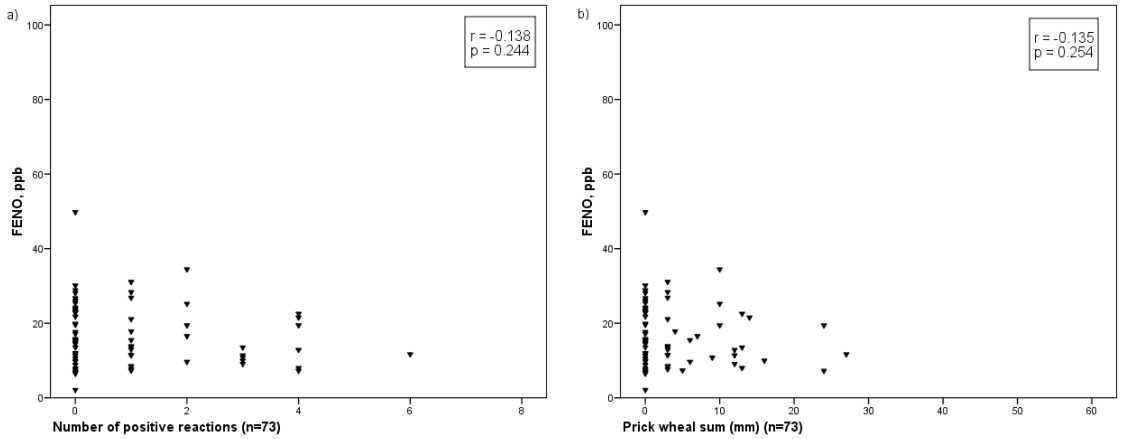

Figure 5 *Correlation between FENO (flow rate 50 ml/s) and number of positive skin prick tests (a) and between FENO and total skin prick test wheal sum (calculated by adding the diameters of each positive reaction together) (b) in healthy nonsmokers with no signs or symptoms of airway disease (n=73). (Reprinted with permission from: Rouhos et al.2008, Clin Respir J, 2:141-148. ©Wiley-Blackwell)*

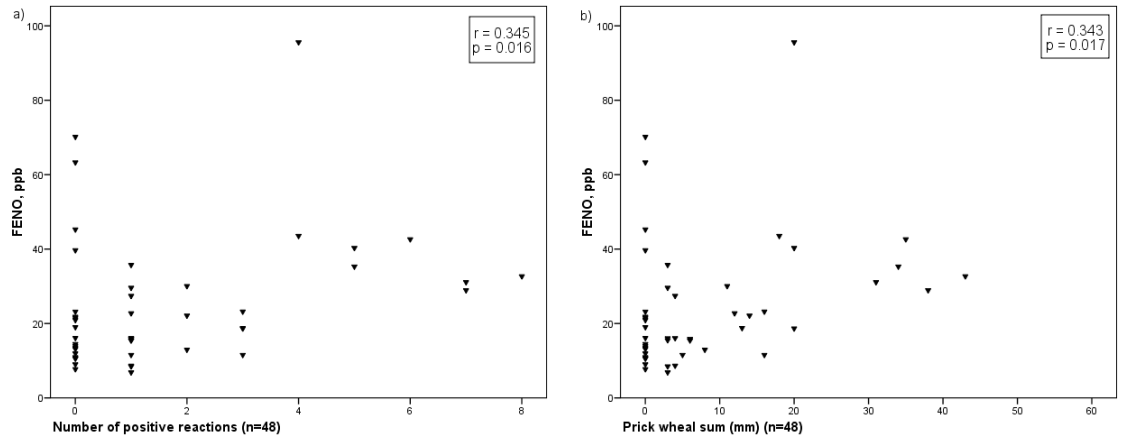

Figure 6 *Correlation between FENO (flow rate 50 ml/s) and number of positive skin prick tests (a) and between FENO and total skin prick test wheal sum (calculated by adding the diameters of each positive reaction together) (b) in nonsmokers with signs, symptoms or history of airway disorder (n=48).*

5.2 Association between bronchial hyperresponsiveness, atopy and FENO

FENO was significantly higher among all 128 atopic than among all 53 nonatopic patients, 21.2 ppb (median) vs. 10.2 ppb, $p < 0.001$, with 12 ppb being the upper normal limit measured by the same method (Ekroos et al. 2000). Among patients with a confirmed diagnosis of asthma, FENO was 29.6 ppb among the 68 atopics and 12.4 ppb among the 19 nonatopics ($p < 0.001$). In atopic patients, the mean fall in PEF after exercise (EIB) was slightly but significantly more severe, but their $PD_{15}FEV1$ in histamine challenge (HIB) did not differ significantly from that of nonatopics. When only subjects with a confirmed diagnosis of asthma were analyzed, no significant difference appeared between atopic and nonatopic groups in EIB or HIB (Table 3). A significant correlation existed between EIB and HIB in both atopic and nonatopic groups, but the correlation was more pronounced in the atopic group ($r = -0.48$, $p < 0.001$) than among nonatopic patients ($r = -0.29$, $p = 0.035$).

Table 3. *FENO (flow rate 90-120 ml/s), exercise-induced bronchoconstriction and histamine-induced bronchoconstriction in young male patients*

	All atopic patients (n = 128)	Atopic asthmatics (n=68)	All nonatopic patients (n=53)	Nonatopic asthmatics (n=19)
FENO, ppb	21.2 (13.2-44.6)*	29.6 (18.4-52.0)†	10.2 (8.4-14.8)	12.4 (8.5-20.0)
ΔPEF%	11.2 (-1.9 - 54.0)**	16.6 (-1.4 - 54.0)	7.1 (-2.1 - 35.5)	12.3 (-2.1 - 35.5)
$PD_{15}FEV1$, mg	1.0 (0.4-2.5)	0.4 (0.2-1.0)	1.5 (0.5-3.2)	0.4 (0.2-0.7)

$PD_{15}FEV1$: provocative dose of histamine causing a 15% fall in FEV1

ΔPEF%: maximum percentage fall in PEF after exercise

$PD_{15}FEV1$ and FENO expressed as median (25-75 percentiles), ΔPEF% as mean (range)

atopics vs nonatopics: * $p < 0.001$; ** $p < 0.01$

atopic asthma vs nonatopic asthma: † $p < 0.001$

The multiple linear regression model showed that atopy ($p < 0.001$), severity of EIB (expressed as ΔPEF%) ($p < 0.001$), and severity of HIB (expressed as log $PD_{15}FEV1$) ($p = 0.006$), all significantly and independently associated with FENO (expressed as logFENO). A significant interaction occurred between atopy and severity of EIB as well as between atopy and HIB in their relationship with FENO; separate regression models were therefore constructed for atopic and nonatopic patients. In these models, EIB and HIB associated with FENO in atopic patients, whereas in nonatopic patients, FENO did not associate with bronchial hyperresponsiveness measured by either an indirect or direct method (Table 4, Figure 7). These associations were similar when only patients with confirmed diagnosis of asthma were analyzed.

Table 4. *Multiple linear regression model with log(FENO) as the dependent variable and ΔPEF% and log(PD15FEV1) as independent variables in atopic and nonatopic patients. (Reprinted with permission from: Rouhos et al.2005, Allergy, 60:1493-1498. ©Wiley-Blackwell)*

	Atopic patients (n = 128)			Nonatopic patients (n = 53)		
Variable	B	95% CI	p value	B	95% CI	p value
ΔPEF%	0.029	0.015 to 0.042	< 0.001	0.012	-0.011 to 0.035	0.290
log (PD15FEV1)	-0.166	-0.28 to -0.051	0.005	-0.037	-0.180 to 0.105	0.600

B = regression coefficient; CI = confidence interval; ΔPEF%: maximum percentage fall in PEF after exercise; PD_{15}FEV1: provocative dose of histamine causing a 15% fall in FEV1

Adjusted coefficient of determination (R^2) for all variables is 0.27 among atopic patients

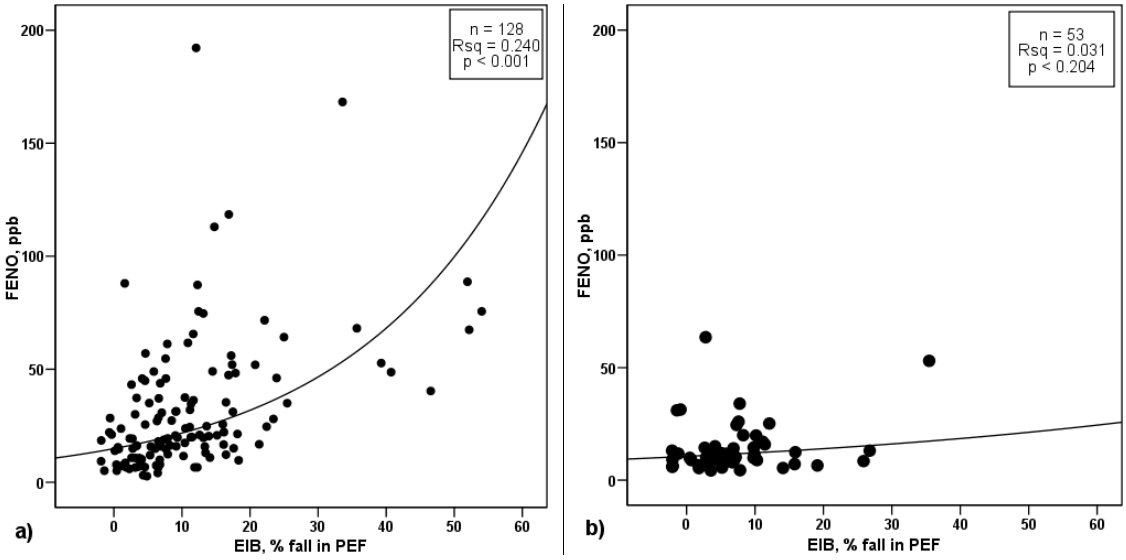

Figure 7 *Association between exercise-induced bronchoconstriction (EIB, % fall in PEF) and exhaled nitric oxide (FENO, ppb) in (a) atopic and (b) nonatopic patients. The curve is exponentially fitted. Rsq = r2 (coefficient of determination) (Reprinted with permission from: Rouhos et al.2005, Allergy; 60:1493-1498. ©Wiley-Blackwell)*

5.3 Influence of smoking and atopy on FENO in asthma

The total study population of 135 conscripts of Study III comprised 123 men and 12 women (7 women among the 54 atopic nonsmoking asthmatics, 3 women among the 16 nonatopic nonsmoking asthmatics, and 2 women among the 16 nonatopic smoking asthmatics). Of the 116 asthmatic subjects, 40% were smokers. Based on skin prick tests, 30 (65%) smoking asthmatics and 54 (77%) nonsmoking asthmatics were atopic.

Median FENO among smoking asthmatics (13.5 ppb) was significantly higher than in smoking (7.3 ppb) and non-smoking (6.7 ppb) healthy controls (p = 0.001, both

comparisons). Among nonsmoking asthmatics, FENO was significantly higher (24.0 ppb) than among smoking asthmatics (p = 0.002) (Figure 8). Among atopic asthmatics, FENO was significantly higher in nonsmoking (29.2 ppb) than in smoking subjects (14.3 ppb) (p = 0.002), whereas nonatopic asthmatics showed no difference between those nonsmoking (13.1 ppb) and those smoking (12.9 ppb) (p = 0.89) (Figure 9). Further, among nonatopic asthmatics, FENO was significantly higher than in healthy controls (p = 0.003), even among smoking nonatopic asthmatics (p = 0.01).

The results of all these comparisons remained unchanged even with the female conscripts (n=12) excluded from the analyses. Among atopic asthmatics, 89% of nonsmokers and 57% of smokers had FENO levels exceeding 12 ppb – the upper limit measured by the same method (Ekroos et al. 2000), whereas among nonatopic asthmatics this level was exceeded by 56% of both nonsmokers and smokers.

No significant correlations appeared between FENO and spirometric variables in any of the groups studied. A significant correlation between FENO and the degree of BHR to direct and indirect stimuli appeared only among nonsmoking atopic asthmatics (r = -0.41, p = 0.003 between FENO and $PD_{15}FEV1$, and r = 0.40, p = 0.003 between FENO and ΔPEF%).

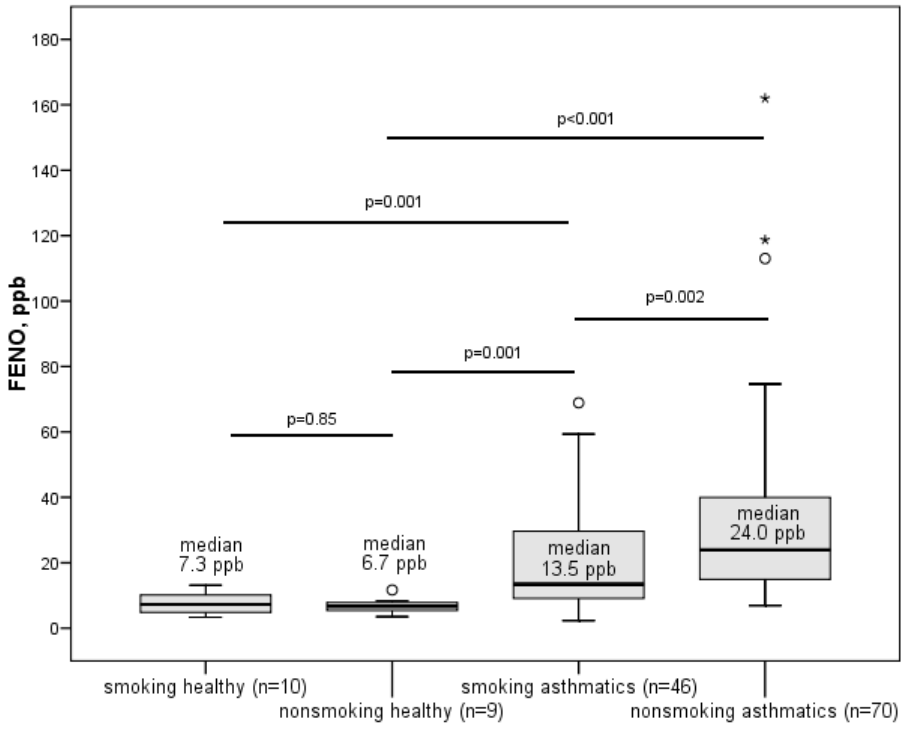

Figure 8 *FENO (flow rate 90-120 ml/s) in healthy and asthmatic smokers (atopic and nonatopic) and nonsmokers (atopic and nonatopic). Data expressed as medians with interquartile range (box) and range (whiskers) excluding outliers (circle) and extremes (asterisk). (Reprinted with permission from: Rouhos et al.2010, Int Arch Allergy Immunol; 152:226-232. ©S. Karger AG, Basel)*

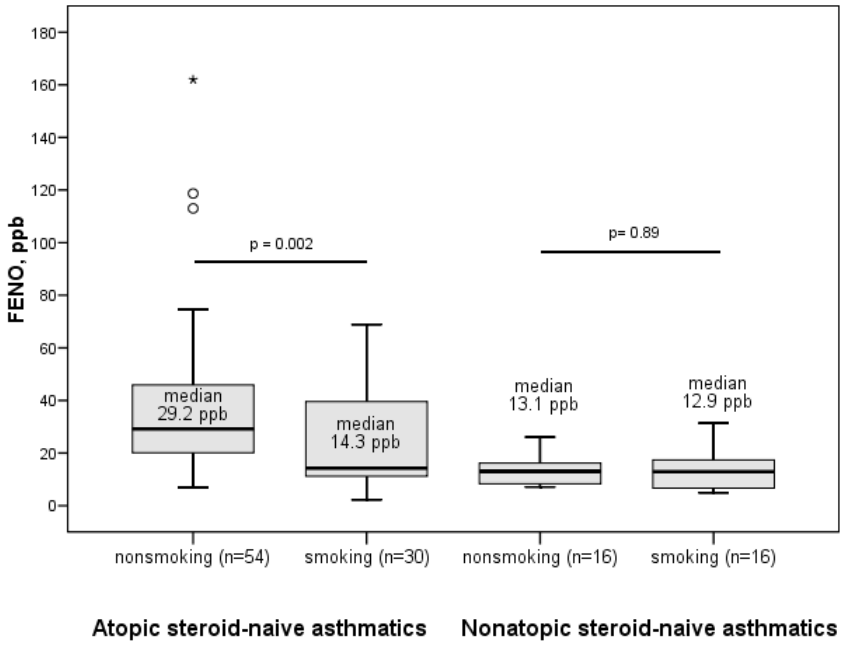

Figure 9 *FENO (flow rate 90-120 ml/s) in atopic and nonatopic steroid-naive asthmatic smokers and nonsmokers. Data expressed as medians with interquartile range (box) and range (whiskers) excluding outliers (circle) and extremes (asterisk). (Reprinted with permission from: Rouhos et al.2010, Int Arch Allergy Immunol; 152:226-232. ©S. Karger AG, Basel)*

5.4 Repeatability of FENO in COPD

Of the 20 COPD patients in Study IV, 12 had moderate (GOLD stage 2), 5 had severe (GOLD stage 3) and 3 had very severe COPD (GOLD stage 4). One subject (female, 73 yrs) with stage 4 COPD was unable to perform technically acceptable FENO measurements and thus was excluded from the analysis. All 20 healthy subjects were able to perform such measurements.

No significant difference appeared between FENO values at baseline and at +24 h either among COPD patients ($p = 0.62$) or among healthy subjects ($p = 0.68$). FENO values at +10 min measurement (when the subjects did not rinse their mouths with sodium bicarbonate) in COPD patients as well as in healthy subjects were slightly but significantly higher than at baseline ($p = 0.008$ and $p = 0.002$, respectively). Within an interval of 24 hours, CoV of FENO was 12.4 in COPD patients and 15.9 in healthy subjects. Intrasession repeatability for COPD patients and healthy subjects ranged from 5.5 to 6.9%, the requirement, according to current guidelines (ATS/ERS 2005), being $\leq 10\%$.

Median FENO values for baseline and +24 h measurements, CoV and ICC between baseline and +24 h measurements for healthy subjects, for all COPD patients, and separately for COPD patients with stage 2 and stage 3-4 disease are shown in Table 5. The

repeatability between baseline and +24 h measurements of FENO in COPD patients and in healthy subjects is presented by Bland Altman plots, where the mean of baseline and +24 h FENO value is plotted against the difference between the FENO values of the two respective sessions (Figure 10).

Table 5. *Median FENO values (flow rate 50 ml/s) and their short-term variability in the groups studied*

	FENO baseline (ppb)	FENO +24 h (ppb)	CoV (%)	ICC (95% CI)
Healthy (n=20)	15.2 (10.1-21.6)	14.5 (7.7-22.3)	15.9	0.90 (0.77-0.96)
COPD (n=19)	15.6 (12.8-22.5)	15.7 (11.1-22.8)	12.4	0.88 (0.72-0.95)
GOLD stage 2 (n=12)	18.2 (14.5-25.7)	15.5 (11.1-29.3)	13.7	0.89 (0.65-0.97)
GOLD stage 3-4 (n=7)	13.6 (9.2-18.6)	15.9 (11.0-20.5)	10.5	0.83 (0.37-0.97)

FENO expressed as medians (25-27% quartiles)
FENO +24 h: FENO measurement 24 h after baseline
CoV: coefficient of variance; ICC: intraclass correlation coefficient; CI: confidence interval

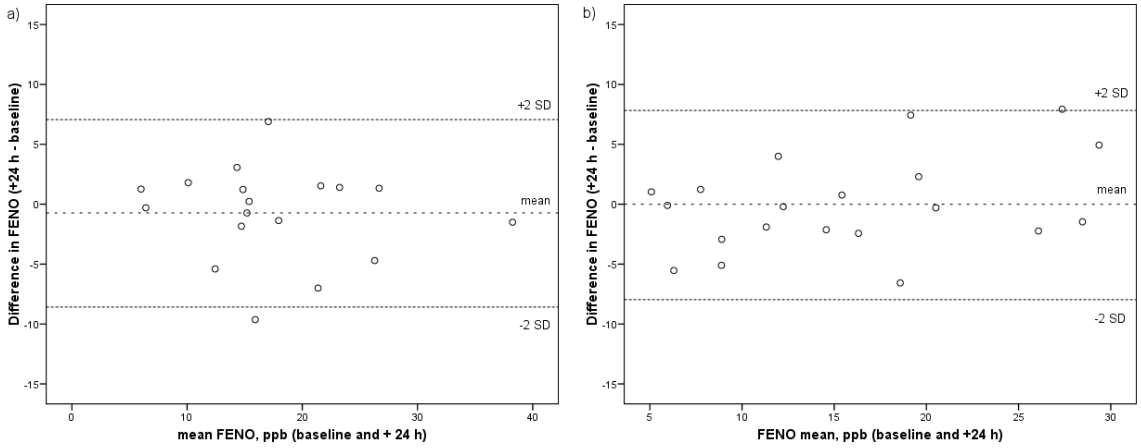

Figure 10 *Day-to-day (24 h) repeatability of FENO presented as Bland-Altman plots in COPD patients (a) and in healthy subjects (b).*

6 DISCUSSION

6.1 Study populations and methods

6.1.1 Study populations

The subjects selected by a randomized procedure from the adult population in Study I were thoroughly characterized by objective tests and a detailed structured interview. This allowed further selection of nonsmoking healthy subjects with no signs, symptoms, or history of airway disorders for assessment of the influence of allergic sensitization per se on FENO. The age group of 27 to 61 years represents a relevant adult target group for FENO measurements in clinical practice.

In Studies II and III, the army conscripts are an ideal group for assessment of associations between bronchial hyperresponsiveness and FENO. In addition to being currently symptomatic and steroid-naive, they formed a homogenous, sufficiently large group of subjects of the same age and with no interfering diseases. Since the majority of the subjects were males, the influence of gender could not be assessed. Whether these young adults with nonatopic asthma represented the typical adult onset phenotype of the disease or rather the childhood phenotype of diseases with good prognosis is unclear, and follow-up studies should investigate this issue.

In Study IV, COPD patients represented differing degrees of disease severity, from moderate to very severe. Only ex-smokers (defined by the commonly used criterion of smoking cessation at least one year earlier) were included in order to avoid any influence of current smoking on FENO. These results may thus be inapplicable to current smokers with possible smoking-induced variations in FENO, but they reflect the variation caused by chronic obstruction per se. In order to obtain a “real-life” population, no subjects with comorbidities were excluded if they were clinically stable. This is essential for clinical application of these results, as COPD patients frequently have comorbidities (Bourdin et al. 2009).

6.1.2 Methods

6.1.2.1 FENO measurement

FENO concentration is dependent on flow rate, with higher flow rates producing lower FENO values and vice versa. During the 1990s, development of and research on FENO measurement was rapid, with several flow rates used. Because the army conscript study began in 1998, FENO measurement in Studies II and III was performed according to that suggested in ERS guidelines from 1997 (Kharitonov et al. 1997), except that the flow rate

was slightly lower (90-120 ml/s) than suggested in the guidelines (167-250 ml/s). The study was already underway by the time of the guideline update, so the flow rate was not altered in the middle of the study. The flow rate used resulted in lower FENO concentrations than would have been obtained with the currently recommended flow rate of 50 ml/s. Since flow rate remained unchanged throughout the study, comparisons between FENO concentrations in various groups in Studies II and III are probably not markedly affected. FENO measurements in Studies I and IV were performed at the flow rate of 50 ml/s, valid since 1999 (ATS 1999). Since this flow rate is identical to that of the current guidelines (ATS/ERS 2005), reference material for FENO for the Finnish adult population could be obtained from Study I. Because the spirometric maneuver and bronchial hyperreactivity tests may temporarily reduce FENO (Silkoff et al. 1999, de Gouw et al. 1998), FENO measurements in all studies were performed either before spirometry (Study IV) or on a separate study day (Studies I-III). Histamine challenge in Studies I to III was performed after FENO measurements, and the exercise challenge one day prior to FENO measurement (Studies II and III). The exercise challenge has been reported to have no effect on eosinophilic airway inflammation or on airway responsiveness (Gavreau et al. 2000, Scollo et al. 2000, El-Halawani et al. 2003).

6.1.2.2 Skin prick tests

Allergic sensitization in Studies I to III was assessed by skin prick testing performed by trained and experienced nurses according to recommendations of the European Academy of Allergology and Immunology (Dreborg 1989), except for single tests performed instead of duplicates. The tests included a large number of common aeroallergens relevant to the Finnish population (Pallasaho et al. 2006b). In Study I, quantification of skin prick test results was also applied by analysis of mean wheal sizes, number of positive reactions, and prick wheal sum, calculated by adding up the diameters of each positive reaction (Miles et al. 1995, Koh et al. 2002). Many studies have assessed allergic sensitization by measuring serum IgE instead of performing skin prick tests. IgE, however, significantly varies by gender, age, and smoking, as well as showing considerable overlap in normal and atopic ranges (Baldacci et al. 2001).

6.1.2.3 Assessment of bronchial hyperresponsiveness and lung function

Bronchial responsiveness was assessed by direct (Studies I to III) and indirect (Studies II and III) stimuli. Histamine challenge was performed by a dosimetric method with controlled tidal breathing, which is a validated method with good reproducibility (Sovijärvi et al. 1993). This method has been in routine use in the laboratory of Clinical Physiology of Helsinki University Central Hospital and in the Central Military Hospital since the 1990's.

The exercise challenge was carried out in the form of a free-running test outdoors. This is appropriate for a study population of young adults, and has been part of routine

assessment of subjects with suspected asthma in the Central Military Hospital since the 1980's. According to the guidelines (Crapo et al. 2000, Joos et al. 2003), a valid test requires target exercise intensity to be sustained for 4 to 6 minutes, and to be achieved within 4 minutes, in order to avoid the potential refractoriness to exercise-induced bronchoconstriction (EIB) induced by prolonging warm-up time. These requirements were fulfilled, because the 8-minute free-running test was heart-rate controlled, with its target intensity achieved within the first 2 minutes.

The tests were performed in various weather conditions, so the results were temperature-adjusted according to a previously reported method (Latvala et al. 2000). No adjustments were made for air humidity, which might have caused some variation. Although measurement of FEV1 is recommended for evaluation of EIB severity (Crapo et al. 2000, Joos et al. 2003), we performed the evaluation using PEF measurements which had been standard at the Central Military Hospital for over 20 years. A decrease in PEF of $\geq$ 10% instead of 15% was regarded as diagnostic of asthma in this heart-rate controlled exercise challenge. Only 4% of the subjects had their asthma diagnosis based only on a PEF decrease of 10 to 14% after exercise challenge.

Flow-volume spirometry was used in all studies for assessment of lung function and was performed according to guidelines valid at the time of the studies. Differences between guidelines were, however, only minor (Miller et al. 2005). Results were expressed as a percentage of current Finnish reference values (Viljanen et al. 1982).

6.1.2.4 Structured interview and assessment of smoking status

In Study I, information from the structured interview served to characterize the subjects. Results from the FinEsS study based on the data from this structured interview have already appeared (Kotaniemi 2005, Pallasaho 2006a, Kainu 2008).

Smoking status and smoking history in all four studies was assessed by interview: in Study I by a study physician using a structured interview, in Studies II and III upon admission to the hospital, with a nurse using open questions, and in Study IV by a study physician or a nurse. As the COPD patients in Study IV had already been diagnosed, their smoking history was also available from their hospital records. Even though smoking status was not biochemically verified, this assessment can be regarded as reliable, since interviewer-assessed smoking status has been reported to yield higher estimates of sensitivity and specificity than do self-administered questionnaires, and observational studies to show a higher level of sensitivity than do intervention studies (Patrick et al. 1994).

6.2 Discussion of the main results

6.2.1 Allergic sensitization without signs of airway disorders does not increase FENO

We observed similar levels of FENO in skin prick test-positive and -negative healthy asymptomatic nonsmoking adults with no signs, symptoms, or history of airway disorders, indicating that atopic sensitization per se does not elevate FENO. A similar finding was reported in a slightly smaller group of 28 skin prick test-positive and 22 skin prick test-negative healthy subjects who had no symptoms or signs of airway disease, no signs of airway obstruction in spirometry, and no bronchial hyperresponsiveness in methacholine challenge (Berlyne et al. 2000). Such results are not necessarily contradictory to previous findings of elevated FENO in atopic compared to nonatopic subjects, since those studies have included subjects with increased bronchial hyperresponsiveness, previous symptoms, or even a previous diagnosis of asthma (Horváth & Barnes 1999, Franklin et al. 2004, Olin et al. 2006). Although those subjects were asymptomatic at the time of the study, such airway disorders may influence assessment of the relationship between FENO and allergic sensitization; subclinical airway inflammation may be present and be reflected in FENO levels. Higher FENO has appeared in atopic asthmatics in clinical remission — defined as complete absence of symptoms without any medication during the preceding year — than in healthy subjects, whereas FENO levels were similar with those of subjects with steroid-naive mild atopic asthma (van den Toorn et al. 2000). Repeated low-dose allergen challenge in asymptomatic subjects with mild atopic asthma has resulted in an asymptomatic increase in airway inflammation measured by FENO and by several indices in sputum samples (de Kluijver et al. 2002); higher FENO has appeared in asthmatics both sensitized and exposed to a relevant allergen than in those sensitized but not exposed (Simpson et al. 1999). A recent longitudinal study with a 4-year follow up of 1 506 subjects showed that baseline FENO > 30 ppb in an asymptomatic subject was associated with increased risk for wheeze during follow-up (Olin et al. 2010). This result further supports the hypothesis that increased FENO in an asymptomatic subject is related to subclinical eosinophilic airway inflammation.

Among healthy nonsmoking subjects, we found no significant correlation between FENO and degree of atopy defined either as number of positive reactions or as total sum of wheal diameters. This is not contrary to previous findings that a significant correlation appears between FENO and number of positive skin prick tests, since those studies included subjects with physician-diagnosed asthma and even recent respiratory symptoms suggesting asthma (Ho et al. 2000, Franklin et al. 2004). Similarly, in the present study, a positive correlation between FENO and degree of atopy did appear among subjects with history, symptoms, or signs of an airway disorder.

The findings of this study suggest that the same reference range can be applied to both skin prick test-positive and -negative adult subjects. The largest study defining reference values of FENO for adults suggests that FENO be adjusted according to height and age, whereas atopic status, defined as increased serum IgE levels, was of minor importance,

suggesting the same upper normal limits (24-53 ppb, depending on age and height) for both atopic and nonatopic healthy nonsmoking subjects (Olin et al. 2007).

6.2.2. Association between FENO and bronchial hyperresponsiveness appears only in atopics

Studies addressing the association between eosinophilic airway inflammation and bronchial hyperresponsiveness have yielded conflicting results. The association between these components of airway pathology is complex. Bronchial responsiveness itself is thought to consist of a variable component reflecting the current inflammatory status and of a more persistent component reflecting structural changes of airway remodeling. These features may be influenced by genetic background, by atopic constitution, and by duration of disease. Intensity of airway inflammation is reflected to some extent in symptom status, with more intense inflammation being more likely in a subject with current respiratory symptoms than in an asymptomatic subject with mild, intermittent disease, although symptom perception varies considerably. In addition, steroid treatment, influencing both bronchial responsiveness and airway inflammation, affects the correlation between these aspects. Smoking influences both hyperresponsiveness and the composition of airway inflammation, and although after smoking cessation many changes are reversible, permanent influences may remain (Willemse et al. 2004a). Methods for assessment of bronchial hyperresponsiveness also influence the reported associations between airway inflammation and bronchial hyperresponsiveness, challenges by indirect stimuli being more likely to reflect airway inflammation than would challenges by direct stimuli (Joos et al. 2003).

The present study included both atopic and nonatopic subjects, with the study population's being very homogenous in other aspects: all being nonsmokers, steroid-naive, and with current respiratory symptoms suggesting asthma. We also included patients not fulfilling the functional criteria of asthma, in order to obtain a wider range of variety in airway inflammation and BHR. We found a statistically significant correlation between airway inflammation (assessed by FENO) and severity of bronchial hyperresponsiveness to indirect stimulus (exercise) among atopic subjects but not among nonatopics. A significant correlation between FENO and BHR to a direct stimulus, histamine, also appeared among atopics but not among nonatopics. These results did not even change when only those subjects with an unequivocal diagnosis of asthma were included. Although median FENO was higher among atopic subjects, increased levels of FENO appeared also among nonatopic subjects. No correlation, however, existed between FENO and bronchial hyperresponsiveness (EIB or HIB) among nonatopic subjects, despite the fact that almost 40% of them showed elevated FENO.

We also found EIB to be slightly but significantly more pronounced in atopic than in nonatopic patients despite similar reactivity in histamine challenge in these groups. Presence of atopy seems to facilitate the development of exercise-induced airway hyperresponsiveness. In a group of mainly atopic asthmatics, a significant correlation appeared between atopy score and EIB severity (Koh et al. 2002), and EIB severity has

also been shown to correlate with markers of eosinophilic airway inflammation in sputum and blood (Yoshikawa et al. 1998). Atopic patients have also been reported to be more reactive to another indirect stimulus, AMP, than are nonatopic patients (Lúdvíksdóttir et al. 2000). No reports were available comparing the association between FENO and bronchial responsiveness to exercise between atopics and nonatopics. A significant association emerged between baseline FENO and EIB in 24 asthmatic children, most of whom were atopic (Scollo et al. 2000). El-Halawani and co-workers (2003) demonstrated the role of FENO as a predictor of exercise-induced bronchoconstriction in subjects with suspected diagnosis of EIB, but their study did not verify atopic status by objective tests. In concordance with our results, a recent pediatric study found increased FENO levels in both atopic and nonatopic wheezy children with EIB, but a significant correlation between FENO and EIB only among atopic subjects (Malmberg et al. 2009).

Grönke and co-workers (2002) found their correlation between FENO and BHR methacholine to depend on duration of asthma, with a significant correlation for those with shorter duration of the disease. Further evidence suggesting that the influence of disease duration may be essential is provided by a recent study involving 267 asthmatic children and adolescents, mainly atopics, in whom BHR to a direct stimulus, acetylcholine, associated significantly with airway inflammation, assessed by FENO, in children aged 5-11 years; among adolescents (12-20 yrs), however, BHR associated with structural changes, and the association between BHR and FENO was weaker (Motomura et al. 2009). In contrast to these findings, Zietkowski and co-workers (2006) reported a significant correlation between FENO and BRH to histamine in both atopic and nonatopic subjects in a study population of 101 nonsmoking steroid-naive asthmatics older (mean age 32-40 yrs) than were our participants (mean age 20). Atopic sensitization was assessed by skin prick tests as in our study, but no criterion for positive response was mentioned, and BHR to histamine was expressed as PC_{20}FEV1, in contrast to our PD_{15}FEV1.

6.2.3 Smoking attenuates increase in FENO only in atopic asthma

Smoking is known to alter the composition of asthmatic airway inflammation. Compared to figures for asthmatic nonsmokers, the proportion of eosinophils in smokers is lower (Chalmers et al. 2001, Broekma et al. 2009), and number of neutrophils is higher (Chalmers et al. 2001). Expression of IL-8, a potent neutrophil attractant, is higher in asthmatic smokers than in nonsmokers (St-Laurent et al. 2008). IL-8 correlates positively with number of neutrophils in sputum, and increases significantly with increasing pack-years (Chalmers et al. 2001). An increased number of neutrophils correlates inversely with FENO (Rytilä et al. 2006). Smoking is also known to reduce FENO by several mechanisms: by reduction in cytokine-induced NO-production, iNOS protein, and iNOS mRNA, or by smoke-induced toxic damage to NO-producing epithelial cells, or by capture of NO from the airways by peroxynitrite formation caused by reactive oxygen species in cigarette smoke (Hoyt et al. 2003, Horváth et al. 2004, Wei et al. 2005).

To our knowledge, the present study was the first to assess whether this influence of smoking on FENO differs between those with atopic and nonatopic steroid-naive asthma.

Studies addressing the influence of smoking on FENO in asthmatics have either included only atopic asthmatics (Horváth et al. 2004) or have not assessed their atopic status (Verleden et al. 1999). One study that did assess both atopic (n=65) and nonatopic (n=33) asthmatics found increased FENO in atopic and nonatopic steroid-naive asthmatic never-smokers, but detected no difference from non-asthmatics when current- and ex-smokers were included (Malinovschi et al. 2009). No comparison of FENO between atopic and nonatopic smokers was performed, and the number of smokers within groups remained unreported. The present study was the first to report attenuation of FENO formation only in atopic but not in nonatopic smokers with steroid-naive asthma. This finding may result from differences in the mechanisms of FENO formation as well as in the sensitivity of these mechanisms to smoking in atopic and nonatopic asthma, and demands further study.

Since smoking reduces FENO, it may compromise the diagnostic value of FENO in asthmatic smokers. Although current guidelines for FENO measurement state that smokers with asthma do have elevated FENO (ATS/ERS 2005), data supporting this conclusion are very limited. As smoking prevalence among adult asthmatics is reported to be at the same level as among the general population (Pietinalho et al. 2009) and as reference values are based on levels in nonsmokers (Olin et al. 2007), it is essential to know whether FENO in steroid-naive asthmatic smokers differs significantly from FENO in healthy nonsmokers in order to evaluate the usefulness of FENO measurement in distinguishing airway inflammation in smokers with respiratory symptoms. Horváth and co-workers (2004) found, among 22 steroid-naive atopic asthmatic smokers, higher FENO than in healthy smokers but did not report any comparison to healthy nonsmokers. Study by Verleden and co-workers (1999) reported higher FENO among 13 steroid-naive asthmatic smokers than among healthy smokers, but no difference when compared to healthy nonsmokers. In the present study, FENO among asthmatic smokers, even among nonatopic subjects, was significantly higher than FENO among healthy subjects, suggesting that FENO measurement may prove useful in assessment of asthmatic airway inflammation in young adult smokers.

Whether FENO in smokers with steroid-naive asthma exceeds the levels in healthy subjects eventually depends on the balance between FENO-producing eosinophilic inflammation and the FENO-reducing effect of smoking. This balance may be affected by age and by length of smoking history. In studies reporting lower FENO in healthy and steroid-naïve asthmatic smokers compared to their nonsmoking counterparts, subjects' mean ages have ranged from 29 to 33 years (Horváth et al. 2004) to 53 to 64 years (Rytilä et al. 2006). Sundy and co-workers (2007) reported an age-related decline in FENO among smokers, and found a significant difference in FENO between healthy smokers and nonsmokers in the age group of 21 to 40 years, whereas no difference appeared in those aged 18 to 20, corresponding to the recruits in the present study. Smoking status of the subjects in Sundy's study was verified by measurement of serum cotinine, and an inverse correlation appeared between serum cotinine and FENO. Accordingly, other studies have reported a negative correlation between FENO and number of pack-years (Persson et al. 1994, Kharitonov et al. 1995a, Corradi et al. 1999).

6.2.4 Repeatability of FENO in COPD patients and in healthy subjects is equally good

The rationale for measuring FENO in COPD patients is based on the association reported between increased FENO or sputum eosinophilia and response to steroid treatment (Fabbri et al. 2003, Leigh et al. 2006, Kunisaki et al. 2008, Lehtimäki et al. 2010), and thus on the potential for FENO measurement in identifying potential steroid responders. Prior to assessment of the magnitude of any significant change in FENO in response to therapy, however, the repeatability of FENO measurements in chronically obstructed patients needs study. Severe obstruction in particular may impair the subject's ability to maintain the required flow or impair the diffusion of NO into the exhaled air due to collapse of the bronchioles during exhalation; this would influence the repeatability of FENO measurement.

The present study demonstrated that the repeatability of FENO between successive days in COPD patients is equal to that in healthy subjects. A FENO CoV of 12.4% in our COPD patients is in agreement with the figure Bhowmik and co-workers (2005) reported: a CoV of 13.1% for the short-term repeatability of FENO in a group of 79 patients with moderate to severe COPD, although their flow rate for the FENO measurements (5 l/min) differed from current recommendations (ATS/ERS 2005), and they mentioned no exact interval for repeated measurements. Another study assessed day-to-day variation as well as diurnal variation in FENO in 8 COPD patients with stage 2 disease as part of a larger study of 81 subjects (nonsmokers and smokers with or without airway obstruction); they used a multiple flow technique and reported a high degree of reproducibility (ICC 0.993 for a flow rate of 50 ml/s) in FENO measurements for the whole study group, and concluded that their results are applicable for COPD of differing severity (Brindicci et al. 2008). According to the ATS/ERS guidelines (2005), for acceptable FENO measurement, constant expiratory flow is required to continue for at least 6 seconds. This requirement might not be easy for patients with severe airway obstruction to fulfill, thus the good repeatability of FENO measurements reported in healthy subjects or asthma patients (Ekroos et al. 2000, Ekroos et al. 2002, Kharitonov et al. 2003) cannot be directly applied to COPD patients.

In spirometry, of our 20 COPD patients in the present study, 8 had severe or very severe airway obstruction. Only one of these 8 was unable to maintain the flow rate required to produce repeated FENO recordings fulfilling requirements for an acceptable measurement. Bhowmik and co-workers (2005) reported that in a sample of 98 patients with airflow obstruction of various degrees, 19 subjects were unable to perform acceptable measurements. Those subjects were older (mean age 71) and had a lower FEV1 (mean 0.84 liters) than did those capable of producing technically acceptable measurements. The COPD patients and healthy subjects in our study were not age- or gender-matched: The healthy subjects were more often female and younger than the COPD patients. Furthermore, we also included COPD patients with comorbidities, representing the usual real-life situation. Despite these inequalities, the older disabled subjects with airflow obstruction performed FENO measurements as well as did the younger healthy ones. Because only ex-smokers were included in our study in order to avoid the influence of

current smoking on FENO, the results are thus not applicable to COPD patients who smoke, and further studies are needed in that patient group.

Our results suggest that a change in FENO exceeding 24% is likely to reflect a measurable change in the inflammatory process in COPD. A recent study measuring FENO (flow rate 50 ml/s) once a month over a period of one year in 59 patients with COPD (mean age 66 years and mean GOLD stage 2.6) found a significant correlation between individual exacerbation rate and FENO CoV; patients with a FENO CoV of > 40% during the year reported a two-fold increase in exacerbation rate over that of patients with a FENO CoV of < 40% (de Laurentiis et al. 2008). The design of our study does not permit conclusions about clinically significant changes.

Because FENO measured by the conventional technique reflects airway inflammation mainly in the larger airways, it thus may not fully represent the more peripheral inflammation present in COPD. NO production from the more peripheral airways can be better detected by a technique utilizing multiple flow rates. This peripheral component of FENO has been shown to be insensitive to steroid treatment, however, and thus may be inapplicable when the purpose of FENO measurement is to identify possible steroid responders. A high level of bronchial NO flux has been reported to relate to symptom relief and improvement in FEV1 after steroid treatment, whereas no such association has been detectable in relation to alveolar NO (Lehtimäki et al. 2010).

7 CONCLUSIONS

In nonsmoking healthy subjects with no signs, symptoms, or history of chronic or recurrent airway diseases allergic sensitization per se does not influence FENO levels; this supports the view that elevated FENO is not just a feature of atopy, but indicates NO-producing inflammation in the airways related to airway disease. The upper normal limit of FENO was found to be 30 ppb in both skin prick test-positive and -negative subjects.

FENO was associated with bronchial hyperresponsiveness to both direct (histamine) and indirect (exercise) stimuli in atopic but not in nonatopic nonsmoking steroid-naive subjects with current asthmatic symptoms. In both groups, a significant correlation appeared between histamine- and exercise-induced bronchoconstriction, but this correlation was more pronounced among the atopic subjects. These findings support the view that the pathogenesis of bronchial hyperresponsiveness in atopic asthma is strongly involved in airway inflammation reflected by FENO, and they suggest that in nonatopic asthma other mechanisms may dominate or coexist in the development of bronchial hyperresponsiveness.

Smoking attenuated the increase in FENO in atopic but not in nonatopic steroid-naive symptomatic asthma. This finding suggests differences in the mechanisms of FENO formation as well as in the sensitivity of these mechanisms to smoking in atopic and nonatopic asthma. Compared to healthy subjects, FENO was elevated in both smoking and nonsmoking subjects with steroid-naive asthma regardless of atopic status, suggesting that FENO measurements may be useful in assessing airway inflammation also in young adult smokers.

The short-term repeatability of FENO in COPD patients and in healthy subjects was equally good, although subjects with very severe disease may have difficulties in maintaining the required flow rate. Change in FENO exceeding 24% is likely to reflect a minimum measurable change in the NO-associated inflammatory process in COPD.

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References

Agusti AGN, Villaverde JM, Togores B, with the technical collaboration of M. Bosch. Serial measurements of exhaled nitric oxide during exacerbations of chronic obstructive pulmonary disease. Eur Respir J 1999;14:523-528

Alving K, Weitzberg E, Lundberg JM: Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6:1368-1370

Alving K, Janson C, Nordvall L. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. Resp Research 2006:7:67

American Thoracic Society. Standardization of spirometry, 1994 Update. Am J Respir Crit Care Med 1995;152:1107-1136

American Thoracic Society. Recommendations for standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children – 1999. Am J Respir Crit Care Med 1999;160:2104-2117

Amin K, Lúdvíksdóttir D, Janson C, Nettelbladt O, Björnsson E, Roomans GM, Boman G, Seveus L, Venge P. Inflammation and structural changes in the airways of patients with atopic and non-atopic asthma. Am J Respir Crit Care Med 2000;162:2295-2301

Amin K, Ekberg-Jansson A, Löfdahl CG, Venge P. Relationship between inflammatory cells and structural changes in the lungs of asymptomatic and never smokers: a biopsy study. Thorax 2003;58:135-142

Amin K, Janson C, Boman G, Venge P. The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma. Allergy 2005;60:1241-1247

Ansarin K, Chatkin JM, Ferreira IM, Gutierrez CA, Zamel N, Chapman KR. Exhaled nitric oxide in chronic obstructive pulmonary disease: relationship to pulmonary function. Eur Respir J 2001;17:934-938

Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, Buist AS, Conway WA Jr, Enright PL, Kanner RE, O'Hara P. Effects of a smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. JAMA 1994; 272:1497-1505

Anthonisen NR, Connett JE, Murray RP. Smoking and lung function of Lung Health Study participants after 11 years. Am J Respir Crit Care Med 2002;166:675-679

ATS/ERS recommendations for standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-930

Baldacci S, Omenaas E, Oryszczyn MP. Allergy markers in respiratory epidemiology. Eur Respir J 2001;17:773-790

Baraldo S, Turato G, Badin C, Bazzan E, Beghé B, Zuin R, Calabrese F, Casoni G, Maestrelli P, Papi A, Fabbri LM, Saetta M. Neutrophilic infiltration within airway smooth muscle in patients with COPD. Thorax 2004;59:308-312

Barbee RA, Brown WG, Kaltenborn W, Halonen M. Allergen skin-test reactivity in a community population sample: correlation with age, histamine skin reactions and total serum immunoglobulin E. J Allergy Clin Immunol 1981;68:15-19

Barnes PJ, Chowdhury B, Kharitonov SA, Magnussen H, Page CP, Postma D, Saetta M. Pulmonary biomarkers in chronic obstructive pulmonary disease. Am J Resp Crit Care Med 2006a;174:6-14

Barnes PJ. Reduced histone deacetylase in COPD. Chest 2006b;129:151-155

Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J 2009;33:1165-1185

Batra J, Sing TP, Mabalijaran U, Sinha A, Prasad R, Gosh B. Association of inducible nitric oxide synthase with asthma severity, total serum immunoglobulin E and blood eosinophil levels. Thorax 2007;62:16-22

Bednarek M, Maciejewski J, Wozniak M, Kuca P, Zielinski J. Prevalence, severity and underdiagnosis of COPD in primary care setting. Thorax 2008;63:402-407

Beg MFS, Alzoghaibi MA, Abba AA, Habib SS. Exhaled nitric oxide in stable chronic obstructive pulmonary disease. Ann Thorac Med 2009;4:65-70

Beier J, Beeh KM, Kornmann O, Buhl R. Sputum induction leads to a decrease of exhaled nitric oxide unrelated to airflow. Eur Respir J 2003;22:354-357

Bergeron C, Boulet LP, Page N, Laviolette M, Zimmerman N, Rothenberg ME, Hamid Q. Influence of cigarette smoke on the arginine pathway in asthmatic airways: increased expression of arginase I. J Allergy Clin Immunol 2007;119:391-397

Berkman N, Avital A, Breuer R, Bardach E, Springer C, Godfrey S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. Thorax 2005;60:383-388

Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. J Allergy Clin Immunol 2000;106:638-644

Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavrod ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. Clin Exp Allergy 2005;35:1175-1179

Bettiol J, Bartsch P, Louis R, de Groote D, Gevaerts Y, Louis E, Malaise M. Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: relationship with blood and sputum eosinophilia and serum IgE levels. Allergy 2000;55:1134-1141

Bhowmik A, Seemungal TAR, Donaldson GC, Wedzicha JA. Effects of exacerbations and seasonality on exhaled nitric oxide in COPD. Eur Respir J 2005;26:1009-1015

Bisgaard H, Loland L, Anhøj J. NO in exhaled air of asthmatic children is reduced by the leukotriene receptor antagonist montelukast. Am J Respir Crit Care Med 1999;160:1227-1231

Bohadana A, Michealy J-P, Teculescu D, Wild P. Reproducibility of exhaled nitric oxide in smokers and non-smokers: relevance for longitudinal studies. BMC Pulm Med 2008;8:4

Borrill Z, Clough D, Truman N, Morris J, Langley S, Singh D. A comparison of exhaled nitric oxide measurements performed using three different analyzers. Resp Med 2006;100:1392-1396

Boulet LP. Asymptomatic airway hyperresponsiveness: A curiosity or an opportunity to prevent asthma? Am J Respir Crit Care Med 2003;167:371-378

Bourdin A, Burges PR, Chanez P, Garcia G, Perez T, Roche N. Recent advances in COPD: pathophysiology, respiratory physiology and clinical aspects, including comorbidities. Eur Respir Rev 2009;18:198-212

Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;161:1720-1745

Bousquet PJ, Hooper R, Kogevinas M, Jarvis D, Burney P. Number of allergens to be tested to assess allergenic sensitization in epidemiologic studies: results of the European Community Respiratory Health Survey I. Clin Exp Allergy 2007;37:780-787

Bousquet PJ, Chatzi L, Jarvis D, Burney P. Assessing skin prick tests reliability in ECRHS-I. Allergy 2008;63:341-346

Bradding P, Green RH. Subclinical phenotypes of asthma. Curr Opin Allergy Clin Immunol 2010;10:54-59

Brannan JD, Koskela H, Anderson SD. Monitoring asthma therapy using indirect bronchial provocation tests. Clin Resp J 2007;1:3-15

Brightling CE, Monteiro W, Ward R, Parker D, Morgan MDL, Wardlaw AJ, Pavrod ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. Lancet 2000;356:1480-1485

Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med 2002;346:1699-1705

Brightling CE, McKenna S, Hargadon B, Birring S, Green R, Siva R, Berry M, Parker D, Monteiro W, Pavrod ID, Bradding P. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. Thorax 2005;60:193-198

Brindicci C, Ito K, Resta O, Pride N, Barnes PJ, Kharitonov SA. Exhaled nitric oxide from lung periphery is increased in COPD. Eur Respir J 2005;26:52-59

Broekema M, ten Hacken NHT, Volbeda F, Lodewijk ME, Hylkema MN, Postma DS, Timens W. Airway epithelial changes in smokers but not in ex-smokers with asthma. Am J Respir Crit Care Med 2009;180:1170-1178

Bruce CT, Zhao D, Yates DH, Thomas PS. L:-arginine reverses cigarette-induced reduction of fractional exhaled nitric oxide in asthmatic smokers. Inflammopharmacology 2010;18:9-16

Brutsche MH, Downs SH, Schindler C, Gerbase MW, Schwartz J, Frey M, Russi EW, Ackermann-Liebrich U, Leuenberger P, for the SAPALDIA Team. Bronchial hyperresponsiveness and the development of asthma and COPD in asymptomatic individuals: SAPALDIA Cohort Study. Thorax 2006;61:671-677

Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, Menezes AM, Sullivan SD, Lee TA, Weiss KB, Jensen RL, Marks GB, Gulsvik A, Nizankowska-Mogilnicka E. BOLD Collaborative Research Group. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. Lancet 2007;370:741-750

Carra S, Gagliardi L, Zanconato S, Scollo M, Azzolin N, Zacchello F, Baraldi E. Budesonide but not nedocromil sodium reduces exhaled nitric oxide levels in asthmatic children. Resp Med 2001;95:734-739

Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez R, Pinto Plata V, Cabral HJ. The body-mass index, airflow obstruction, dyspnea and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med 2004;350:1005-1012

Chalmers GW, Macleod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. Chest 2001;120:1917-1922

Chalmers GW, Macleod KJ, Little SA, Thomson LJ, McSharry CP, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. Thorax 2002;57:226-230

Chatkin JM, Ansarin K, Silkoff PE, McClean P, Gutierrez C, Zamel N, Chapman KR. Exhaled nitric oxide as a non-invasive assessment of chronic cough. Am Respir J Crit Care Med 1999;159:1810-1813

Chaudhuri R, McSharry C, McCoard A, Livingston E, Hothersall E, Spears M, Lafferty J, Thomson NC. Role of symptoms and lung function in determining asthma control in smokers with asthma. Allergy 2008:63;132-135

Clini E, Bianchi L, Paganini M, Ambrosino N. Endogenous nitric oxide in patients with stable COPD: correlates with severity of disease. Thorax 1998;53:881-883

Coca AF, Cooke RA. On the classification of the phenomena of hypersitiveness. J Immunol 1923;8:163-182

Cockcroft DW, Davis BE. Mechanisms of airway hyperresponsiveness. J Allergy Clin Immunol 2006;118:551-559

Corradi M, Majori M, Cacciani GC, Consigli GF, de'Munari E, Pesci A. Increased exhaled nitric oxide in patients with stable chronic obstructive pulmonary disease. Thorax 1999;54:572-575

Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, Macintyre NR, McKay RT, Wanger JS, Anderson SD, Cockroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing – 1999. Am J Respir Crit Care Med 2000;161:309-329

Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. Am J Respir Crit Care Med 1998;157:4-9

de Gouw HWFM, Hendriks J, Woltman AM, Twiss IM, Sterk PJ. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. Am J Respir Crit Care Med 1998;158:315-319

de Kluijver J, Evertse CE, Schrumpf JA, van der Veen H, Zwinderman AH, Hiemstra PS, Rabe KF, Sterk PJ. Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma. Protection by inhaled steroids. Am J Resp Crit Care Med 2002;166:294-300

de Laurentiis G, Maniscalco M, Cianciulli F, Stanziola A, Marisco S, Lundberg JO, Weitzberg E, Sofia M. Exhaled nitric oxide monitoring in COPD using a portable analyzer. Pulm Pharmacol Ther 2008;21:689-693

Delen FM, Sippel JM, Osborne ML, Law S, Thukkani N, Holden WE. Increased exhaled nitric oxide in chronic bronchitis. Comparison with asthma and COPD. Chest 2000;117:695-701

Deykin A, Halpern O, Massaro AF, Drazen JM, Israel E. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. Am J Respir Crit Care Med 1998;157;769-775

Deykin A, Massaro AF, Coulston E, Drazen JM, Israel E. Exhaled nitric oxide following repeated spirometry or repeated plethysmography in healthy individuals. Am J Respir Crit Care Med 2000;161:1237-1240

Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, Holgate ST. Mucosal inflammation in asthma. Am Rev Respir Dis 1990;142:434-457

Donaldson GC, Seemungal TAR, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax 2002;57:847-852

Dreborg S. Sub-Committee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Skin tests used in type I allergy testing. Position paper. Allergy 1989; 44 (Suppl.10):13-59

Dreborg S, Frew AJ. Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. Position Paper. Allergy 1993;48 (Suppl 14):48-82

Drummond MB, Dasenbrook EC, Pitz MW, Murphy DJ, Fan E. Inhaled corticosteroids in patients with stable chronic obstructive pulmonary disease: a systematic review and meta-analysis. JAMA 2008;300:2407-2416

Dupont LJ, Rochette F, Demedts M: Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naive patients with asthma. Am J Respir Crit Care Med 1998;157:894-898

Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. Chest 2003;123:751-756

Ekberg-Jansson A, Amin K, Bake B, Rosengren A, Tylen U, Venge P, Löfdahl CG. Bronchial mucosal mast cells in asymptomatic smokers relation to structure, lung function and emphysema. Resp Med 2005;99:75-83

Ekroos H, Tuominen J, Sovijärvi ARA: Exhaled nitric oxide and its long-term variation in healthy non-smoking subjects. Clin Phys 2000;20:434-439

Ekroos H, Karjalainen J, Sarna S, Laitinen LA, Sovijärvi ARA. Short-term variability of exhaled nitric oxide in young male patients with mild asthma and in healthy subjects. Resp Med 2002;96:895-900

Ekroos H, Rouhos A, Pallasaho P, Karjalainen J, Sarna S, Sovijärvi ARA. Equally elevated concentrations of exhaled nitric oxide in nonatopic and low-sensitized atopic asthmatics. Resp Med 2009;103:152-158

El-Halawani SM, Ly NT, Mahon RT, Amundson DE. Exhaled nitric oxide as a predictor of exercise-induced bronchoconstriction. Chest 2003;124:639-643

European Lung White Book. The first comprehensive survey on respiratory health in Europe. European Respiratory Society 2003

Fabbri LM, Romagnoli M, Corbetta L, Casoni G, Busljetic K, Turato G, Ligabue G, Ciaccia A, Saetta M, Papi A. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;167:418-424

Ferreira IM, Hazari MS, Gutierrez C, Zamel N, Chapman KR. Exhaled nitric oxide and hydrogen peroxide in patients with chronic obstructive pulmonary disease: effects of inhaled beclomethasone. Am J Respir Crit Care Med 2001;164:1012-1015

Fletcher C, Peto R. The natural history of chronic airway obstruction. BMJ 1977;1:1645-1648

Franklin PJ, Stick SM, LeSouëf PN, Ayres JG, Turner SW. Measuring exhaled nitric oxide levels in adults. The importance of atopy and airway hyperresponsiveness. Chest 2004;126:1540-1545

Franklin PJ, Turner S, Mutch R, Stick SM. Parental smoking increases exhaled nitric oxide in young children. Eur Respir J 2006;28:730-733

Gade E, Thomsen SF, Porsbjerg C, Backer V. The bronchial response to mannitol is attenuated by a previous methacholine test: but not vice versa. Clin Exp Allergy 2009;39:966-971

Gauvreau GM, Ronnen GM, Watson RM, O'Byrne PM. Exercise-induced bronchoconstriction does not cause eosinophilic airway inflammation or airway hyperresponsiveness in subjects with asthma. Am J Respir Crit Care Med 2000;162:1302-1307

Gelb AF, Flynn Taylor C, Shinar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict asthma exacerbations in treated asthmatics. Chest 2006;129:1492-1499

Gergen PJ, Arbes SJ, Calatroni A, Mitchell HE, Zeldin DC. Total IgE levels and asthma prevalence in the US population: Results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 2009;124:447-453

Global Initiative for Chronic Obstructive Lund Disease (GOLD). www.gold.com (accessed February 2010)

Global Initiative for Asthma. www.ginasthma.com (accessed February 2010)

Godtfredsen NS, Lam TH, Hansel TT, Leon ME, Gray N, Dresler C, Burns DM, Prescott E, Vestbo J. Eur Respir J 2008;32:844-853

Gold DR, Wang X, Wypij D, Speizer FE, Ware JH, Dockery DW. Effects of cigarette smoking on lung function in adolescent boys and girls. N Engl J Med 1996;335:931-937

Gratziou CH, Lignos M, Dassiou M: Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. Eur Respir J 1999;14:897-901

Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavrod ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002a;360:1715-1721

Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavrod ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 2002b;57:875-879

Grönke L, Kanniess F, Holz O, Jörres RA, Magnussen H. The relationship between airway hyperresponsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. Clin Exp All 2002;32:57-63

Gustafsson LE, Leone AM, Persson MG, Wiklund NO, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Comm 1991;181:852-857

Haahtela T, Heiskala M, Suoniemi I. Allergic disorders and immediate skin test reactivity in Finnish adolescents. Allergy 1980;35:433-441

Haahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Persson T, Selroos O, Sovijärvi A. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. N Engl Med J 1994;331:700-705

Haahtela T, Kaila M, Ahonen E, Kava T, Kinnula V, Klaukka T, Mäkelä M, Remes S, Sovijärvi A, Valovirta E. Astma. (Asthma). Käypä hoito –suositus (Evidence based recommendation). Duodecim 2006

Haahtela T, Tamminen K, Kava T, Malmberg LP, Rytilä P, Nikander K, Persson T, Selroos O. Thirteen-year follow-up of early intervention with an inhaled corticosteroid in patients with asthma. J Allergy Clin Immunol 2009;124:1180-1185

Hahn PY, Morgenthaler TY, Lim KG. Use of exhaled nitric oxide in predicting response to inhaled corticosteroids for chronic cough. Mayo Clin Proc 2007;81:1350-1359

Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, Bousquet J, Godard P, Holgate S, Polak JM. Induction of nitric oxide synthase in asthma. Lancet 1993;342:1510-1513

Helakorpi S, Paavola M, Prättälä R, Uutela A. Health Behaviour and Health among the Finnish Adult Population, Spring 2008, National Institute for Health and Welfare, Report 2/2009, http:/www.thl.fi/avtk

Henriksen AH, Sue-Chu M, Lingaas Holmen T, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. Eur Respir J 1999;13:301-306

Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in large population survey. Eur Respir J 2000;15:849-855

Hewitt RS, Modrich CM, Medlicott T, Cowan JO, Taylor DR. Supporting the diagnosis of non-specific respiratory symptoms in primary care: the role of exhaled nitric oxide measurement and spirometry. Prim Care Respir J 2008;17:97-103

Ho L-P, Wood FT, Robson A, Innes A, Greening AP. Atopy influences exhaled nitric oxide levels in adult asthmatics. Chest 2000;118:1327-1331

Holloway JW, Yang IA, Holgate ST. Genetics of allergic disease. J Allergy Clin Immunol 2010;125:S91-94

Horváth I, Donnelly LE, Kiss A, Kharitonov SA, Lim S, Chung KF, Barnes PJ. Combined use of exhaled hydrogen peroxide and nitric oxide in monitoring asthma. Am J Respir Crit Care Med 1998;158:1042-1046

Horváth I, Barnes PJ. Exhaled monoxides in asymptomatic atopic subjects. Clin Exp All 1999;29:1276-1280

Horváth I, Donnelly LE, Kiss A, Balint B, Kharitonov SA, Barnes PJ. Exhaled nitric oxide and hydrogen peroxide concentrations in asthmatic smokers. Respiration 2004;71:463-468

Hoyt JC, Robbins RA, Habib M, Springall DR, Buttery LDK, Polak JM, Barnes PJ. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. Exp Lung Res 2003;29:17-28

Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, Kay AB. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. Immunol Today 1999;11:528-533

Hurwitz KM, Roach JM, Argyros GJ, Eliasson AH, Philips YY. Refractory period during bronchoprovocation with eucapnic hyperventilation and methacholine. Am J Respir Crit Care Med 1994;149:1452-1456

Hylkema MN, Sterk PJ, de Boer WI, Postma DS. Tobacco use in relation to COPD and asthma. Eur Respir J 2007; 29:438-445

Högman M, Holmkvist T, Wålinder R, Meriläinen P, Lúdvíksdóttir D, Håkansson L, Hedenström H. Increased nitric oxide elimination from the airways after smoking cessation. Clin Sci 2002;103:15-19

Ichinose M, Takahashi T, Sugiura H, Endoh N, Miura M, Mashito Y, Shirato K. Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre. Eur Respir J 2000;15:248-253

Jaakkola MS, Jaakkola JJK. Effects of environmental tobacco smoke on the respiratory health of adults. Scand J Work Environ Health 2002;20 suppl 2:52-70

Jaakkola MS. Smoke and dust get in your eyes: what does it mean in the work place? Thorax 2009;64:1-2

Jain B, Rubinstein I, Robbins RA, Leise KL, Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem Biophys Res Commun 1993;191:83-88

Jatakanon A, Lim S, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax 1998;53:91-95

Jatakanon A, Kharitonov A, Lim S, Barnes PJ. Effect of differing doses of inhaled budesonide on markers of airway inflammation in patients with mild asthma. Thorax 1999a;54:108-114

Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophil inflammation in severe persistent asthma. Am J Respir Crit Care Med 1999b;160:1532-1539

Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. Am J Resp Crit Care Med 2000;161:64-72

Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemière C, Pizzichini E, Cartier A, Hussack P, Goldsmith CH, Laviolette M, Parameswaran K, Hargreave FE. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J 2006;27:483-494

Jeffery PK. Bronchial biopsies and airway inflammation. Eur Respir J 1996;9:1583-1587

Johansson SGO, Hourihane JO'B, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, Kowalski ML, Mygind N, Ring J, van Cauwenberge P, van Hage-Hamsten M, Wüthrich B. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;56:813-824

Johansson SGO, Bieber T, Dahl R, Friedman PS, Lanier BQ, Lockey RF, Motala C, Ortega Martell JA, Platts-Mills TAE, Ring J, Thien F, Van Cauvenberge P, Williams HC. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol 2004;113:832-836

Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control: Am J Respir Crit Care Med 2001;164:738-743

Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J 2002;20:601-608

Jones WA, Fransson M, Maldonado-Holmertz E. Does consumption of ethanol distort measurements of exhaled nitric oxide? Resp Med 2005;99:196-199

Joos GF, O'Connor B, on behalf of the Task Force. Indirect airway challenges. Eur Respir J 2003;21:1050-1068

Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. Thorax 1978;33:705-710

Juusela M, Poussa T, Kotaniemi J, Lundbäck B, Sovijärvi A. Bronhcial hyperresponsiveness in a population of North Finland with no previous diagnosis of asthma or chronic bronchitis assessed with histamine and methacholine tests. Int J Circumpolar Health 2008;67:308-317

Kainu A. Spirometric studies on the adult general population of Helsinki – bronchodilatation responses, determinants, and intrasession repeatability of FEV1, FEV6, FVC, and forced expiratory time. A report from the FinEsS-Helsinki II study. Dissertation, Helsinki University 2008

Kanazawa H, Shoji S, Yoshikawa T, Hirata K, Yoshikawa J. Increased production of endogenous nitrite oxide in patients with bronchial asthma and chronic obstructive pulmonary disease. Clin Exp Allergy 1998;28:1244-1250

Karjalainen EM, Lindqvist A, Laitinen LA, Kava T, Altraja A, Halme M, Laitinen A. Airway inflammation and basement membrane tenascin in newly detected atopic and nonatopic asthma. Resp Med 2003;97:1045-1051

Karjalainen J. Exercise response in 404 young men with asthma: no evidence for late asthmatic reaction. Thorax 1991;46:100-104

Khalili B, Boggs PB, Bahna SL. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. Allergy 2007;62:1171-1174

Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. Am J Respir Crit Care Med 1995a; 152:609-612.

Kharitonov SA, O´Connor B, Evans D, Barnes PJ: Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. Am J Respir Crit Care Med 1995b;151:1894-1899

Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J 1995c;8:295-297

Kharitonov SA, Yates D, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. Eur Respir J 1996a;9:196-201

Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1996b;153:454-457

Kharitonov S, Alving K, Barnes PJ. ERS Task Force Report: Exhaled and nasal nitric oxide measurements: recommendations. Eur Respir J 1997;10:1683-1693

Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. Am J Respir Crit Care Med 2001;163:1693-1722

Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax 2002;57:889-896

Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. Eur Respir J 2003;21:433-438

Kilpeläinen M, Terho EO, Helenius H, Koskenvuo M. Validation of a new questionnaire on asthma, allergic rhinitis, and conjunctivitis in young adults. Allergy 2001;56:377-384

Koh Y, Choi I, Lim H. Atopy may be related to exercise-induced bronchospasm in asthma. Clin Exp All 2002;32:532-536

Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signaling in inflammation. Current Drug Targets – Inflammation and Allergy 2005;4:471-479

Kotaniemi JT, Lundbäck B, Nieminen MM, Laitinen LA, Sovijärvi ARA. Increase of asthma in adults in Northern Finland? a report from the FinEsS study. Allergy 2001;56:169-174

Kotaniemi JT, Sovijärvi A, Lundbäck B. Chronic obstructive pulmonary disease in Finland: prevalence and risk factors. COPD 2005;3:331-339

Kotaniemi J. Asthma, chronic obstructive pulmonary disease and respiratory symptoms among adults: prevalence and risk factors – The FinEsS study in Northern Finland. Dissertation, Helsinki University 2006

Kunisaki KM, Rice KL, Janoff EN, Rector TS, Niewoehner DE. Exhaled nitric oxide, systemic inflammation, and spirometric response to inhaled fluticasone propionate in severe chronic obstructive pulmonary disease: A prospective study. Ther Adv Respir Dis 2008;2:55-64

Kupczyk M, Haahtela T, Cruz AA, Kuna P. Reduction of asthma burden is possible through National Asthma Plan. Allergy 2010; 65:415-419

Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis 1993;147:697-704

Laitinen LA, Koskela K and the expert advisory group. Chronic bronchitis and chronic obstructive pulmonary disease: Finnish National Guidelines for Prevention and Treatment 1998-2007. Resp Med 1999;5:297-332

Laitinen T, Daly MJ, Rioux JD Kauppi P, Laprise C, Petäys T, Green T, Cargill M, Haahtela T, Lander ES, Laitinen LA, Hudson TJ, Kere J. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. Nat Genet 2001;28:87-91

Langley SJ, Goldthorpe S, Custovic A, Woodcock A. Relationship among pulmonary function, bronchial reactivity, and exhaled nitric oxide in a large group of asthmatic patients. Ann Allergy Asthma Immunol 2003;91:398-404

Laoudi Y, Nikasinovic L, Saharoui F, Grimfeld A, Momas I, Just J. Passive smoking is a major determinant of exhaled nitric oxide levels in allergic asthmatic children. Allergy 2010;65:491-497

Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: a three-year follow-up. Am J Respir Crit Care Med 1997;156:403-409

Latvala J, Karjalainen J, Remes J, Hassi J. Exercise-induced asthma and weather (abstract). XXXIII International Congress of Military Medicine. Helsinki, Finland, 25-30 June 2000

Lee JJ, Liu D, Lee JS, Kurie JM, Khuri FR, Ibarguen H, Morice RC, Walsh G, Ro JY, Broxson A, Hong WK, Hittelman WN. Long-term impact of smoking on lung epithelial proliferation in current and former smokers. J Natl Cancer Inst 2001;93:1081-1088

Lehtimäki L. Measurement of exhaled nitric oxide. Differentiation between alveolar and bronchial inflammation by using multiple exhalation flow rates. Dissertation. Tampere University 2003

Lehtimäki L, Kankaanranta H, Saarelainen S, Annila I, Nieminen R, Moilanen E. Bronchial nitric oxide is related to symptom relief during fluticasone treatment in COPD. Eur Respir J 2010;35:72-78

Leigh R, Pizzichini MMM, Morris MM, Maltais F, Hargreave FE, Pizzichini E. Stable COPD: predicting benefit from high-dose inhaled corticosteroid treatment. Eur Respir J 2006;27:964-971

Leuppi JD, Salome CM, Jenkins CR, Koskela H, Brannan JD, Anderson SD, Andersson M, Chan HK, Woolcock AJ. Markers of airway inflammation and airway hyperresponsiveness in patients with well-controlled asthma. Eur Respir J 2001;18:444-450

Lim S, Jatakanon A, John M, Gilbey T, O'Connor BJ, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation. Am J Respir Crit Care Med 1999;159:22-30

Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. Thorax 2000;55:184-188

Lim S, Tomita K, Carramorri G, Jatakanon A, Oliver B, Keller A, Adcock I, Chung KF, Barnes PJ. Low-dose theophylline reduces eosinophilic inflammation but not exhaled nitric oxide in mild asthma. Am J Respir Crit Care Med 2001;164:273-276

Liu J, Sandrini A, Thurston MC, Yates DH, Thomas PS. Nitric oxide and exhaled breath nitrite/nitrates in chronic obstructive pulmonary disease patients. Respiration 2007;74:617-623

Lúdvíksdóttir D, Janson C, Högman M, Hedenström H, Björnsson E, Boman G on behalf of the BHR-Study Group. Exhaled nitric oxide and its relationship to airway responsiveness and atopy in asthma. Resp Med 1999;93:552-556

Lúdvíksdóttir D, Janson C, Björnsson E, Stålenheim G, Boman G on behalf of the BHR Study Group, Hedenström H, Venge P, Gudbjörnsson B, Valtysdottir S. Different airway responsiveness profiles in atopic asthma, nonatopic asthma and Sjögren's syndrome. Allergy 2000;55:259-265

Lundbäck B, Nyström K, Rosen hall L, Stjernberg N. Obstructive lung disease in northern Sweden: respiratory symptoms assessed in a postal survey. Eur Respir J 1991;4:257-266

Lundbäck B, Stjernberg N, Nyström L, Lundbäck K, Lindström M, Rosenhall L. An interview study to estimate prevalence of asthma and chronic bronchitis. The obstructive lung disease in northern Sweden study. Eur J Epidemiol 1993;9:123-133

Lundbäck B, Lindberg A, Lindström M, Rönmark E, Jonsson AC, Jönsson E, Larsson LG, Andersson S, Sandström T, Larsson K. Not 15 but 50% of smokers develop COPD? – Report from the Obstructive Lung Disease in Northern Sweden Studies. Resp Med 2003;97:115-122

Lundberg JON, Weitzberg E, Lundberg JM, Alving K: Nitric oxide in exhaled air. Eur Respir J 1996;9:2671-2680

Malinovschi A, Janson C, Högman M, Rolla G, Torén K, Norbäck D, Olin A-C. Both allergic and nonallergic asthma are associated with increased FENO levels, but only in never-smokers. Allergy 2009;64:55-61

Malmberg LP, Turpeinen H, Rytilä P, Sarna S, Haahtela T. Determinants of increased exhaled nitric oxide in patients with suspected asthma. Allergy 2005;60:464-468

Malmberg LP, Pelkonen AS, Mattila PS, Hammarén-Malmi S, Mäkelä MJ. Exhaled nitric oxide and exercise-induced bronchoconstriction in young wheezy children – interactions with atopy. Pediatr Allergy Immunol 2009;20:673-678

Mannino DM, Gagnon RC, Petty TL, Lydick E. Obstructive lung disease and low lung function in adults in the United States: data from the National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 2000;160:1683-1689

Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007;370:765-773

Marsh SE, Travers J, Weatherall M, Williams MV, Aldington S, Shirtcliffe PM, Hansell AL, Nowitz MR, McNaughton AA, Soriano JB, Beasley RW. Proportional classifications of COPD phenotypes. Thorax 2008;63:761-767

Marteus H, Törnberg DC, Weitzberg E, Schedin U, Alving K. Origin of nitrite and nitrate in nasal and exhaled breath condensate and relation to nitric oxide formation. Thorax 2005;60:219-225

McFadden ER Jr, Gilbert IA. Current concepts: Exercise-induced asthma. N Engl J Med 1994;330:1362-1367

McSharry CP, McKay IC, Chaudhuri R, Livingston E, Fraser I, Thomson NC. Short and long-term effects of cigarette smoking independently influence exhaled nitric oxide concentration in asthma. J Allergy Clin Immunol 2005;116:88-93.

Meren M. Asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD) and respiratory symptoms among adults in Estonia: Prevalence and risk factors – comparison with Sweden and Finland. The "FinEsS" Studies – Estonia I. Dissertation, Karolinska Institutet, Stockholm 2005

Michils A, Baldassarre S, Van Muylem A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. Eur Respir J 2008;31:539-546

Michils A, Louis R, Peché R, Baldassarre S, Van Muylem A. Exhaled nitric oxide as a marker of asthma control in smoking patients. Eur Respir J 2009;33:1295-1301

Miles J, Cayton R, Ayres J. Atopic status in patients with brittle and non-brittle asthma: a case-control study. Clin Exp Allergy 1995;25:1074-1082

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. Eur Respir J 2005;26:319-338

Miller MR, Pedersen OF, Pellegrino R, Brusasco V. Debating the definition of airflow obstruction: time to move on? Eur Respir J 2009; 34: 527-528

Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human epithelial cells. Am J Respir Crit Care Med 1997;155:1770-1776

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev 1991;43:109-142

Motomura C, Odajima H, Tezuka J, Murakami Y, Moriyasu Y, Kando N, Taba N, Hayashi D, Okada K, Nishima S. Chest 2009;136:519-525

Murphy AW, Platts-Mills TAE, Lobo M, Hayden F. Respiratory nitric oxide levels in experimental human influenza. Chest 1998;114:452-456

Murray CJL, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997;349:1498-1504

Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 1991;3:65-70

O'Byrne PM, Pedersen S, Lamm CK, Tan WC, Busse WW on behalf of the START Investigators Group. Severe exacerbations and decline in lung function in asthma. Am J Respir Crit Care Med 2009;179:19-24

O'Shaugnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. An J Respir Crit Care Med 1997;155:852-857

O'Sullivan S, Roquet A, Dahlen B, Larsen F, Eklund A, Kumlin M, O'Byrne PM, Dahlen S-E. Evidence for mast cell activation during exercise-induced bronchoconstriction. Eur Respir J 1998;12:345-350

Oh MJ, Lee, JY, Lee BJ, Choi DC. Exhaled nitric oxide measurement is useful for the exclusion of nonasthmatic eosinophilic bronchitis in patients with chronic cough. Chest 2008;134:990-995

Olin AC, Alving K, Torén K. Exhaled nitric oxide: relation to sensitization and respiratory symptoms. Clin Exp Allergy 2004;34:221-226

Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest 2006;130:1319-25

Olin AC, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 ml/s: reference values for adult lifelong never-smokers. Chest 2007;131:1852-1856

Olin AC, Rosengren A, Thelle DS, Lissner L, Torén K. Increased fraction of exhaled nitric oxide predicts new-onset wheeze in a general population. Am J Respir Crit Care Med 2010;181:324-327

Olivieri M, Talamini G, Corradi M, Perbellini L, Mutti A, Tantucci C, Malerba M. Reference values for exhaled nitric oxide (reveno) study. Resp Res 2006;7:94

Pallasaho P, Lundbäck B, Läspä SL, Jönsson E, Kotaniemi J, Sovijärvi ARA, Laitinen LA. Increasing prevalence of asthma but not of chronic bronchitis in Finland? Report from the FinEsS-Helsinki study. Resp Med 1999;93:798-809

Pallasaho P, Lundbäck B, Meren M, Kiviloog J, Loit H-M, Larsson K, Laitinen LA. Prevalence and risk factors for asthma and chronic bronchitis in the capitals Helsinki, Stockholm and Tallinn. Resp Med 2002;96:759-769

Pallasaho P. Prevalence and determinants of respiratory symptoms, asthma, chronic bronchitis and allergic sensitization in Helsinki. A comparison between Finland, Sweden and Estonia. The FinEsS studies – Helsinki I. Dissertation, Helsinki University 2006a

Pallasaho P, Rönmark E, Haahtela T, Sovijärvi ARA, Lundbäck B. Degree and clinical relevance of sensitization to common allergens among adults: a population study in Helsinki, Finland. Clin Exp Allergy 2006b;36:503-509

Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A, Fabbri LM. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;162:1773-1777

Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. Am J Public Health 1994;84:1086-1093

Pauwels RA, Pedersen S, Busse WW, Tan WC, Chen YZ, Ohlsson SV, Ullman A, Lamm CJ, O'Byrne PM; START Investigators Group. Early intervention with budesonide in mild persistent asthma; a randomized, double-blind trial. Lancet 2003;361:1071-1076

Payne DNR, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. Am J Respir Crit Care Med 2001;164:1376-1381

Pedersen B, Dahl R, Karlström R, Peterson CG, Venge P. Eosinophil and neutrophils activity in asthma in a one-year trial with inhaled budesonide. The impact of smoking. Am J Respir Crit Care Med 1996;153:1519-1529

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CPM, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. Eur Respir J 2005;26:948-968

Pellegrino R, Brusasco V, Viegi G, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CPM, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Definition of COPD: based on evidence or opinion? Eur Respir J 2008;31:681-682

Pérez de Llano A, González FC, Añón OC, Perea MP, Gómez RG, Villar AB, Caruncho MV, Boquete Paris M. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. Eur Respir J 2009; doi: 10.1183/09031936.00118809

Persson MG, Zetterström O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurement in asthmatic patients and smokers. Lancet 1994;343:146-147.

Petsky HL, Cates CJ, Li A, Kynaston JA, Turner C, Chang AB. Tailored interventions based on exhaled nitric oxide versus clinical symptoms for asthma in children and adults. Cochrane Database of Systematic Reviews 2009, Issue 4. Art. No.: CD006340.DOI: 10.1002/14651858.CD006340.pub3

Piacentini G, Bodini A, Costella S, Vicentini L, Mazzi A, Sperandio S, Boner A. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. Eur Respir J 1999;13:1386-1390

Piccillo G, Caponnetto P, Barton S, Russo C, Origlio A, Bonaccorsi A, Di Maria A, Olivieri C, Polosa R. Changes in airway hyperresponsiveness following smoking cessation. Resp Med 2008;102:256-265

Pietinalho A, Pelkonen A, Rytilä P. Linkage between smoking and asthma. Allergy 2009; 64:1722-1727

Polosa R, Ciamarra I, ManganoG, Prosperini G, Pistorio MP, Vancheri C, Crimi N. Bronchial hyperresponsiveness and airway inflammation markers in nonasthmatics with allergic rhinitis. Eur Respir J 2000;15:30-35

Porsbjerg C, Brannan JD, Anderson SD, Backer V. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. Clin Exp Allergy 2007;38:43-50

Porsbjerg C, Lund TK, Pedersen L, Backer V. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. J Asthma 2009;46:606-612

Prieto L, Gutiérrez V, Uixera S, Bruno L. Concentrations of exhaled nitric oxide in asthmatics and subjects with allergic rhinitis sensitized to the same pollen allergen. Clin Exp Allergy 2002a;32:1728-1733

Prieto L, Uixera S, Gutiérrez V, Bruno L. Modifications of airway responsiveness to adenosine 5'-monophosphate and exhaled nitric oxide concentrations after the pollen season in subjects with pollen induced rhinitis. Chest 2002b;122:940-947

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Official statement of the European Respiratory Society. Eur Respir J 1993;6 (Suppl. 16):5-40

Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. GOLD Executive Summary. Am J Respir Crit Care Med 2007;176:532-555

Rackemann FM. A working classification of asthma. Am J Med 1947;33:601-606

Rennard SI, Vestbo J. COPD: the dangerous underestimate of 15%. Lancet 2006:367:1216-1219

Robbins RA, Millatmal T, Lassi K, Rennard S, Daughton D. Smoking cessation is associated with an increase in exhaled nitric oxide. Chest 1997;112:313-318.

Romanet-Manet S, Charpin D, Magnan A, Lanteaume A, Vervloet D, and the EGEA Cooperative Group. Allergic vs nonallergic asthma: what makes the difference? Allergy 2002;57:607-613

Rutgers SR, van der Mark TW, Coers W, Moshage H, Timens W, Kauffman HF, Koeter GH, Postma DS. Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease. Thorax 1999;54:576-580

Rutgers SR, Postma DS, ten Hacken NH, Kauffman HF, van Der Mark TW, Koëter GH, Timens W. Ongoing airway inflammation in patients with COPD who do not currently smoke. Thorax 2000;55:12-18

Rytilä P, Metso T, Heikkinen K, Saarelainen P, Helenius IJ, Haahtela T. Airway inflammation in patients with symptoms suggesting asthma but with normal lung function. Eur Respir J 2000;16:824-830

Rytilä P, Rehn T, Ilumets H, Rouhos A, Sovijärvi A, Myllärniemi M, Kinnula VL. Increased oxidative stress in asymptomatic current chronic smokers and GOLD stage 0 COPD. Respir Res 2006;7:69

Saetta M, Di Stefano A, Maestrelli P, Turato G, Ruggieri MP, Roggeri A, Calcagni P, Mapp CE, Ciaccia A, Fabbri LM. Airway eosinophilia in chronic bronchitis during exacerbations. Am J Resp Crit Care Med 1994;150:1646-1652

Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, Maestrelli P, Ciaccia A, Fabbri LM. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. Am J Resp Crit Care Med 1998;157:822-826

Salome CM, Roberts AM, Brown NJ, Dermand J, Marks GB, Woolcock A. Exhaled nitric oxide measurements in a population sample of young adults. Am J Respir Crit Care Med 1999;159:911-916

Sandrini A, Ferreira IM, Gutierrez C, Jardim JR, Zamal N, Chapman KR. Effect of montelukast on exhaled nitric oxide and nonvolatile markers of inflammation in mild asthma. Chest 2003;124:1334-1340

Sato S, Saito J, Sato Y, Ishii T, Xintao W, Tanino Y, Ishida T, Munakata M. Clinical usefulness of fractional exhaled nitric oxide for diagnosing prolonged cough. Resp Med 2008;102:1452-1459

Scanlon PD, Connett JE, Waller LA, Altose MD, Bailey WC, Buist AS, Tashkin DP for the Lung Health Study Research Group. Smoking cessation and lung function in mild-to moderate chronic obstructive disease. Am J Respir Crit Care Med 2000;161:381-390

Scollo M, Zanconato S, Ongaro R, Zaramella C, Zacchello F, Baraldi E. Exhaled nitric oxide and exercise induced bronchoconstriction in asthmatic children. Am J Respir Crit Care Med 2000;161:1047-1050

Settipane RJ, Hagy GW, Settipane GA. Long-term risk factors for developing asthma and allergic rhinitis: a 23-year follow-up of study of college students. Allergy Proc 1994;15:21-25

Shafey O, Dolwick S, Guindon GE. The Tobacco Control Country Profiles (2nd edition). American Cancer Society, Inc., World Health Organization, and International Union Against Cancer 2003

Shaw DE, Berry MA, Green RH, Brightling CE, Wardlaw AJ, Pavrod ID. The use of exhaled nitric oxide to guide asthma management. A randomized controlled trial. Am J Respir Crit Care Med 2007;176:231-237

Silkoff PE, Wakita S, Chatkin J, Ansarin K, Gutierrez C, Caramori M, McClean P, Slutsky S, Zamal N, Chapman KR. Exhaled nitric oxide after b2-agonist inhalation and spirometry in asthma. Am J Respir Crit Care Med 1999;159:940-44

Silkoff PE, Romero FA, Gupta N, Townley RG, Milgrom H. Exhaled nitric oxide in children with asthma receiving Xolair (omalizumab), a monoclonal anti-immunoglobulin E antibody. Pediatrics 2004;113:e308-312

Simpson A, Custovic A, Pipis S, Adisesh A, Faragher B, Woodcock A. Exhaled nitric oxide, sensitization and exposure to allergens in patients with asthma who are not taking inhaled steroids. Am J Respir Crit Care Med 1999;160: 45-49

Siva R, Green RH, Brightling CE, Shelley M, Hargadon B, McKenna S, Monteiro W, Berry M, Parker D, Wardlaw AJ, Pavrod ID. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J 2007;29:906-913

Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, Taylor R. Diagnosing asthma. Comparisons between exhaled nitric oxide measurements and conventional tests. Am J Respir Crit Care Med 2004;169:473-478

Smith AD, Cowan JO, Brasset KP, Herbison GP, Taylor RD. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. NEJM 2005;352:2163-2173

Smith AD, Cowan JO, Taylor DR. Exhaled nitric oxide levels in asthma: Personal best versus reference value. J Allerg Clin Immunol 2009;124:714-718

Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ and the AMPUL Study Group. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long term treatment. Am J Respir Crit Care Med 1999;159:1043-1051

Sovijärvi A, Malmberg LP, Reinikainen K, Rytilä P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge. Chest 1993;104:164-170

Sovijärvi ARA, Haahtela T, Ekroos HJ, Lindqvist A, Saarinen A, Poussa T, Laitinen LA. Sustained reduction in bronchial hyperresponsiveness with inhaled fluticasone propionate within three days in mild asthma: time course after onset and cessation of treatment. Thorax 2003;58:500-504

Sterk PJ. Bronchial hyperresponsiveness today. Respir Med 1993a; Suppl B:27-29

Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, Malo JL. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party standardization of lung function tests, European Community for Steel and Coal. Official statement of the European Respiratory Society. Eur Respir J suppl 1993b;16:53-83

St-Laurent J, Bergeron C, Page N, Couture C, Laviolette M, Boulet L-P. Influence of smoking on airway inflammation and remodeling in asthma. Clin Exp Allergy 2008; 38:1582-1589

Sundy JS, Hauswirth DW, Mervin-Blake S, Fernandez CA, Patch KB, Alexander KM, Allgood S, McNair PD, Levesque MC. Smoking is associated with an age-related decline in exhaled nitric oxide. Eur Respir J 2007;30:1074-1081

Svanes C, Omenaas E, Jarvis D, Chinn S, Gulsvik A, Burney P. Parental smoking in childhood and adult obstructive lung disease: results from the European Community Respiratory Health Survey. Thorax 2004;59:295-302

Szefler SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, Kattan M, Pongracic JA, Teach SJ, Bloomberg GR, Eggleston PA, Gruchalla RS, Kercsmar CM, Liu AH, Wildfire JJ, Curry MD, Busse WW. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults. a randomised controlled trial. Lancet 2008;372:1065-1072

Söderström L, Kobar A, Ahlstedt S, de Groot H, Lange CE, Paganelli R, Roovers MHWM, Sastre J. A further evaluation of the clinical use of specific IgE antibody testing in allergic diseases. Allergy 2003;58:921-928

Takalo R, Piirilä P, Sovijärvi ARA. Repeatability of successive measurements with a portable nitric oxide analyser in patients with suggested or diagnosed asthma. Scand J Clin Lab Invest 2008;68:830-832

Tateishi K, Motojima S, Kushima A, Fukuda T, Makino S. Comparison between allergen-induced and exercise-induced asthma with respect to the late asthmatic response, airway responsiveness, and Creola bodies in sputum. Ann Allergy Asthma Immunol 1996;77:229-237

Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. Thorax 2006;61:817-827

Taylor DR, Mandhane P, Greene JM, Hancox RJ, Filsell S, McLachlan CR, Williamson AJ, Cowan JO, Smith AD, Sears MR. Factors affecting exhaled nitric oxide measurements: the effect of sex. Respir Res 2007;8:82

Taylor ES, Smith AD, Cowan JO, Herbison GP, Taylor DR. Effect of caffeine ingestion on exhaled nitric oxide measurements in patients with asthma. Am J Respir Crit Care Med 2004;169:1019-1021

Thomson NC, Chaudhuri R, Livingston E. Asthma and cigarette smoking. Eur Respir J 2004;24:822-833

Thomson NC, Spears M. The influence of smoking on the treatment response in patients with asthma. Curr Opin Allergy Clin Immunol 2005;5:57-63

Torén K, Brisman J, Järvholm B. Asthma and asthma-like symptoms in adults assessed by questionnaires. A literature review. Chest 1993;104:600-608

Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A, Weatherall M, Beasley R. Reference ranges for exhaled nitric oxide derived from random community survey of adults. Am J Respir Crit Care Med 2007;176:238-242

Tsang KW, Ip SK, Leung R, Tipoe GL, Chan SL, Shum IH, Ip MS, Yan C, Fung PC, Chan-Yeung M, Lam W. Exhaled nitric oxide: the effects of age, gender and body size. Lung 2001;179:83-91

US Public Health Service. The health consequences of smoking. Chronic obstructive lung disease. A report of the Surgeon General. Rockville, MD: US Department of Health and Human Services, Public Health Service, Office of Smoking and Health, 1984:1–512.

van den Berge M, Meijer RJ, Kerstjens AHM, de Reus DM, Koëter GH, Kauffman HF, Postma DS. PC20 adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC20 methacholine. Am J Respir Crit Care Med 2001;163:1546-1550

van den Nieuwenhof L, Schermer T, Heijdra Y, Bottema B, Akkermans R, Folgering H, van Weel C. Are asymptomatic airway hyperresponsiveness and allergy risk factors for asthma? A longitudinal study. Eur Respir J 2008;32:70-76

van den Toorn LM, Prins J-B, Overbeek SE, Hoogsteden HC, de Jongste JC. Adolescents in clinical remission of atopic asthma have elevated exhaled NO levels and bronchial hyperresponsiveness. Am J Respir Crit Care Med 2000;162:953-957

van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present in clinical remission of atopic asthma. Am J Respir Crit Care Med 2001;164:2107-2113

van Rensen EL, Straathof KC, Veselie-Charvat MA: Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 1999;54:403-408

van Schoor J, Joos GF, Pauwels RA. Indirect bronchial responsiveness in asthma: mechanisms, pharmacology and implications for clinical research. Eur Respir J 2000;16:514-533

van Schoor J, Pauwels R, Joos G. Indirect bronchial hyper-responsiveness: the coming of age of a specific group of bronchial challenges. Clin Exp Allergy 2005;35:250-261

Verleden GM, Dupont LJ, Verpeut AC, Demedts MG. The effect of cigarette smoking on exhaled nitric oxide in mild steroid-naive asthmatics. Chest 1999;116:59-64.

Viljanen AA, Halttunen PK, Kreus K-E, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest Suppl. 1982;159:5-20

Vollmer WM, Gislason P, Burney P, Enright PL, Gulsvik A, Kocabas A and Buist AS for the BOLD Collaborative Research Group. Comparison of spirometry criteria for the diagnosis of COPD: results from the BOLD study. Eur Respir J 2009;34:588-597

Ward C, Pais M, Bish R, Reid D, Feltis B, Johns W, Walters EH. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax 2002;57:309-316

Warke T, Fitch P, Brown V: Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. Thorax 2002;57:383-387

Wei XM, Kim HS, Kumar RK, Heywood GJ, Hunt JE, McNeil HP, Thomas PS. Effects of cigarette smoke on degranulation and NO production by mast cells and epithelial cells. Respir Res 2005;6:108

Wenzel SE. Asthma: defining of the persistent adult phenotypes. Lancet 2006;368:804-813

WHO The global burden of disease: 2004 update. World Health Organization, 2008a

WHO Report on the Global Tobacco Epidemic, 2008: The MPOWER package. Geneva, World Health Organization, 2008b

Willemse BWM, Postma DS, Timens W, ten Hacken NHT. The impact of smoking cessation on respiratory symptoms, lung function, airway hyperresponsiveness and inflammation. Eur Respir J 2004a;23:464-476

Willemse BWM, ten Hacken NHT, Rutgers B, Lesman-Leegte IGAT, Timens W, Postma DS. Smoking cessation improves both direct and indirect airway hyperresponsiveness in COPD. Eur Respir J 2004b;24:391-396

Wise RA, Kanner RE, Lindgren P, Connett JE, Altose MD, Enright PL, Tashkin P, for the Lung Health Study Group. The effect of smoking intervention and an inhaled bronchodilator on airways reactivity in COPD. Chest 2003;124:449-458

Yang IA, Fong KM, Sim EH, Black PN, Lasserson TJ. Inhaled corticosteroids for stable chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2007;(2):CD002991

Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. The effect of alcohol ingestion on exhaled nitric oxide. Eur Respir J 1996;9:1130-1133

Yates DH, Kharitonov SA, Barnes PJ. Effect of short- and long-acting inhaled β2-agonists on exhaled nitric oxide in asthmatic patients. Eur Respir J 1997;10:1483-1488

Yates DH. Role of exhaled nitric oxide in asthma. Immunol Cell Biol 2001a;79:178-190

Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. Am J Respir Crit Care Med 2001b;164:1043-46

Yoshikawa T, Shoji S, Fujii T, Kanazawa H, Kudoh S, Hirata K, Yoshikawa J. Severity of exercise-induced bronchoconstriction is related to airway eosinophilic inflammation in patients with asthma. Eur Respir J 1998;12:879-884

Zetterquist W, Pedroletti C, Lundberg JON, Alving K. Salivary contribution to exhaled nitric oxide. Eur Respir J 1999;13:327-333

Zhang X, Moilanen E, Lahti A, Hämäläinen M, Giembycz MA, Barnes PJ, Lindsay MA, Kankaanranta H. Regulation of eosinophil apoptosis by nitric oxide: role of c-Jun-N-terminal kinase and signal transducer and activation of transcription 5. J Allergy Clin Immunol 2003;112:93-101

Zietkowski Z, Kucharewicz I, Bodzenta-Lukaszyk A. The influence of inhaled corticosteroids on exhaled nitric oxide in stable chronic obstructive disease. Resp Med 2005;99:816-824

Zietkowski Z, Bodzenta-Lukaszyk A, Tomasiak MM, Skiepko R, Szmitkowski M. Comparison of exhaled nitric oxide measurement with conventional tests in steroid-naive asthma patients. J Investig Allergol Clin Immunol 2006;16:239-246

Ziora D, Dworniczak S, Kaczmarczyk G, Jastrzebski D, Krzywiecki A, Kozielski J. Correlation of exhaled nitric oxide with nitrogen oxides and selected cytokines in induced sputum of chronic obstructive pulmonary disease patients. J Physiol Pharmacol 2007;58 (Suppl 5):791-799