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Development of allergic and respiratory symptoms in adolescence and early adulthood

Risk factors and gender differences

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Abstract

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Background: Asthma and allergic diseases have increased in prevalence for several decades and affect a substantial number of individuals in everyday life, as well as their families and public healthcare resources. Subjects with asthma report impaired self-rated health. Fractional exhaled nitric oxide (FeNO) is a marker of type 2 inflammation in the airways and higher levels may precede the development of allergic and respiratory disease.

Aims: To investigate the development of allergic and respiratory symptoms in adolescence and early adulthood, and related baseline risk factors. Further, to study self-rated health in young adults with reported asthma.

Methods: A total of 959 schoolchildren completed a standardized respiratory questionnaire and underwent lung function and FeNO measurements at baseline (12–15 years; early adolescence). Four (late adolescence) and sixteen (early adulthood) years later, 921 (96%) and 502 (52%) of these individuals completed a similar questionnaire. A total of 491 subjects participated in all three examinations. Nineteen clinically assessed non-asthmatic subjects with elevated FeNO and 28 control subjects with low FeNO and without symptoms of asthma or allergy in early adolescence were identified. Their FeNO, IgE sensitization, airway responsiveness, and inflammatory markers in blood and sputum were measured.

Results: The main finding was that higher FeNO in early adolescence was associated with an increased risk of developing allergic symptoms to cat and dog, but not pollen allergens, during adolescence. Gender-stratified data showed that obesity at baseline in girls and an atopic constitution in boys were associated with increased risk of developing wheeze during adolescence. The prevalence of asthma and wheeze had increased in early adulthood, but the increase was significant only in females. Reduced lung function at baseline in females and higher FeNO in males were associated with an increased risk of incident asthma sixteen years later. The increase in allergic symptoms during this period was significant but without sex differences. Asthmatic females rated their health worse than non-asthmatic females, a difference not observed in males. Non-asthmatic adolescents with higher FeNO at baseline were to a higher extent sensitized, had more reactive airways, higher blood eosinophil counts, and lower systemic activation of neutrophils, compared with controls.

Conclusions: It is important to detect risk factors for the development of allergic and respiratory diseases at an early stage to optimize health and wellbeing. Gender differences in respiratory development, associated risk factors, and treatment of respiratory symptoms must be taken into account.

Keywords: Adolescents, allergy, asthma, breath test, epidemiology, gender, health status indicator, incidence, nitric oxide, prevalence, wheeze.

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To Mike, Alexander and Hanna

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Kalm-Stephens, P., Nordvall, L., Janson, C., Neuman Å., Malinovschi, A., Alving, K. (2019) Elevated exhaled nitric oxide in adolescents is associated with incident allergic symptoms: a prospective cohort study. *Journal of Investigational Allergology and Clinical Immunology,* 29(3):231-238.

II Kalm-Stephens, P., Malinovschi, A., Janson, C., Venge, P., Nordvall, L., Alving, K. (2020) Concurrence of elevated FeNO and airway hyperresponsiveness in nonasthmatic adolescents. *Pediatric Pulmonology,* 55(3):571-579.

III Kalm-Stephens, P., Nordvall, L., Janson, C., Neuman, Å., Malinovschi, A., Alving, K. (2020) Different baseline characteristics are associated with incident wheeze in female and male adolescents. *Acta Paediatrica,* 109:2324-2331.

IV Kalm-Stephens, P., Nordvall, L., Engvall, G., Janson, C., Malinovschi, A., Alving, K. Incidence of asthma between adolescence and adulthood: early risk factors and gender differences. *Submitted.*

Contents

Abbreviations

ACT: asthma control test

AHR: airway hyperresponsiveness

BMI: body mass index

CI: confidence interval

ECP: eosinophilic cationic protein

FEV_1: forced expiratory volume in one second

FeNO: fraction of exhaled nitric oxide

$FeNO_{0.1}$: fraction of exhaled nitric oxide measured at 100 mL/s

HRQoL: health-related quality of life

HNL: human neutrophil lipocalin

IFN: interferon

IgE: immunoglobulin E

IL: interleukin

ISAAC: international study of asthma and allergies in childhood

LLN: lower limit of normal

MCT: methacholine challenge test

MPO: myeloperoxidase

NO: nitric oxide

ppb: parts per billion

PD_{20}: provocative dose causing a fall in FEV_1 by 20%

SPAIS: screening project asthma in schools

SPT: skin prick test

SRH: self-rated health

Th1: T helper cell type 1

Th2: T helper cell type 2

Introduction

Allergy, rhinitis and asthma

Hypersensitivity

According to the Nomenclature Review Committee of the World Allergy Organization, the term hypersensitivity should be used to describe objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by healthy people. Furthermore, allergy is a hypersensitivity reaction initiated by specific immunological mechanisms. When other mechanisms are proven, the term non-allergic hypersensitivity should be used (1).

Allergic sensitization

The sensitization process is complex, but can be described as an inappropriate response by the immune system, which produces IgE antibodies against common environmental substances, usually proteins, normally tolerated by healthy individuals.

The sensitization process starts when an allergen, entering through the skin or the mucosal membrane, is taken up by antigen-presenting cells. It is then transported to the lymph nodes, where naïve T cells are activated and stimulated to develop into T helper type 2 (Th2) cells (2). Th2 cells produce the cytokines interleukin (IL)-4 and IL-13, and induce naïve B cells to become plasma cells, activated to produce allergen-specific IgE antibodies.

The IgE antibodies bind to receptors on mast cells and basophils. When the sensitized subject is re-exposed to the specific allergen, cross-linking occurs between the allergen and two IgE antibodies, causing cell degranulation with the release of preformed inflammatory mediators such as histamine (3). Th2 cells also produce IL-5, which activates eosinophilic granulocytes. Altogether, this is referred to as type 2 inflammation, previously known as allergic inflammation.

T helper type 1 (Th1) cells also develop from naïve T cells and produce the cytokines IL-2, interferon-gamma (IFN-γ), and tumor necrosis factor-β in response to intracellular bacteria and viruses (4). Neutrophils are activated in Th1 inflammation, also known as non-type 2 inflammation.

The most commonly used methods to verify sensitization are skin-prick tests (SPT) and measurements of specific IgE antibodies in serum.

Disease prevalence

Atopic diseases are among the most common chronic diseases in children and adolescents, and their prevalence has been observed to increase in the last several decades (5, 6).

The prevalence of allergic sensitization in children has increased over time and also increases with age. In a Swedish longitudinal cohort study, children were investigated at 7–8 years and again four years later (7). The prevalence of positive SPT against inhaled allergens had increased from 20.6% to 30.4% in four years. A similar study was performed 10 years later, studying the same ages (8). At this time, the prevalence had increased from 30% to 41% at follow-up and a family history of allergy was concluded to be the main risk factor for incident sensitization. A similar degree of sensitization, 43%, was seen in a recent cross-sectional study with adolescents, aged 13–14 years (9). At greater ages (21–86 years), sensitization decreases (10).

The International Study of Asthma and Allergies in Childhood (ISAAC) was established in 1991, performed in three investigation phases, and was formally closed in 2012 (11). The purpose of the study was to assess the worldwide prevalence of asthma, allergic rhinitis and eczema in children from the general population, at ages six to seven years and 13 to 14 years, and to identify possible risk factors that could influence these three disorders using a standardized questionnaire. The questionnaires were designed to be completed by parents when the children were at lower ages and self-reported by the adolescents themselves at 13 to 14 years of age (12, 13).

According to ISAAC, the prevalence of asthma symptoms in Sweden is around 10%, in both children aged six to seven years and those aged 13 to 14 years. The corresponding prevalence of allergic rhinitis is about 7% and 10%, and for eczema 22% and 13%, at the same ages, respectively (12). The calculated worldwide prevalence of asthma in children is 12.0%, that of allergic rhinitis is 12.7% and that of eczema is 7.9% (11).

There seems to be a close relationship between these three disorders. For example, for children with asthma, the risk of having all three diseases is over five times higher than for children without asthma (11). At 12 years of age, 58% of the children in the BAMSE (children, allergy, milieu, Stockholm, epidemiology) cohort, had had eczema, asthma and/or rhinitis at some point in time (14). Comorbidity increased with age, and at 12 years, 7.5% of the children were affected by at least two allergy-related diseases – more commonly in children with parental allergy. Co-morbidity is present at adult ages as well. Rhinitis is associated with adult-onset asthma (15) and in a group of asthmatics, mean age 40 years, over 85% had co-existing allergic rhinitis (16).

The global epidemic of asthma and allergies has created a substantial health burden for affected individuals in everyday life, for their families, and for public healthcare resources (17-19).

Disease etiology and development

The etiology of allergic disease is multifactorial and complex. A family history of allergic disease as well as specific timing of environmental exposures to certain stimuli is necessary for allergic diseases to develop (20-22).

The development usually follows a pattern, the *atopic march,* and allergen exposure through inflamed skin is thought to be the primary route by which individuals become initiated. The atopic march involves atopic dermatitis and concomitant sensitization to food as well as aeroallergens in early childhood, progressing to asthma and allergic rhinitis in later childhood or adult life (23, 24).

Rhinitis

Rhinitis is a heterogeneous condition, related to allergic or non-allergic factors, and is characterized by typical symptoms of nasal itching, sneezing, runny nose, and congestion (25). Allergic rhinitis is an IgE-mediated inflammation of the nasal mucous membrane and affects approximately one in four of the population in industrialized countries. Seasonal allergic rhinitis is frequently accompanied by conjunctivitis. The prevalence of rhinitis has not been found to differ between sexes (26).

Asthma

Asthma is by definition an inflammatory disorder and is the most common chronic childhood disease in nearly all industrialized countries (27). Asthma is characterized by a history of obstructive respiratory symptoms that alter over time and in intensity, and by variable expiratory airflow limitation. The airflow obstruction is usually caused by inflammation in the airways (28).

Asthma symptoms are trigged by various stimuli such as allergens, infections, cold air, or exercise. Airway hyperresponsiveness (AHR) is a key feature of asthma and may be used to support a diagnosis of asthma and as an indicator of asthma severity (29).

There are different phenotypes of asthma, such as allergic and non-allergic asthma, which are associated with different risk factors (30). The incidence of allergic asthma is highest in childhood and steadily decreases with advancing age, while the incidence of non-allergic asthma is low in youth and peaks in late adulthood (31).

Clinical measurements in asthma

Biomarkers in asthma

In this thesis, the main focus has been on the biomarker fraction of exhaled nitric oxide (FeNO), which was the original target of the screening project asthma in schools (SPAIS) study and has been described as a marker of type 2 inflammation in the airways. Other biomarkers that are evaluated in this thesis and reflect type 2 inflammation are eosinophil counts in blood and eosinophilic cationic protein measured in serum and sputum. Neutrophil counts in blood, human neutrophil lipocalin measured in both serum and sputum, and myeloperoxidase, measured in serum only, are markers representing non-type 2 inflammation.

Exhaled nitric oxide

Measurement of FeNO is a non-invasive method to monitor airway inflammation (32). In the early 1990s, FeNO was found to be elevated in asthma (33). A majority of the NO measured in exhaled air is produced in the airway epithelium.

Height is the best determinant of FeNO in healthy schoolchildren (34). There are sex differences in FeNO, but these are, to a large extent, explained by the relation to height and the area of the airway epithelium (35). Current cigarette smoking is associated with a 40–60% decrease of FeNO (36).

Several studies have reported correlations between FeNO and the degree of AHR (37, 38), as well as levels of eosinophils in blood and sputum (39, 40). Allergic sensitization relates to increased FeNO in both population-based studies and asthma patients (37, 41-43). FeNO is also a useful method to evaluate steroid-sensitive inflammation of the airways (44). Results from a recent study provided strong support for the use of FeNO measurements to guide decisions on prescribing inhaled corticosteroids to patients without a confirmed asthma diagnosis (45). Further, FeNO is a predictor of accelerated decline in lung function in patients with difficult-to-treat asthma (46).

Increased FeNO has been reported in subjects, both children and adults, without acknowledged respiratory symptoms. It is related to an increased risk of development of asthma, wheeze, and rhinitis and may indicate subclinical inflammation or “early asthma” (47-51).

FeNO is today the most commonly used biomarker in clinical practice. It is considered to be more representative of type 2 inflammation than of general eosinophilic inflammation, and specifically related to the expression of interleukin (IL)-4 and IL-13 in the airway epithelium (52).

Pulmonary function

Spirometry is the most common pulmonary function test. The method is widely used in the assessment of lung function and provides objective information used for diagnosis and monitoring of lung diseases (53). In spirometry, there are several measures available, but in this thesis, only the forced exhaled volume during the first second (FEV_1) has been used. Reference values are used to evaluate the lung function measurements and height, weight, sex, age, and ethnicity are taken into account to adjust for the between-subject variability (54). Therefore, FEV_1 is usually expressed as percent predicted.

Bronchial challenge testing with methacholine is an established method for assessing AHR, which is defined as an increased sensitivity to non-allergenic stimuli that cause airway narrowing (55). Inhaled methacholine causes bronchoconstriction and the patient may experience the test situation as unpleasant. It can also entail environmental problems for the nurse or technician performing the provocation test (56).

Asthma control test

The goal for all asthma healthcare is that the patients achieve full asthma control, which means that they experience no symptoms of asthma and no restrictions in everyday life. The asthma control test (ACT) is a validated questionnaire with five questions for adults and children over 12 years (57) and seven questions for children 4–11 years (58). The test is used clinically to score a patient's level of asthma control.

Health-related quality of life

There are many different validated health-related quality of life (HRQoL) questionnaires available and they cover different aspects and dimension of a subject's perception of health (59). There are both generic and disease-specific instruments. The generic ones have the advantage of enabling comparison between subjects with different diseases and with the general population, while the disease-specific instruments contribute with important data, valuable for clinical practice and treatment evaluation.

Self-rated health

Self-rated health (SRH) is a validated general health status indicator, preferably used in middle-aged populations, in cohort studies and in population health monitoring (60). SRH refers to how a subject responds to the question "How do you rate your general health status?" and there are five response alternatives: "very good," "quite good," "neither good nor poor," "quite poor," and

"very poor". The definitions of HRQoL and SRH are similar, covering a person's integrated perception of health, including biological, psychological, and social dimensions (60).

Factors influencing asthma development

Adolescence

Adolescence is a critical period when a young person transitions from childhood to adulthood. At this stage, the individual becomes biologically, psychologically, and socially mature, and potentially able to live independently (61). Adolescents meet age-specific challenges, for example, expectations from school, separation from parents, establishment of new relationships, and dealing with puberty.

Beginning in the teenage years, adolescents become less reliant on their parents for support. This is a normal part of the developmental process and often means that adolescents, to varying degrees, have more confidence in their friends than in their parents or other adults (62). Risk behaviors such as tobacco and alcohol use are as common among adolescents with a chronic disease as among their healthy peers (63).

Adolescents with asthma experience both physical and psychological changes that affect their health and well-being (64). They are at an increased risk of morbidity, explained by the adolescents' non-adherence to their medical regimens, poor asthma control, and poor treatment outcomes. Further, asthma seems to impair school performance in adolescence (65).

Obesity

Obesity in children and adolescents is today a priority health concern globally, and is clearly associated with both obesity in adulthood and a large health burden (66). The occurrence of wheeze and asthma increases slightly with body mass index (BMI) in ages 2–18 years (67). Overweight and obesity have been suggested to affect asthma in several ways, for example through effects on lung function and through the release of inflammatory mediators from adipose tissue (68, 69). Forno et al. (70) have suggested that obesity is primarily related to anatomical and developmental aberrations, leading to airway obstruction that may not be related to airway inflammation or bronchospasm. This view is strengthened by results from another study, which found no association between BMI and FeNO (71).

Obesity and asthma also share common consequences that complicate and strengthen the interactions between them. Both asthma and obesity are related to gastro-esophageal reflux, impaired physical activity, and sleep disturbances (72). Vigorous physical activity and watching television five or more hours

per day have both been associated with an increased risk of symptoms of asthma in adolescents (73).

Gender differences

A number of studies have shown gender differences in the prevalence of wheeze and asthma, and a relationship to age, with males being more affected in childhood, and females more affected in adolescence and adulthood (74-77).

Male sex is strongly associated with IgE sensitization to airborne allergens from early childhood up to young adulthood (78). Further, a greater influence of allergic heredity on the development of childhood wheezing and allergic sensitization was confirmed in boys, compared with girls, in a large prospective birth cohort study (79). The greater susceptibility of females to asthma, mainly non-allergic, may be explained by their narrower airway caliber (80). Another explanation is that female sexual hormones play a role. This hypothesis is strengthened by the fact that asthma and respiratory symptoms start to increase during puberty in young females and that these symptoms vary during the menstrual cycle (80, 81).

Smoking

Cigarette smoke is a well-known harmful exposure for the lung tissue. An individual can be exposed by own or second-hand smoking. Worldwide, 40% of children, 33% of male non-smokers, and 35% of female non-smokers were exposed to second-hand smoke in 2004 (82). Maternal smoking, although only during pregnancy, appears to be associated with an increased risk of wheeze and asthma in offspring at pre-school ages (83).

Smoking in asthma has been associated with poorer disease control, impaired response to corticosteroid therapy, accelerated decline in lung function, and increased healthcare utilization (84).

Data from the population-based BAMSE cohort, at the 16-year follow-up, suggested that smoking habits did not differ between adolescents with asthma and those without asthma (85). Further, subjects with adolescent-onset asthma tended to be active smokers more often than those with persistent asthma, and more were females than males. Smoking has also been shown to be more related to chronic nasal symptoms than to allergic rhinitis (86).

Epidemiology of asthma

Epidemiological studies are observational, and assess the relationship between factors of interest and disease in a population (87). A cohort study has been

considered as the best way to identify the incidence and natural history of a disease, and can be used to examine multiple outcomes and exposures (88).

The SPAIS study

In 1997, when designing the **S**creening **P**roject **A**sthma **I**n **S**chools (SPAIS, described on page 21) study, FeNO measurement was a new method still undergoing methodological development. The aims of the SPAIS study were to investigate FeNO in schoolchildren as part of the general population, to evaluate FeNO as a screening method for asthma and allergy, and to compare FeNO and lung function measurements in relation to questionnaire-assessed symptoms of asthma and allergy. Results from the baseline study showed that there was a strong relationship between FeNO and self-reported allergic and asthma symptoms, but no association between symptoms of asthma and FEV_1 (49).

In a clinical reinvestigation within two months of the screening at school, allergen exposure was found to be the major determinant of elevated FeNO in IgE-sensitized children, while respiratory infections and home window pane condensation were the major determinants in non-sensitized children (89). Furthermore, the risk of both allergic and non-allergic asthma increased with increasing BMI, and associations between both early-life and current environmental exposures were primarily found in non-allergic asthma (30).

A follow-up study four years later showed that increased FeNO at baseline predicted new-onset rhinitis and persistent rhinitis in adolescents without allergic symptoms at baseline (48).

There are some other ongoing pediatric longitudinal cohort studies in Sweden (90-93). However, to the author's knowledge, none of these studies have looked at early objective risk factors in a long-term perspective.

Objectives

Overall objective

This PhD research project was designed to investigate the development of allergic and respiratory symptoms in adolescence and early adulthood, and risk factors for such development, using data generated from the SPAIS study.

Specific objectives

Study I

To evaluate elevated FeNO as a risk factor for incident allergic symptoms to cat, dog, or pollen within a four-year timeframe, in adolescents from the general population.

Study II

To examine the relationship between elevated FeNO, and airway hyperresponsiveness, IgE sensitization, and other markers of inflammation in a group of adolescents without a clinical diagnosis of asthma. Further, to examine the long-term effects of elevated FeNO on self-reported respiratory and allergic symptoms, in this non-symptomatic sub-group, four and 16 years later.

Study III

To investigate the relationship between baseline characteristics and the development of wheeze within four years in a population-based cohort of adolescents, with particular emphasis on obesity and possible gender differences regarding risk factors associated with wheeze.

Study IV

To investigate the development of respiratory symptoms from early adolescence to young adulthood in relation to baseline risk factors, gender differences, and allergic symptoms. Further, to investigate how respiratory and allergic symptoms associated with self-rated health in young adulthood.

Material and methods

Study subjects

The SPAIS cohort

The SPAIS cohort included 959 subjects, aged 12–15 years, from nine randomized schools in Uppsala, Sweden. Baseline data were collected in 1998–1999 (Figure 1) (49). The adolescents answered the ISAAC questionnaire (13, 94), and underwent lung function, FeNO, height, and weight measurements at their schools. Their parents answered additional written questions. The adolescents were not to have had any sign of respiratory infection in the week preceding when measurements of FeNO and lung function were performed.

Adolescents (n = 238) who fulfilled at least one of the following four criteria, according to screening data, were invited to a clinical reinvestigation at the Uppsala University Hospital within two months of the screening at school:

- parent-reported asthma,
- self-reported symptoms indicative of asthma,
- elevated FeNO measured at 100 mL/s ($FeNO_{0.1}$) defined as either ≥ 15 ppb on two separate occasions or > 20 ppb at one occasion, or
- $FEV_1 \leq 80\%$ predicted (as suggested by Knudson et al. (95)).

SPT and assessment of clinical asthma were carried out together with a pediatrician, specialized in allergology (SPAIS IB). A clinical diagnosis of asthma was assigned to 96 of the 195 investigated adolescents based on their fulfilling both of the following criteria: [i] repeated episodes of wheeze and/or chest tightness upon exercise in the absence of respiratory tract infection, during the preceding year, and [ii] repeated episodes of wheeze and/or chest tightness in conjunction with respiratory tract infection or allergen exposure (furred animals, pollen), during the preceding year.

The follow-up study, SPAIS II (Figure 1), with an identical ISAAC questionnaire and similar additional questions, was performed four years later (2002–2003). During SPAIS II, 96% of the adolescents (aged 16–19 years) completed the questionnaires.

In 2014, sixteen years after the baseline study was initiated, identical ISAAC questionnaires and additional questions were sent out to the entire SPAIS cohort. The subjects were then between 28 and 31 years of age. Out of

the 959 subjects in the cohort, 502 (52%) completed the questionnaire in this third part of the SPAIS study, SPAIS III (Figure 1).

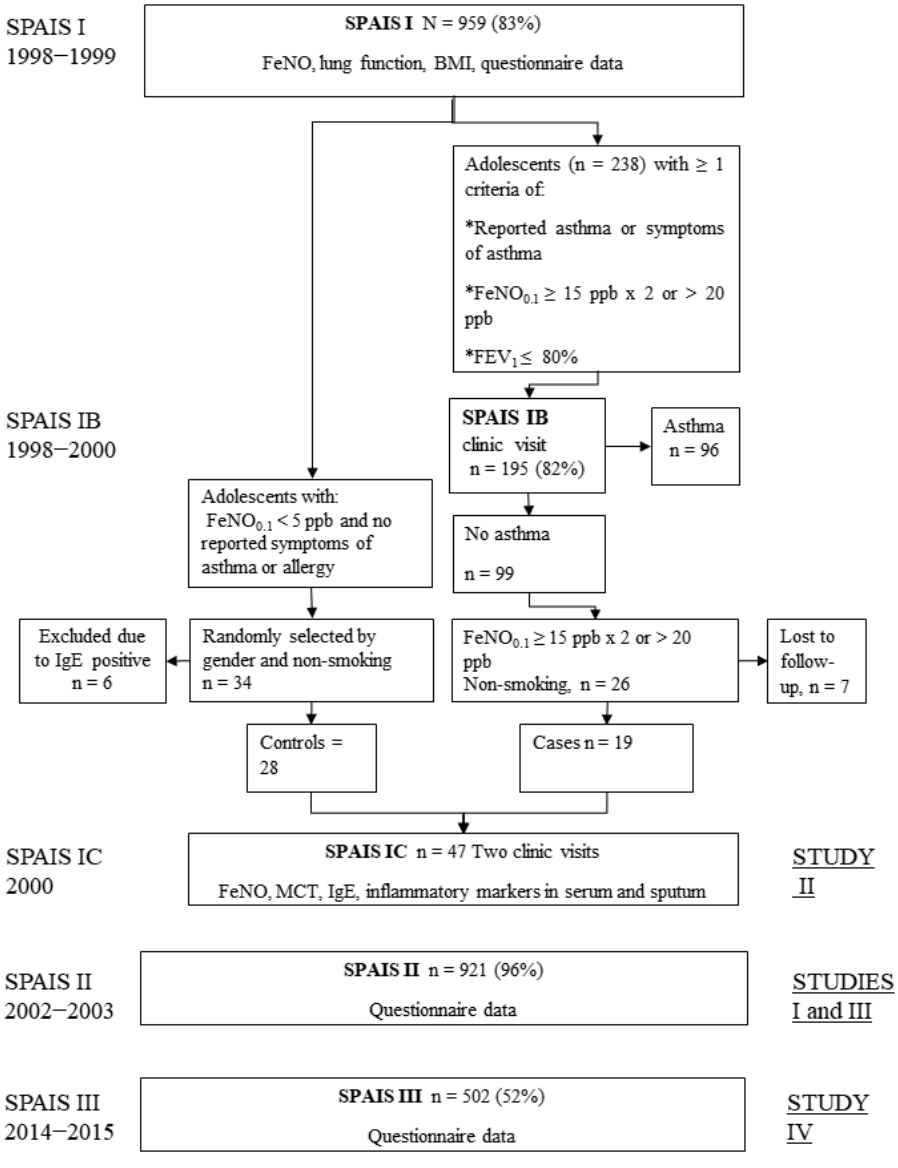

Figure 1. Flowchart of the SPAIS studies.

Data collection

Questionnaire data

The ISAAC questionnaire is a widely used standard for determining outcomes from allergic diseases in epidemiological studies and there is a version adapted for adolescents aged 13–14 years (13, 94). The ISAAC questionnaire was used at all three data collection timepoints, SPAIS I, SPAIS II, and SPAIS III (Figure 1), together with additional questions concerning hypersensitivity to cat, dog, or pollen, asthma diagnosis, asthma medication, family history of asthma and rhinitis, family smoking, and other environmental issues. An additional question concerning own smoking was included at the four-year follow-up. At SPAIS III, the additional part of the questionnaire contained questions concerning self-rated health, asthma diagnosis and medication, allergic symptoms to airborne allergens, environmental issues, physical activity, smoking habits, and work situation. The ISAAC questionnaire was completed by the participants at all three timepoints and the additional questions were answered by a parent at SPAIS I and by the participants themselves at SPAIS II and III.

At SPAIS I, the questionnaires were distributed in person at the schools and at SPAIS II and III by post. At SPAIS III, the subjects had the possibility to choose between using a paper version of the questionnaire or a web version (Webropol).

Definition of exposures

In Studies I and III, *asthma* was defined as ever having had self-reported asthma (ISAAC), in combination with having used inhaled corticosteroid treatment in the preceding year, or having wheezing or whistling in the chest at any time in the preceding year (ISAAC), or having a respiratory infection that caused wheezing or whistling in the chest during the same time period. In Study IV, asthma was defined as above, but instead of only corticosteroid treatment, any asthma medication in the preceding year was included in the definition. *Wheeze* (Studies I–IV) was defined as having had wheezing or whistling in the chest at any time in the preceding year (ISAAC). *Ever wheeze* (Study II) was defined as ever having had wheezing or whistling in the chest (ISAAC). *Rhinitis* (Studies I–IV) was defined as having had sneezing, nasal congestion or rhinorrhea during the preceding twelve months, without having a cold (ISAAC). *Atopic dermatitis* (Study III) was defined as an itchy rash which came and went for at least six months in the preceding twelve months (ISAAC).

At baseline, *allergic symptoms* (Studies I–IV) were defined as the subject's hypersensitivity to cat, dog, or pollen, noticed and reported by the parents. There was also a question regarding parental suspicion of hypersensitivity to cat, dog, or pollen. Allergic symptoms at follow-up were defined as above,

but reported by the participant and only at the level "Have you noticed hypersensitivity to cat, dog, or pollen?" In Study III, allergic symptoms to cat and dog were referred to as perennial allergic symptoms and allergic symptoms to pollen as seasonal allergic symptoms. At SPAIS III (Study IV), allergic symptoms were defined as the subject's experience of ever having had allergic symptoms to cat, dog, or pollen. Due to a missing page in the paper questionnaire, data on allergic symptoms were missing for 30 subjects in SPAIS III. *Exposure to cat and dog allergens at home* was questionnaire-assessed (Studies I and IV).

Asthma and rhinitis in the family were questionnaire-assessed, with separate questions regarding mother, father, and siblings (Studies I and II). *Family asthma and family rhinitis* involved symptoms reported by mother, father, or siblings (Studies III and IV). *Family smoking* (Studies I–IV) and *the subject's own current smoking habits* at follow-up (Studies II–IV) were also questionnaire-assessed – the latter with the question "Do you smoke?" (Studies II and III) and defined as smoking at least one cigarette a day during the preceding six months (Study IV). At baseline, all girls were asked if they had reached *menarche* or not.

At SPAIS III (Study IV), the SRH question "How do you rate your general health status?" was included in the questionnaire, with five response options.

Exhaled NO measurements

Measurement of FeNO was performed using the Aerocrine NO system (Aerocrine AB, Sweden), including the CLD 77 AM chemiluminescence analyzer (Eco Physics AG, Dürnten, Switzerland), and in accordance with the prevailing recommendations of the European Respiratory Society (96). Before measurement, each participant washed their mouth with 25 mL of 10% sodium bicarbonate for 20 seconds. FeNO was measured at 100 mL/s at the baseline visit and at both 100 mL/s and 50 mL/s at SPAIS IC. At each session, three correctly performed exhalations during 10 seconds, at requested flow rates, were recorded. Measurements of FeNO that were performed between March and September were defined as FeNO measurements inside the pollen season.

Measurements of pulmonary function

Pulmonary function measurements were performed in accordance with the criteria of the American Thoracic Society (97), using a Spirolab spirometer (Medical International Research, Rome, Italy).

Using the Excel macro for The Global Lung Function Initiative (GLI) (54) reference values, lower limit of normal (LLN), Z-scores, and percentiles for forced expiratory volume in one second (FEV_1) were calculated for each subject in the study population in Studies I, III, and IV. The lower limit of normal was defined as $FEV_1 < -1.65$ standard deviations and referred to as reduced

FEV_1. In Study II, FEV_1 was measured and Knudson's reference values were used (95).

Measurements of body mass index

Measurements of height and weight were performed at the baseline visit at school and at clinic visit one at SPAIS IC. BMI was calculated [weight (kg) / height $(m)^2$], and BMI values were used to divide subjects into normal weight, underweight, overweight, and obese groups (Study III), based on WHO recommendations (Growth reference data 5–19 years) (98).

Sputum induction and processing

Sputum was induced by inhalation of hypertonic saline using an Omron U1 ultrasonic nebulizer, following a protocol described by Iredale et al. (Study II) (99). Collected sputum was weighed and treated with Sputolysin® (dithiothrietol-DTT) for 15 minutes and then with CTAB (N-Cetyl-N, N, N-trimethylammonium bromide) for one hour, both at room temperature (100). After centrifugation, the supernatant was collected and held at -20 °C until analysis.

Methacholine challenge test

The methacholine challenge test (MCT) was performed by applying a dosimetric method, using an automatic inhalation-synchronized jet nebulizer (Spira Elektro 2, Spira, Finland) and controlled tidal breathing (101). FEV_1 was measured three minutes after each dose of methacholine and the challenge test was terminated when the subject had completed all inhalations or when FEV_1 had fallen by 20% or more from the baseline value. The dose of methacholine (in μmol) that caused a 20% reduction in FEV_1 (PD_{20}) was calculated (102).

Blood, serum, and sputum analyses

Eosinophil and neutrophil counts were analyzed in blood. Eosinophilic cationic protein (ECP) and human neutrophil lipocalin (HNL) were analyzed in both serum and sputum. Myeloperoxidase (MPO) and IgE antibodies were analyzed in serum. IgE antibodies to mite, cat, dog, birch, and timothy were measured using ImmunoCAP (Pharmacia Diagnostics, Uppsala, Sweden). Sensitization was defined as IgE antibody levels ≥ 0.35 kU_A/L.

Analyses of ECP, HNL, and MPO in serum and of ECP and HNL in sputum were performed using in-house radioimmunoassays, and measurements of eosinophils and neutrophils in blood were performed by the Clinical Chemistry Laboratory at the Uppsala University Hospital using an automated cell counter.

Skin-prick test

SPTs were performed in a sub-sample of 374 subjects at baseline (Study I). This sub-sample consisted of all volunteering participants from two of the schools, as well as adolescents from all other schools who had participated in SPAIS IB. The most common airborne allergens in the area – cat, dog, birch pollen, and timothy pollen – were tested (Soluprick, ALK, Horsholm, Denmark), as was mite (*Dermatophagoides pteronyssinus*), which is uncommon in this part of Sweden. A positive SPT was defined as a mean wheal diameter of at least 3 mm after 15 minutes (103).

Study designs

Overview of the included studies

This thesis consists of four studies with quantitative designs. An overview of designs, applied methods, and study subjects is presented in Table 1.

Table 1. Overview of the included studies, I–IV.

Study	Design	Data collection	Participants	Analyses
I	Longitudinal, prospective	Questionnaire FeNO, FEV_1	921 subjects participating in SPAIS II	Descriptive statistics, t-tests, chi-squared tests, logistic regression analyses
II	Case-control study. Longitudinal, prospective	FeNO, FEV_1 blood and sputum biomarkers, IgE, PD_{20} questionnaire	19 cases 28 controls	Descriptive statistics, t-tests, chi-squared tests, Spearman's rank correlation, Mann-Whitney non-parametric U tests
III	Longitudinal, prospective	Questionnaire BMI, FeNO, FEV_1	795 subjects participating in SPAIS II and without wheeze at SPAIS I	Descriptive statistics, t-tests, chi-squared tests, logistic regression analyses
IV	Longitudinal, prospective	Questionnaire self-rated health BMI, FeNO, FEV_1	491 subjects, participating in all three parts of SPAIS	Descriptive statistics t-tests, chi-squared tests, logistic regression analyses

Studies I and III

A total of 959 adolescents from a general population answered a standardized questionnaire, in part together with their parents, and underwent height, weight, lung function, and FeNO measurements at a baseline visit in their schools in 1998–1999 (SPAIS I, Figure 1). Four years later, 921 of these subjects (96%) filled out a similar questionnaire (SPAIS II, Figure 1). In Study III, only adolescents without self-reported wheeze at baseline were included (n = 795).

Study II

Adolescents who had elevated $FeNO_{0.1}$ (defined as either ≥ 15 ppb on two separate occasions or > 20 ppb at one examination) at baseline and were negative in the clinical assessment of asthma at SPAIS IB (cases; n = 26) (Figure 1), were invited to a follow-up study (SPAIS IC, Figure 1). Randomly selected subjects, with a similar sex distribution as the cases, with low FeNO ($FeNO_{0.1}$ < 5 ppb) and without reported symptoms of asthma or allergy at baseline, were selected as controls (n = 34). Neither cases nor controls should have a known pulmonary disease, have started on corticosteroid or $ß_2$-agonist treatment after participation at the baseline examination, be smokers, or have signs of respiratory infection within two weeks prior to participation in any of the two study visits. Cases with reported allergic symptoms to pollen, according to screening data, were invited to perform the study visits outside the pollen season. Six of the eligible cases were lost to follow-up, and one was excluded after the first clinic visit due to initiation of asthma medication.

At the first clinic visit within SPAIS IC, height, weight, FeNO, and lung function were measured, and blood and induced sputum were sampled. Further, data regarding the subjects' medical history and current medication were collected. At the second clinic visit, FeNO measurement and a MCT were carried out. Thirty-four controls participated in the two clinic visits, but six were excluded before data analyses due to confirmed IgE sensitization (≥ 0.35 kU_A/L) to mite, dog, birch, or timothy, in spite of having no parent-reported allergic symptoms to cat, dog, or pollen at baseline.

Questionnaire data from SPAIS II and III were used to study long-term effects of elevated FeNO on the development of respiratory and allergic symptoms.

Study IV

The second follow-up study, SPAIS III, with slightly abbreviated versions of the original questionnaire, was performed sixteen years after the baseline examination (2014–2015). At this timepoint, 502 subjects (52.3%) participated and only subjects who participated in all three parts of SPAIS were included in Study IV, n = 491 (51.2%).

Statistical analyses

Statistical analyses were performed using STATA IC 14 (StataCorp, College Station, Texas, USA).

All studies

Comparisons at the group level were performed using t-tests for normally distributed continuous variables or using chi-squared tests for categorical variables. FeNO was log-transformed to achieve normal distribution before t-tests. McNemar's test was used to assess within-subject changes of categorical variables across two timepoints. A p value < 0.05 was considered statistically significant.

Study I

Multiple logistic regression analyses with incident allergic symptoms to cat and dog as outcomes, were performed to estimate odds ratios with 95% confidence intervals. FeNO, along with relevant confounders identified as significant ($p < 0.05$) in the univariate analyses, were used as predictors. Height and information on if FeNO measurements were performed inside or outside the pollen season, were additional predictors.

Furthermore, a model was created where a FeNO value above an arbitrary level (75^{th} percentile) was used as a predictor for incident allergic symptoms to cat and dog, respectively, after adjustments for confounders identified in previously described univariate analyses. In subjects with SPT results, similar models were also used and adjustments made for sensitization to cat and dog.

Study II

The significance of the differences in serum and sputum ECP, HNL, serum MPO, and cell counts of eosinophils between cases, non-asthmatics with elevated FeNO, and controls with low FeNO, were examined using the Mann-Whitney' non-parametric U test. T-tests were used for corresponding analyses of neutrophil counts. Correlations between FeNO, on the one hand, and the aforementioned biomarkers, on the other hand, were determined using Spearman's rank correlation.

Study III

Multiple logistic regression analyses were performed with incident wheeze as outcome, and obesity, along with relevant confounders identified as significant ($p < 0.05$) for either girls or boys in the univariate analyses, were used as predictors. Menarche at baseline was an additional predictor for girls, as was

current smoking at SPAIS II, for both girls and boys. A stepwise multiple regression model was used, and variables were excluded if no significant association with the outcome was found ($p > 0.05$). All subjects with reported asthma at baseline were excluded in these models.

Study IV

SRH was dichotomized into good (response alternatives: very good and quite good) or poor (response alternatives: neither good nor poor, quite poor, and very poor).

Multiple logistic regressions were performed with incident asthma as the outcome and all variables identified as significant for either females or males in the univariate analyses, used as predictors. A stepwise multiple regression model was used, and variables were excluded if no significant association with the outcome was found ($p > 0.05$).

Ethics

SPAIS I was approved by the Ethical Committee of the Medical Faculty of Uppsala University, Sweden (registration number 243/1998), as were SPAIS IC (registration number 014/2000) and SPAIS II (registration number 499/2001). SPAIS III was approved by the Regional Ethical Review Board in Uppsala, Sweden (registration number 440/2013). The study procedures were in accordance with the Declaration of Helsinki (104).

As described in an information letter appended to the questionnaire at SPAIS I, a completed parental part of the questionnaire was regarded as written informed consent from the parents for participation of their child in the study. The adolescents gave informed consent by completing the ISAAC part of the questionnaire and by verbally agreeing to participate in the study. At SPAIS II and III, a completed and returned questionnaire was seen as written informed consent from the participants, in accordance with an information letter appended to the questionnaires.

Results

Study I

A total of 921 of the 959 subjects (96%) completed the questionnaire in SPAIS II. The prevalence of wheeze, but not of asthma, rhinitis, or allergic symptoms to cat, dog, or pollen, had increased between baseline and follow-up.

Incident allergic symptoms to cat

The adolescents (n = 50) with self-reported incident allergic symptoms to cat had significantly higher FeNO compared with those without parent- and self-reported symptoms to cat at either assessment (Table 2). The group with self-reported incident allergic symptoms to cat had higher FEV_1 % predicted, were shorter, and more often had reported asthma, wheeze, rhinitis, and allergic symptoms to dog and pollen at baseline, compared with adolescents without cat symptoms. Fewer subjects with incident cat symptoms performed measurements of FeNO during the pollen season at baseline, compared with subjects who never reported cat symptoms. During follow-up, 30% of participants with self-reported incident allergic symptoms to cat also self-reported allergic symptoms to dog and 60% to pollen. Further, at follow-up, but not at baseline, there was a significant difference in having a cat at home, with a lower prevalence in the incident group than among individuals who never developed allergic cat symptoms. There were no confirmed differences concerning family asthma or rhinitis.

In the group of participants who had developed allergic symptoms to cat after four years, 25 underwent SPT at baseline. Of these, 14 had a positive SPT result and 11 had a negative SPT result for cat.

Incident allergic symptoms to dog

Participants with self-reported incident allergic symptoms to dog (n = 33) had significantly higher FeNO, and parent- and self-reported asthma, wheeze, rhinitis and allergic symptoms to cat and pollen to a higher extent at baseline, compared with those without allergic symptoms to dog at either timepoint (Table 3). A family history of asthma was more common in the incident group, as was rhinitis reported by the mother. Among participants with self-reported incident allergic symptoms to dog, 76% self-reported allergic symptoms to cat

and 55% to pollen in late adolescence. There was no difference in the number of individuals with reported presence of a dog at home, at baseline or follow-up.

Of the 33 individuals with self-reported incident allergic symptoms to dog, 22 underwent SPT at baseline. Of these, 8 had a positive result and 14 had a negative result for dog.

Incident allergic symptoms to pollen

Subjects (n = 85) who self-reported incident allergic symptoms to pollen more commonly reported asthma, wheeze, rhinitis, allergic symptoms to cat, and allergic rhinitis among siblings at baseline, compared with subjects without reported allergic pollen symptoms at either timepoint. Among participants who self-reported incident allergic symptoms to pollen at follow-up, 29% also self-reported allergic symptoms to cat and 6% to dog. However, there was no significant difference regarding FeNO.

Table 2. Characteristics of study subjects at baseline in relation to incident allergic symptoms to cat at SPAIS II.

	No allergic symptoms to cat at baseline or follow-up (n = 776)	Incident allergic symptoms to cat (n = 50)	p value
Male sex (%)	49.7	42	0.29
$FeNO_{0.1}$ (pbb)	4.17 (3.90, 4.46)	6.89 (4.93, 9.62)	< 0.001
FEV_1 (% predicted)	94.7 ± 10.6	98.0 ± 11.5	0.03
Asthma (%)	3.7	14	0.001
Wheeze (%)	8.5	20	0.006
Rhinitis (%)	18	52	< 0.001
Allergic symptoms to dog (%)	0.4	8	< 0.001
Allergic symptoms to pollen (%)	10.6	30	< 0.001
Cat exposure year 1 (%)	21.3	10	0.06
Cat exposure SPAIS I (%)	30.2	26	0.53
Cat exposure SPAIS II (%)	31.7	18	0.04
FeNO measurements inside pollen season (%)	36.6	22	0.04
Ever asthma (mother) (%)	9.1	14	0.26
Ever asthma (father) (%)	8.1	12	0.34
Ever asthma (siblings) (%)	13.5	12	0.76
Ever allergic rhinitis (mother) (%)	24.4	34	0.13
Ever allergic rhinitis (father) (%)	22.7	22	0.91
Ever allergic rhinitis (siblings) (%)	19.1	20	0.87

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; ppb, parts per billion.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

Table 3. Characteristics of subjects at baseline in relation to incident allergic symptoms to dog at SPAIS II.

	No allergic symptoms to dog at baseline or follow-up (n = 838)	Incident allergic symptoms to dog (n = 33)	p value
Male sex (%)	49.6	45.5	0.64
$FeNO_{0.1}$ (ppb)	4.37 (4.10, 4.67)	9.60 (7.26, 12.69)	< 0.001
FEV_1 (% predicted)	94.9 ± 10.6	94.7 ± 10.2	0.91
Asthma (%)	5	24.2	< 0.001
Wheeze (%)	10.1	39.4	< 0.001
Rhinitis (%)	21.7	60.6	< 0.001
Allergic symptoms to cat (%)	4.7	39.4	< 0.001
Allergic symptoms to pollen (%)	12.9	51.5	< 0.001
Dog exposure year 1 (%)	14.7	18.2	0.58
Dog exposure SPAIS I (%)	21.4	24.2	0.69
Dog exposure SPAIS II (%)	22.8	21.2	0.83
FeNO measurements inside pollen season (%)	35.7	39.4	0.66
Ever asthma (mother) (%)	9.4	21.2	0.03
Ever asthma (father) (%)	8.5	18.2	0.05
Ever asthma (siblings) (%)	13.8	27.3	0.03
Ever allergic rhinitis (mother) (%)	25.4	45.5	0.01
Ever allergic rhinitis (father) (%)	24.1	27.3	0.68
Ever allergic rhinitis (siblings) (%)	19.9	27.3	0.30

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in one second; ppb, parts per billion.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

Multivariate analyses

In multiple logistic regression analyses, elevated FeNO at baseline was independently related to self-reported incident allergic symptoms to cat ($p < 0.001$), after adjustments were made for variables identified as significant in univariate analysis as well as for height (Table 2). Similarly, elevated FeNO

at baseline was independently related to self-reported incident allergic symptoms to dog after the aforementioned adjustments as well as adjustment for FeNO measurements inside the pollen season ($p = 0.048$).

Analyses based on an arbitrary FeNO cut-off showed that having a FeNO above the 75th percentile was related to incident allergic symptoms to cat after the adjustments described above were made. The adjusted odds ratio [aOR (95% confidence interval)] was 4.2 (2.2, 8.0) times higher for incident allergic symptoms to cat if FeNO was > 75th percentile (vs. < 75th percentile) at baseline (Figure 2). This was consistent when looking at subjects without reported asthma, wheeze, or rhinitis at baseline (18 subjects remained with incident self-reported allergic symptoms to cat).

Similarly, having a FeNO above the 75th percentile was related to incident allergic symptoms to dog, after adjustments were made for variables identified as significant in a univariate analysis as well as for height and FeNO measurements inside the pollen season (Table 3). The adjusted odds ratio [aOR (95% confidence interval)] was 3.3 (1.45, 7.58) times higher if FeNO was > 75th percentile (vs. < 75th percentile) at baseline (Figure 2). However, no significant relations were found when looking only at subjects without reported asthma, wheeze, or rhinitis at baseline (9 subjects remained with incident allergic symptoms to dog).

Using the same logistic regression model as above for the subgroup with SPT results available (not excluding individuals with asthma, wheeze, or rhinitis at baseline) and adding adjustment for sensitization to cat, a significant association was found for FeNO > 75th percentile, aOR 4.1 (1.2, 13.9). Adding the adjustment for sensitization to dog in a similar logistic regression model revealed no significant association with FeNO at baseline.

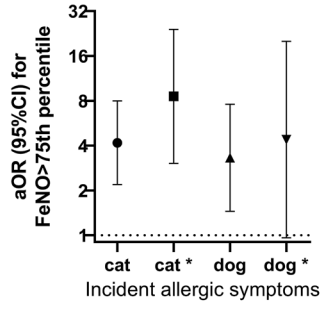

Figure 2. Adjusted odds ratio (aOR) for self-reported incident allergic symptoms to cat and dog. *Subjects with reported asthma, wheeze, and rhinitis at baseline were excluded; subjects remaining in the models: cat, 18 subjects and dog, 9 subjects.

Study II

There were 19 cases out of 26 eligible subjects (73%), and 28 controls who completed the two clinic visits within SPAIS IC (Figure 1). There were no differences in demographic data between cases and controls. Comparing questionnaire data from baseline, the cases significantly more often reported rhinitis in the preceding year, but there were no statistical differences concerning reported wheeze, family asthma, rhinitis, or smoking, between cases and controls.

Respiratory measurements

The cases had significantly higher values of FeNO measured at 50 mL/s than controls at both visits ($p < 0.001$, both), but there was no difference concerning FEV_1 between the two groups at either visit. The cases had significantly lower PD_{20} values ($p < 0.05$) than the controls (Table 4). However, no significant correlation was found between FeNO and PD_{20} (rho = -0.21, p = 0.17).

Analyses of serum, blood and sputum

Most of the cases were IgE-sensitized (79%), most commonly to cat (52.6%) and dog (57.9%), and least frequently to mite (21.1%). Blood eosinophil counts were significantly higher, and serum ECP showed a trend to be higher among the cases (Table 4). HNL and MPO in serum were higher in the controls, whereas blood neutrophil counts did not differ between the two groups. Cases and controls produced similar amounts of sputum and no significant differences in sputum ECP or HNL were found between the two groups.

Table 4. Inflammatory markers, cell counts, FEV_1 and PD_{20} in cases and controls.

	Cases (n = 19)	**Controls** (n = 28)	**p value**
$FeNO_{0.05}$ visit 1 (ppb)	30.98 (24.79, 38.72)	5.87 (4.73, 7.30)	< 0.001
$FeNO_{0.05}$ visit 2 (ppb)	30.56 (22.94, 40.72)	6.11 (4.84, 7.72)	< 0.001
FEV_1 visit 1 (% predicted)	102.21 ± 12.33	101.71 ± 14.43	0.90
FEV_1 visit 2 (% predicted)	99.05 ± 10.25	98.54 ± 12.38	0.88
Sputum (g)	1.59 ±1.76	1.47 ±1.21	0.80
PD_{20} (µmol)	6.94 [1.87, 11.39]	11.42 [6.33, 59.4]	< 0.05
HNL sputum (µg/L)	1 025 [591, 2 096]	1 167 [649, 2 613]	0.81
ECP sputum (µg/L)	417 [157, 671]	187 [61.4, 421]	0.12
ECP serum (µg/L)	13.3 [9.1, 18.3]	8.6 [6.2, 13.7]	0.05
HNL serum (µg/L)	55.3 [47, 60.6]	60.75 [49.8, 76.8]	< 0.05
MPO serum (µg/L)	219 [195, 272]	334 [228, 410]	< 0.05
Eosinophils blood (10^9/L)	0.31 [0.20, 0.44]	0.13 [0.1, 0.22]	< 0.001
Neutrophils blood (10^9/L)	3.28 ±1.04	3.33 ±1.58	0.90

Abbreviations: $FeNO_{0.05}$, fractional exhaled nitric oxide measured at 50 mL/s; FEV_1, forced expiratory volume in one second; ppb, parts per billion; PD_{20}, cumulative dose methacholine causing a fall of 20% in FEV_1.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval or median and interquartile range.

Correlations between FeNO and other inflammatory biomarkers

There was a significant correlation between FeNO and blood eosinophil count (rho = 0.41, p = 0.005) and a negative correlation between FeNO and HNL in serum (rho = -0.31, p = 0.04).

Long-term follow-up of cases and controls

All study subjects completed the questionnaire at SPAIS II and 26 of the subjects (55.3%), 9 cases and 17 controls, did so at SPAIS III. The proportion of cases who reported any allergic symptoms had increased from 21.1% to 63.2% between SPAIS I and II (p = 0.02) and from 21.1% to 77.8% between SPAIS I and SPAIS III (p = 0.01). Self-reported ever wheeze was significantly higher among cases than controls at all assessments and the proportion of cases who reported these symptoms increased from 26.3% to 55.6% between SPAIS I and SPAIS III. However, this increase was not significant (p = 0.21). During these periods, there were no significant changes in the reported symptoms of allergy or ever wheeze among controls.

Study III

In SPAIS II, 921 (96%) subjects completed and returned the questionnaires. Study III included only adolescents without self-reported wheeze at baseline (n = 795). Out of them, 84 subjects (10.6%) had developed self-reported wheeze four years later.

Gender specific incident wheeze

When presenting data stratified by sex, girls with incident wheeze were on average taller, weighed more, and had higher mean BMI than girls who never reported wheeze. Further, they had more often reported asthma, rhinitis and perennial allergic symptoms. Further, it was significantly more common among girls who had incident wheeze to have reached menarche at baseline than among girls who never reported symptoms of wheeze (Table 5). In the incident wheeze group, there were more girls classified as obese compared with in the group without reported wheeze, 22.4% vs. 7.5% (Figure 3).

The boys with incident wheeze did not exhibit higher BMI (Table 6), and there was no difference in the proportion of boys who were obese with regard to incident wheeze (Figure 3). Instead, boys with incident wheeze had higher FeNO, more often had reduced FEV_1, and more often reported rhinitis, allergic symptoms to both perennial and seasonal allergens and family asthma, at baseline, than boys who never reported any symptoms of wheeze.

Both girls and boys with incident wheeze had a higher prevalence of current smoking at follow-up, compared with subjects without symptoms of wheeze, but the difference was larger among girls.

There was no sex difference between the subjects with incident wheeze and those without wheeze at both timepoints with regard to baseline FEV_1 % predicted.

Table 5. Characteristics of girls at baseline, except current smoking, in relation to incident wheeze at SPAIS II.

	No wheeze at baseline or follow-up (n = 345)	Incident wheeze (n = 49)	p value
$FeNO_{0.1}$ (ppb)	4.08 (3.71, 4.49)	3.96 (2.64, 9.96)	0.85
FEV_1 (% predicted)	94.8 ± 10.5	96.0 ± 10.5	0.44
FEV_1 (< -1.65 SD), n (%)	30 (8.7)	5 (10.4)	0.70
Height (cm)	160.3 ± 6.41	162.4 ± 6.26	< 0.05
Weight (kg)	51.1 ± 9.06	55.2 ± 12.3	< 0.01
BMI (kg/m^2)	19.9 ± 3.06	20.9 ± 4.06	< 0.05
Menarche, n (%)	247 (71.6)	43 (87.8)	< 0.05
Asthma, n (%)	4 (1.2)	3 (6.1)	0.01
Rhinitis, n (%)	59 (17.1)	18 (36.7)	< 0.01
Atopic dermatitis, n (%)	75 (21.7)	11 (22.4)	0.91
Perennial allergic symptoms, n (%)	16 (4.6)	7 (14.3)	< 0.01
Seasonal allergic symptoms, n (%)	39 (11.3)	9 (18.4)	0.16
Current smoking, n (%)	36 (10.4)	12 (24.5)	< 0.01
Family asthma, n (%)	91 (26.4)	19 (38.8)	0.07
Family smoking, n (%)	126 (36.5)	18 (36.7)	0.98
Family allergic rhinitis, n (%)	169 (49.0)	30 (61.2)	0.11

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; n, number; ppb, parts per billion; SD, standard deviation.

All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

Table 6. Characteristics of boys at baseline, except current smoking, in relation to incident wheeze at SPAIS II.

	No wheeze at baseline or follow-up (n = 366)	Incident wheeze (n = 35)	p value
$FeNO_{0.1}$ (ppb)	4.52 (4.08, 5.00)	6.38 (4.76, 8.55)	< 0.05
FEV_1 (% predicted)	95.3 ± 10.6	93.1 ± 10.5	0.24
FEV_1 (< -1.65 SD), n (%)	20 (5.5)	5 (14.3)	< 0.05
Height (cm)	164.4 ± 9.07	164.1 ± 8.19	0.84
Weight (kg)	53.5 ± 10.9	55.9 ± 11.9	0.22
BMI (kg/m^2)	19.7 ± 2.92	20.5 ± 3.43	0.09
Asthma, n (%)	11 (3.0)	1 (2.9)	0.96
Rhinitis, n (%)	68 (18.6)	13 (37.1)	< 0.01
Atopic dermatitis, n (%)	55 (15.0)	8 (22.9)	0.22
Perennial allergic symptoms, n (%)	18 (4.9)	7 (20.0)	< 0.001
Seasonal allergic symptoms, n (%)	47 (12.8)	15 (42.9)	< 0.001
Current smoking, n (%)	18 (4.9)	5 (14.3)	< 0.05
Family asthma, n (%)	73 (20.0)	14 (40.0)	< 0.01
Family smoking, n (%)	106 (29.0)	10 (28.6)	0.96
Family allergic rhinitis, n (%)	179 (48.9)	21 (60.0)	0.21

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; n, number; ppb, parts per billion; SD, standard deviation.
All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

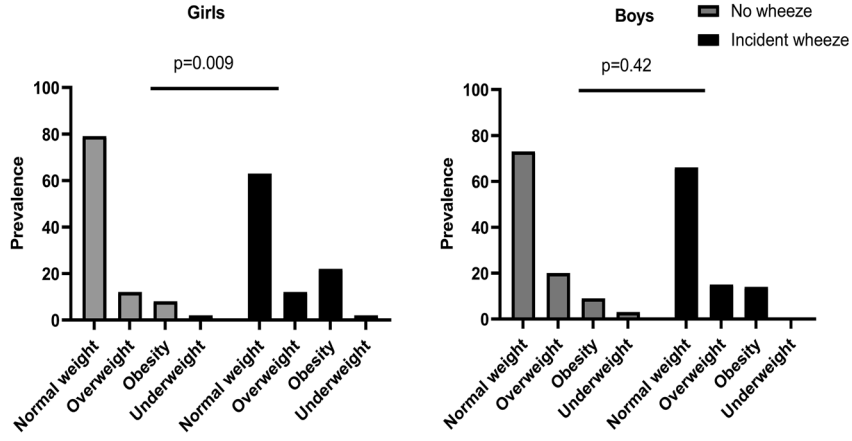

Figure 3. Prevalence (%) of BMI groups in girls and boys with no reported wheeze (grey bars) and with incidence wheeze (black bars).

Multivariate analyses

In multiple logistic regression analyses, obesity was independently related to self-reported incident wheeze in girls after adjustments for confounders (see Statistical analyses) (Table 7). These analyses were performed after exclusion of the three girls with reported asthma but no reported wheeze at baseline. Further, incident wheeze in girls was associated with self-reported rhinitis at baseline and current smoking at follow-up. Corresponding logistic regression analyses in boys showed that allergic symptoms to seasonal allergens, family asthma, and reduced FEV_1 at baseline were independently associated with incident wheeze (Table 7). These analyses were performed after exclusion of the one boy with reported asthma but no wheeze at baseline.

Table 7. Independent risk factors (recorded at baseline, except current smoking) for incident wheeze in adolescent girls and boys.

		aOR	95% CI	p value
Girls:	Normal weight	1.0	1.0	1.0
	Obesity versus normal	2.84	1.17, 6.86	0.02
	Rhinitis	3.04	1.53, 6.03	0.001
	Current smoking	2.60	1.16, 5.82	0.02
Boys:	Seasonal allergic symptoms	5.61	2.56, 12.27	< 0.001
	FEV_1 (< -1.65 SD)	3.20	1.04, 9.79	0.04
	Family asthma	3.16	1.46, 6.86	0.004

Abbreviations: aOR: adjusted odds ratio, BMI, body mass index; CI, confidence interval; FEV_1, forced expiratory volume in one second; SD, standard deviation.
aOR: Variables adjusted for in all models: BMI groups, rhinitis, perennial and seasonal allergic symptoms, family asthma, $FeNO_{0.1}$ and FEV_1 < -1.65 SD, at baseline and current smoking at follow-up. Additional variable in females: menarche at baseline. All subjects with asthma at baseline were excluded.

Study IV

Subjects who had participated in all three parts of SPAIS were included in the study (n = 491; 51.2%) and data on allergic symptoms were available for 461 subjects. The prevalence rates of asthma, wheeze, rhinitis, and allergic symptoms to cat, dog, and pollen had all increased significantly between baseline and follow-up at SPAIS III (Figure 4). The overall prevalence of asthma tended to be reduced at SPAIS II and, at the same timepoint, more girls than boys reported symptoms of wheeze: 17.9% vs. 11.9% (p = 0.07). Further, current smoking at SPAIS III was reported by 53 subjects, or 11.5% of the females and 10.4% of the males (p = 0.69).

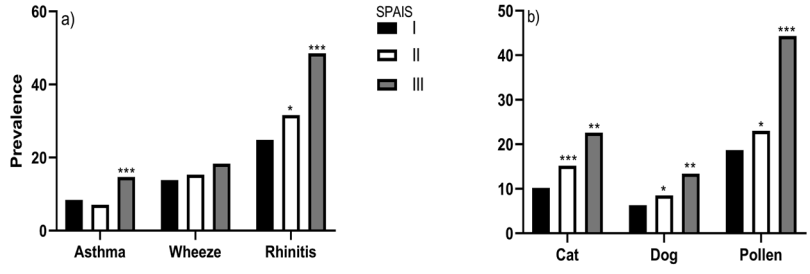

Figure 4. Prevalence (%) of a) respiratory symptoms and b) allergic symptoms reported at SPAIS I–III. *** $p < 0.001$, ** $p = 0.01$, * $p < 0.05$, significant increase in reported symptoms.

Gender-specific prevalence of respiratory and allergic symptoms to cat

At SPAIS III, the prevalence of asthma had increased significantly for females, but not for males (Figure 5a). The prevalence of allergic symptoms to cat had increased significantly over sixteen years, with no sex differences, and remission was low (Figure 5b).

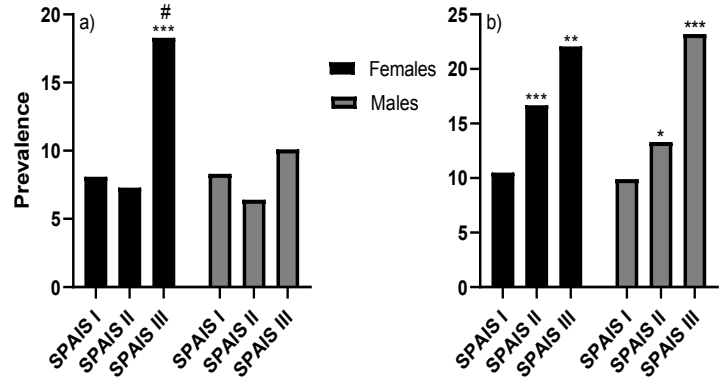

Figure 5. Prevalence (%) of a) asthma and b) allergic symptoms to cat reported at SPAIS I–III among female and male subjects. *** $p < 0.001$, ** $p = 0.01$, * $p < 0.05$, significant increase in reported symptoms. # $p = 0.01$, significant sex difference in reported symptoms.

Risk factors in early adolescence

As the development of respiratory symptoms showed clear gender differences, a sex-specific analysis of baseline risk factors for incident asthma at SPAIS III was performed.

Females with incident asthma more often had reduced FEV_1 and reported more rhinitis, allergic symptoms to cat, and family rhinitis at baseline than females with no reported asthma at SPAIS I or SPAIS III (Table 8).

Males who developed asthma had higher FeNO and more reported wheeze, rhinitis, family asthma and allergic symptoms to both cat and dog at baseline than males who did not report asthma symptoms at any timepoint (Table 8). Generally, males with incident asthma reported more allergic symptoms at baseline than females. At SPAIS III, this difference was reduced and 53.9% of the males and 46.9% of the females with incident asthma reported allergic symptoms to cat. There was no significant difference in reported current smoking at SPAIS III, for either females or males, compared with subjects who never reported any symptoms of asthma (Table 8).

Table 8. Characteristics of subjects at baseline, except current smoking and pets at home*, in relation to incidence of asthma at SPAIS III.

	Females			**Males**		
	No asthma at baseline or at SPAIS III (n = 216)	Incident asthma at SPAIS III (n = 35)	p value	No asthma at baseline or at SPAIS III (n = 186)	Incident asthma at SPAIS III (n = 13)	p value
$FeNO_{0.1}$ (ppb)	4.46 (3.99, 4.99)	3.37 (2.12, 5.35)	0.09	4.39 (3.79, 5.10)	9.69 (4.58, 20.48)	0.009
FEV_1 (% predicted)	95.02 ± 8.98	92.88 ± 9.60	0.20	95.67 ± 11.08	93.36 ± 12.18	0.47
FEV_1 (< -1.65 SD) (%)	5.1	14.3	0.04	7.0	15.4	0.27
BMI ($kg/m^{2)}$	19.66 ± 2.94	20.12 ± 3.20	0.39	19.80 ± 2.99	21.18 ± 2.73	0.11
Height (cm)	160.7 ± 6.51	161.0 ± 5.43	0.83	163.6 ± 9.52	164.4 ± 10.6	0.76
Wheeze (%)	7.4	17.1	0.06	5.4	30.8	0.001
Rhinitis (%)	18.5	42.9	0.001	17.2	53.9	0.001
Allergic symptoms to cat (%)	4.2	17.1	0.003	4.8	30.8	< 0.001
Allergic symptoms to dog (%)	1.4	5.7	0.09	3.2	15.4	0.03
Allergic symptoms to pollen (%)	12.0	17.1	0.40	16.1	23.1	0.52
Family asthma (%)	29.6	42.9	0.12	20.4	69.2	< 0.001
Family rhinitis (%)	51.9	74.3	0.01	45.7	69.2	0.21
Family smoking (%)	31.9	25.7	0.46	25.3	30.8	0.66
Current smoking (%)	11.3	11.8	0.93	11.6	8.3	0.73
Cat at home (%)	17.8	22.9	0.47	15.1	8.3	0.52
Dog at home (%)	12.2	17.1	0.42	9.2	7.7	0.85

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; ppb, parts per billion; SD, standard deviation.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval. * self-reported at SPAIS III.

Multivariate analyses

In the adjusted logistic regression (adjustments, see Statistical analyses), reduced FEV_1, reported rhinitis, and family rhinitis at baseline were related to incident asthma in females (Table 9). Corresponding risk factors for males were higher FeNO, reported rhinitis, and family asthma at baseline. There were no effects on the results when data on current smoking at SPAIS III, a non-significant variable for both sexes, (Table 8), were added into the female model. However, in the male model, reduced FEV_1, wheeze, and allergic symptoms to cat at baseline became related exposures, and rhinitis at baseline ceased being a related exposure.

Table 9. Independent baseline risk factors for incident asthma at SPAIS III.

Risk factors	Incident asthma Females (aOR [95% CI])	Incident asthma Males (aOR [95% CI])
FEV_1 < -1.65 SD	4.11 (1.27, 13.24)	4.51 (0.59, 34.69)
$FeNO_{0.1}$	0.98* (0.92, 1.05)	1.13* (1.06, 1.20)
Rhinitis	3.34 (1.54, 7.25)	7.39 (1.78, 30.78)
Family asthma	1.47 (0.66, 3.25)	12.74 (2.88, 56.31)
Family rhinitis	2.89 (1.25, 6.68)	0.73 (0.14, 3.72)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; SD, standard deviation.
aOR: Variables adjusted for in incident asthma models: $FeNO_{0.1}$, FEV_1 < -1.65 SD, rhinitis, wheeze, allergic symptoms to cat and dog, family asthma and rhinitis, at baseline.
*per 1 ppb increase in FeNO.

Self-rated health in early adulthood

The proportion of females with current asthma who rated their health as poor was significantly higher than that of females without asthma at SPAIS III, 20.0% vs. 7.7% ($p = 0.008$). Similar findings were seen for females with reported symptoms of wheeze or allergic symptoms to cat at SPAIS III: 20.3% vs. 7.0% ($p = 0.003$) and 21.1% vs. 7.0% ($p = 0.002$), respectively. Corresponding differences in poor SRH were not observed for males concerning any of these reported symptoms. However, there was no significant sex difference when comparing the proportion of males and females with poor SRH in the group with reported asthma at SPAIS III ($p = 0.86$).

Discussion

Development of allergic symptoms

The main finding was that the prevalence of reported allergic symptom to cat, dog, and pollen had increased significantly between early adolescence and early adulthood, with no gender differences. Objective measurements at baseline revealed that higher FeNO was an independent risk factor for incident allergic symptoms to cat and dog four years later.

Prevalence and gender differences

The prevalence of allergic symptoms in early adulthood was in line with results from a Swedish cross-sectional study for individuals of comparable ages, 22–40 years (10). That study showed that 35.1% of the subjects were IgE-sensitized to pollen, 23.4% to cat, and 22.7% to dog. In Study IV, which took self-reported allergic symptoms into account, the prevalence rates at early adulthood were similar, except that fewer subjects reported allergic symptoms to dog, 13.4%. There were no sex differences in the prevalence of any reported allergic symptoms at any timepoint in the study.

A part of the explanation for this continuous increase in reported allergic symptoms could be the low degree of remission. Studies on natural remission of sensitization to aeroallergens are infrequent. Results from past studies indicated that complete remission of aeroallergen sensitization was very rare in children with atopic disease (105) and that remission of sensitization to pollen and furry animals in adult subjects with rhinitis was 11% and predicted by low IgE levels at baseline (106, 107).

In late adolescence, there was a trend towards a lower risk of developing allergic symptoms to cat for individuals who had been exposed to cat during the first year of life; similar results have been reported previously (108). No such effect of early exposure to dogs was seen in Study I. Further, in subjects who had developed cat symptoms in late adolescence, significantly fewer were exposed to cat at home. There was no increased exposure to dog or cat at home, either at baseline or at follow-up in early adulthood, for those who developed allergic symptoms, compared with subjects without reported pet symptoms at any assessment.

Elevated exhaled nitric oxide as a risk factor for development of allergic symptoms

The main finding in Study I was that increased levels of FeNO predicted the onset within four years of self-reported allergic symptoms to cat and dog, but not to pollen. Elevated FeNO at baseline probably signaled subclinical Th2-driven inflammation in the airways that preceded the development of allergic symptoms, which had occurred at follow-up in late adolescence. This finding is supported by results from another study showing that low-grade IgE sensitization (IgE antibody concentrations < 0.35 kU_A/L) may precede symptoms (109).

Subjects with self-reported incident allergic symptoms to cat or dog more often reported asthma, wheeze, and rhinitis at baseline compared with subjects without these allergic symptoms. All these conditions are related to higher FeNO and have been discussed in the introduction. Still, the result concerning an association with FeNO was consistent for incident allergic symptoms to cat when excluding all subjects with reported asthma, wheeze, or rhinitis at baseline.

In a Swedish cross-sectional study, cat, dog, and mite sensitization were found to be related to wheeze, whereas timothy sensitization was related to rhinoconjunctivitis (9). Further, sensitization to perennial but not seasonal allergens has been found to be an important determinant of FeNO (42). These results strengthen the results in Study I on differences in the impact of perennial and seasonal allergens on airway inflammation and, consequently, on measured FeNO.

Less than one third of the participants who reported incident allergic symptoms to cat in late adolescence also reported allergic symptoms to dog, while more than three quarters of those with reported incident allergic symptoms to dog also reported allergic symptoms to cat. Thus, it seems that cat is a more common initial sensitizer than dog, and that dog-sensitized individuals are sensitized to more allergens. Further, FeNO correlates to the degree of IgE sensitization, in terms of both the number of positive skin-prick tests (89, 110) and IgE antibody concentrations (111, 112). This may explain why the group with incident allergic symptoms to dog had higher FeNO compared with the cat and pollen groups.

Most of the findings in Study II will be discussed below, but concerning FeNO and the association with IgE sensitization, nearly 80% of the cases, with elevated FeNO, were confirmed to be sensitized, most commonly to cat and dog. At baseline, only 21.1% of the cases reported any allergic symptoms, whereas the proportion had increased to 63.2% about two years later, in late adolescence. There was an imbalance between reported symptoms of allergy and objective measurement of sensitization to specific IgE antibodies in se-

rum. The long-term effect of elevated FeNO in early adolescence was a significant higher prevalence of allergic symptoms in early adulthood compared with controls, 77.8% vs. 5.9%.

Development of respiratory symptoms

The main finding was that the incidence of respiratory symptoms had increased significantly between early adolescence and early adulthood. When stratifying for sex, the incidence of both asthma and wheeze had increased significantly in females but not in males. Objective measurements at baseline revealed that reduced FEV_1 in females and higher FeNO in males were independent risk factors for having developed asthma sixteen years later.

Prevalence and gender differences

In Study IV, the prevalence of asthma, wheeze, and rhinitis in young adulthood was similar to a previously mentioned Swedish cross-sectional study including individuals of comparable ages, 22–40 years (10). Similar findings concerning sex differences in the development of respiratory symptoms through life have been reported previously. In a Finnish population-based questionnaire study, the incidence of asthma peaked in young boys (0–9 years) and in middle-aged women (40–49 years) (113). These results are further confirmed in several birth cohort studies where a male predominance in asthma prevalence was seen before puberty, and a sex shift towards females was seen around puberty (114). This was particularly apparent in females with concurrent asthma and rhinitis (115). Further, three out of four school-aged children with asthma have outgrown the disease by middle age. The risk of persistence of asthma increases with severity, IgE sensitization, smoking, female sex, and a family history of asthma (116-118).

Most subjects with childhood-onset asthma are allergic, whereas most subjects with asthma onset after 40 years of age are non-allergic (31). In a large population-based cohort study of people aged 20–44 years, 65% of the females with incident asthma at follow-up were non-sensitized, compared with 37% of the males. There was no sex difference concerning incidence of allergic asthma during the same period (119).

Several hypotheses have been put forward to explain the increased female susceptibility to the development of respiratory symptoms after puberty. The effect of female hormones, increased AHR (120) and resulting increased vulnerability to environmental exposures like tobacco smoke, and exercise causing exercise-induced dyspnea (121), have been proposed. In a cohort of children with asthma, a significant decrease in bronchial responsiveness was observed after age 11 years in boys, but not in girls (122). The persistent AHR

in girls may be due to hormonal changes, for example, the levels of progesterone and estrogen; one study has shown that exogenous administration of estrogen may reduce AHR (123). However, there have been differences in reported results concerning menopause and respiratory symptoms. Troisi et al. reported that the incidence of asthma tended to decrease after menopause (124) while Triebner et al. reported increased incidence of asthma and respiratory symptoms in postmenopausal women (125). Furthermore, looking at girls with incident wheeze in late adolescence (Study III), a higher proportion had started to menstruate at baseline, compared with girls who neither in early nor late adolescence reported symptoms of wheeze.

Obesity and generally smaller airway caliber (80) have been proposed as other explanatory factors for development of respiratory disease. A few studies have investigated the asthma-obesity association in relation to gender and the results have been mixed, which may be explained by different study designs and ages of study populations (76, 126, 127). Obesity is associated with systemic low-grade inflammation, with, for example, blood leukocytosis and increased serum levels of C-reactive protein, but negatively associated with sputum eosinophils and FeNO (68, 69). In a Swedish study, higher levels of circulating neutrophils were associated with overweight and obesity in girls but not in boys (71). Further, obesity has been associated with neutrophilic airway inflammation in female but not in male adults with asthma, and asthma in obese children has been reported to be mainly non-eosinophilic in girls but not in boys (68, 128).

In Study III, obesity at baseline was found to be an independent risk factor for incident wheeze in girls in late adolescence. Additional risk factors were reported rhinitis at baseline and current smoking at follow-up. In a German follow-up study of adolescents, aged 16–18 years, who had completed an ISAAC questionnaire at two timepoints, active smoking was associated with increased incidence of wheeze within five years (129). Corresponding analyses of boys in Study III showed that allergic symptoms to seasonal allergens, family asthma, and reduced FEV_1 at baseline were risk factors for incident wheeze. Thus, an atopic constitution was associated with development of wheeze in boys, while factors related to non-type 2 inflammation and lifestyle components were important for the development of wheeze in girls.

A review study has concluded that asthma after childhood was more severe in females than in males, and was underdiagnosed and undertreated in female adolescents (74). Results from another study showed that adolescent girls with asthma had lower ACT scores than boys with asthma (63). In a Norwegian study among adolescents with current wheeze, the likelihood of having a doctor's diagnosis of asthma was lower in girls compared with boys, although more girls than boys with current wheeze had AHR. A doctor's diagnosis of asthma was strongly related to increased FeNO (130). Consequently, the results in Study IV showing that the prevalence of wheeze was higher in females than in males, at all three study timepoints, indicated that some of the wheeze

reported by females could be due to undiagnosed and (therefore) untreated asthma, especially during adolescence.

In Study IV, the females with incident asthma did not have higher BMI at baseline and were not more often current smokers compared with females who had no asthma in early adolescence or early adulthood. Nor was there a sex difference concerning smoking at this timepoint. As previously reported, there is no gender difference in incidence of allergic asthma in adult ages. Female asthma is a combination of both the allergic and the non-allergic phenotypes of asthma, while male asthma is mainly allergic. Rhinitis may also be allergic or non-allergic. In Study III, current smoking and higher BMI and rhinitis at baseline were independent risk factors for incident wheeze in females in late adolescence. A decreased study population, approximately 50%, and a more heterogeneous asthma disease development, both allergic and non-allergic, may explain why the risk factors, except rhinitis, were not confirmed as independently related to incident asthma in females in early adulthood in Study IV.

Elevated exhaled nitric oxide as a risk factor for development of respiratory symptoms

In Study II, adolescents with elevated FeNO but without a clinical diagnosis of asthma were significantly more reactive to methacholine than controls. The relationship between FeNO and reactivity to methacholine have been confirmed in other studies (39, 131), but this was the first study to confirm this relationship in subjects where asthma had been excluded after thorough clinical examination. Furthermore, a majority of the cases were IgE-sensitized and had higher blood eosinophil counts than controls. Interestingly, they also had significantly lower serum levels of both HNL and MPO, which are markers of neutrophil activity, compared with controls.

There was a significant correlation between FeNO and eosinophils in blood, which signals systemic inflammation. A limitation of blood cell counts is that they do not provide much information on the cells' involvement in local processes or on the activity of the cells (132). The protein ECP, released when eosinophils are activated, may better reflect the degree of eosinophilic inflammation in the airways and is related to recent asthma exacerbations (133, 134). In Study II, adolescents with elevated FeNO but without a clinical diagnosis of asthma had significantly higher eosinophil counts in blood, but their serum ECP showed only a trend to be higher than in the controls. This can be explained by that their airway inflammation had not yet developed into symptomatic asthma. Furthermore, there were no differences in blood neutrophil counts between the groups, but the cases had significantly lower serum levels of both HNL and MPO, markers of neutrophil activity. There was also a significant negative correlation between FeNO and HNL. IFN-γ is a Th1 cytokine

and is important in both innate and acquired immune responses and seems to prime neutrophils in vivo, leading to an upregulated release of granule proteins upon stimulation (135). Taken together, these results may support the Th1/Th2 dichotomy, with assumed suppressed IFN-γ levels and, consequently, lower neutrophil activity in the pre-asthma group. These results further strengthen FeNO as a sensitive screening method for subjects with pre-asthma or mild asthma.

In Study III, data concerning incident wheeze were stratified by sex and showed that boys, but not girls, with incident wheeze, had higher levels of FeNO at baseline, compared with those who neither at baseline nor at the follow-up in late adolescence reported symptoms of wheeze. Objective measurements of allergic disease (FeNO, AHR, SPT, blood and sputum eosinophil counts, total IgE) have been studied previously in relation to weight, but no associations were found (127, 136, 137). These results strengthen the view that development of wheeze is related to different risk factors in boys and girls, mainly allergy-related ones in boys and non-allergy-related ones in girls.

In Study IV, females with incident asthma did not have elevated FeNO, but instead showed a statistical trend for lower FeNO, compared with females without asthma at baseline and follow-up. Males with incident asthma had significantly higher FeNO than males without asthma at all assessments. The risk factors for incident asthma in early adulthood in males were all related to an atopic constitution, including higher FeNO, rhinitis, and family asthma. All these data support the view that female and male respiratory symptoms are, at least in part, related to different mechanisms.

Reduced lung function as a risk factor for development of respiratory symptoms

There was a strong relationship between FeNO and self-reported allergic and asthma symptoms at baseline, but no association between these symptoms and FEV_1 (49).

In Study II, cases and controls had similar FEV_1 values at both study visits, despite confirmed airway inflammation in cases, as evidenced by significantly higher FeNO. A likely explanation is that elevated FeNO is an early sign of airway disease, with no impact on lung function at this stage.

In Study III, there was no difference, for either sex, in FEV_1 % predicted for those with and those without incident wheeze in late adolescence. When instead studying the number of subjects with reduced FEV_1 at baseline, more boys with incident wheeze than without had reduced FEV_1, and reduced FEV_1 was an independent risk factor for incident wheeze in males in the multiple logistic regression analyses.

In the 16-year follow-up, presented in Study IV, no difference concerning FEV_1 % predicted was found in relation to development of asthma. Again,

reduced FEV_1 seemed more specific for identifying subjects with impaired lung function. Females with incident asthma significantly more often had reduced FEV_1 at baseline compared with females who did not develop asthma. Reduced FEV_1 at baseline was a confirmed risk factor for having developed asthma sixteen years later in female adults. Other risk factors were rhinitis and family rhinitis. The greater susceptibility of females to develop respiratory symptoms may be caused by their generally narrower airways. Irreversible airflow obstruction, developed in early childhood during periods of bronchial obstruction, and with symptom recurrence in adult life, may be another explanation (138). Therefore, in Study IV, a period of remission in early adolescence in females, characterized by reduced FEV_1 but no reported symptoms of asthma, may be an explanation for the finding that reduced lung function at baseline was a confirmed independent risk factor for the development of symptomatic asthma sixteen years later.

Self-rated health

Results from studies using asthma-specific instruments have shown that adolescents with asthma have impaired HRQoL compared with adolescents without asthma (139, 140). In one of the studies, adolescents with symptomatic asthma reported worse HRQoL compared with those with non-symptomatic asthma and those without asthma. Further, subjects with non-allergic asthma rated their quality of life lower than subjects with allergic asthma (140). In similar studies, when taking impact of gender into account, girls with current asthma had lower HRQoL than girls without asthma, but this difference was not seen among boys (141). Further, risk factors for decreased HRQoL were female sex, decreased ACT, severe asthma, and having current eczema (63).

In a Swedish population-based study, SRH was associated with asthma and the association was at least as strong as that for asthma-related quality of life, with the advantage of being easier to apply (142). Other factors that have been found to be related to poor SRH are lower education and unemployment in women. Further, tobacco use in younger ages, and overweight and low physical activity in all ages relate to poor SRH (143). The impact that allergy have on SRH has also been studied and poor self-rated health was found to be associated with high levels of IgE against food allergens, and seasonal and perennial aeroallergens (144).

All these findings are in line with the results in Study IV: females with current asthma, wheeze, and allergic symptoms to cat in early adulthood, rated their health lower than females without these symptoms, while such results were not seen for males.

Methodological considerations

Strengths and limitations

Study population and participation rates

The major strength of this thesis is that all data were based on the SPAIS cohort, a population-based study of nearly 1,000 schoolchildren, with the relatively long follow-up period of sixteen years. The nine schools involved were randomized and turned out to be from different socio-economic neighborhoods, as well as representing both urban and rural settings. At the four-year follow-up, SPAIS II, 921 of the adolescents (96%) completed and returned the questionnaire, and, at SPAIS III, 502 did (52%). Results from data at SPAIS I and II are quite well generalizable to adolescents aged 12–15 years and 16–19 years as the participation rates were comparatively high, and the loss to follow-up at SPAIS II was very limited. At SPAIS III, the group lost to follow-up included 48% of the subjects in the baseline cohort and may have introduced some selection bias. However, this response rate is similar to other cross-sectional, epidemiological studies (113, 145) and longitudinal studies with similar follow-up times (146). When data from Study IV were analyzed, non-responders did not differ significantly in any baseline characteristics when compared with responders, with the exception of a higher proportion of males and subjects with family smoking history; similar findings have been reported previously (147).

A strength of the case-control study may be the homogenous group of participants. They were all of the same age, matched by sex, all non-smokers, and all exhibited normal lung function values, both at baseline and at the two study visits. Already at study inclusion of participants, some known subject-related factors (sex, age, lung function, respiratory infection, smoking habits) that affect both FeNO and methacholine responsiveness were controlled for (36, 148). A limitation of the study may be that, in spite of the fact that a large material was screened (959 subjects), only a small group of interest (subjects without asthma and with elevated FeNO) was identified. In Study II, long-term effects of elevated FeNO on the development of respiratory and allergic symptoms were studied. In SPAIS II, all subjects participated, but in SPAIS III, only 9 cases and 17 controls completed the questionnaires, which may affect the validity of the results.

Questionnaire data and definitions

Another strength is the use of the well-validated, globally used ISAAC questionnaire, at all three timepoints. Self-reported symptoms of rhinitis and wheeze were assessed using the ISAAC questionnaire, while allergic symptoms were assessed using study-specific additional questions, which, however, were not validated. ISAAC does not include any questions concerning current asthma. The definition of asthma used in this thesis was based on a combination of included questions (see page 23) and was largely in line with the recommendations of a review study concerning different definitions of asthma, based on the results from 117 studies where nearly 50% had used the questions in the ISAAC questionnaire (149).

Use of questionnaires has limitations as results will depend on how individual participants interpret the questions and how they grade experienced symptoms. Furthermore, some additional bias might have been introduced by the fact that parents reported their children's allergic symptoms at baseline, while the participants themselves reported their allergic symptoms at follow-ups. There is a risk of report bias, as the parents may not perceive the child's symptoms appropriately. Another bias may have been introduced at SPAIS III when a question was changed to "have you ever experienced any allergic symptoms to cat, dog, or pollen?", instead of asking for *current* symptoms. To obtain larger groups for statistical analyses, the variables family asthma and family rhinitis were created and included data on mother's, father's, and siblings' reported symptoms.

Objective measurements

Another strength was the availability of FeNO and lung function measurements for all subjects in the cohort at baseline. Measurements of FeNO, lung function, height, and weight, and performance of SPT, MCT, blood sampling, and induced sputum processing were carried out by the same person, the author of this thesis, providing favorable conditions for high consistency of data. No measurements of FeNO or lung function were performed when the participants had respiratory infections or signs of allergic reactions, mainly during the pollen season. Further, adolescents with $FeNO_{0.1}$ between 15 and 20 ppb were asked to undergo an additional FeNO measurement during the same month that the examinations were performed at school, to confirm the FeNO results.

A limitation may be the use of 100 mL/s as exhalation flow rate for FeNO measurements; this was the standard flow rate at the time of SPAIS I. The FeNO values in these studies may not be adapted to current clinical practice. Still, the validity of the findings of an association between FeNO and incident allergic symptoms to cat and dog in a four-year timeframe, and incidence of

asthma in males after 16 years should not be impaired. In Study II, FeNO measurements were performed using flow rates of both 50 mL/s and 100 mL/s.

Furthermore, the current reference values of the Global Lung Function Initiative are preferred when performing lung function measurements and were used in Studies I, III, and IV. In Study II, reference values published by Knudson were used.

A limitation of Study I may be the lack of information on IgE sensitization and that SPT results were available only for a subpopulation. However, the study focused on allergic symptoms and not on IgE-mediated sensitization, and the SPT results were used only to validate the specificity of the questions regarding parent- or self-reported allergic symptoms. The available data showed a poor relationship between a positive SPT result and parent-reported ongoing allergic symptoms at baseline, or incident self-reported symptoms at follow-up.

Data on weight and height at baseline were obtained through direct physical measurements, using the same equipment for all participants, which should yield greater reliability than the use of self-reported values. Unfortunately, no data on height and weight were reported at any follow-up included in this thesis.

Conclusions

Between early adolescence and early adulthood, females and males follow different paths in the development of respiratory symptoms, which are associated with different risk factors in the two sexes. No such difference was observed concerning the development of allergic symptoms to cat, dog, or pollen, at any assessment.

The following specific conclusions can be drawn from the studies included in the thesis:

- Increased levels of exhaled nitric oxide in early adolescence seemed to indicate an increased risk of development of allergic symptoms to cat and dog within a four-year timeframe.
- Elevated levels of exhaled nitric oxide in non-asthmatic adolescents were associated with a higher degree of sensitization, higher blood eosinophil count, airway hyperresponsiveness, and reduced systemic neutrophil activity. The long-term effect of elevated FeNO, in this group of non-asthmatic adolescents, was increased prevalence of ever wheeze and allergic symptoms compared with in controls.
- Data stratified by gender showed that obesity in girls and an atopic constitution in boys were independently associated with the development of wheeze within four years during adolescence.
- The incidence of asthma and wheeze between early adolescence and early adulthood was higher in females than males, whereas the incidence of allergic symptoms showed no sex difference. Reduced FEV_1 at baseline was related to incidence of asthma in females, whereas higher FeNO was associated with incidence of asthma in males.
- Females with current asthma, wheeze, and allergic symptoms to cat in early adulthood, more often rated their health as poor compared with females without these symptoms. There was no significant difference in ratings between males with and without asthma, wheeze, and allergic symptoms to cat.

Clinical implications and future research

It is important to detect risk factors for the development of respiratory and allergic disease at an early stage, because these diseases constitute a major burden for many individuals, their families and, not least, for society.

In these studies, higher FeNO among younger adolescents was found to signal an increased risk of development of perennial allergies among both females and males in late adolescence, and of asthma among males in early adulthood. FeNO screening of schoolchildren at certain critical ages, aimed at identifying those at risk of developing allergic asthma, followed by a proper clinical investigation of subjects with elevated FeNO, may be advantageous for public health. This needs to be investigated in the context of health economy aspects.

It would be beneficial if healthcare providers possessed adequate knowledge of health in adolescence and were familiar with psychosocial developments during this period. Awareness of the sex differences concerning development of respiratory symptoms through life and that risk factors may differ between the sexes is important. Asthma in males is mainly allergic and objective measurements, such as IgE antibodies in serum and FeNO, as well as effective medical treatments are available. In females, who more often have a non-allergic phenotype of asthma, other investigations should be considered, such as tests of airway hyperresponsiveness and exercise-induced bronchoconstriction. Obesity and smoking should not be assumed as explanations for breathing problems in females, without thorough examination. Further studies are needed, for example to increase the understanding of how respiratory symptoms vary during the menstrual cycle as well as understanding of the relationship between higher BMI and the development of respiratory symptoms in females.

Both women and adolescent girls rate their quality of life, generally and in case of asthma, lower than males and boys. In order to optimize health and well-being, this knowledge is important and should influence the priorities of healthcare providers in their work with these patient groups. Adolescent girls and young women should be offered appropriate support. ACT, as a measurement of asthma control, is a well-validated questionnaire related to asthma-specific quality of life and should be used frequently at all levels of healthcare. For optimal data, it should be combined with an asthma-specific HRQoL questionnaire.

Analyses of risk factors related to the development of allergic symptoms, in a sixteen-year time frame, were not included in these studies. Such analyses would be of interest for future research and might add valuable information and understanding on the development of such symptoms.

A subgroup of subjects (n = 193), who participated in the third questionnaire part of SPAIS, performed a clinical follow-up visit at the Uppsala University Hospital during 2015–2016. Measurements of height, weight, waist

circumference, FeNO (measured at 50–300 mL/s), nasal NO, forced oscillation technique, single breath nitrogen wash out, lung function, and mannitol airway provocation tests were performed and blood was sampled. The collection of these data will enable further studies in this field and comparisons on changes in objective measurements of FeNO, FEV_1, and BMI, over a period of eighteen years. Additionally, data on airway responsiveness, IgE sensitization, and inflammatory markers are available and may also be studied in relation to self-reported symptoms of allergic and respiratory disease. Furthermore, data from measurements of FeNO (measured at multiple exhalation flow rates), nasal NO, forced oscillation technique, and single breath nitrogen washout may contribute with in-depth knowledge on the long-term effects of elevated FeNO on airway inflammation and lung function.

Populärvetenskaplig sammanfattning

Bakgrund

Allergiska sjukdomar är mycket vanliga och antalet barn och ungdomar som drabbas ökar både i Sverige och i övriga världen. Andelen barn som är sensibiliserade, det vill säga att immunförsvaret har bildat IgE-antikroppar mot olika allergiframkallande ämnen, har också ökat. Idag är 30–40 % av alla yngre tonåringar sensibiliserade mot något luftburet allergen. Alla har dock inte symptom, trots sensibilisering, och idag har 10 % astma, 10 % allergiska näsbesvär och 13 % eksem i den åldersgruppen.

Astma är den vanligaste kroniska sjukdomen under barndomen i nästan alla industrialiserade länder. Sjukdomsbilden karaktäriseras av perioder av andningsbesvär med pipande och väsande andning. Grundorsaken till astma anses vara inflammation i luftvägsslemhinnan. Denna inflammation kan orsakas av till exempel allergen eller infektioner. På sikt kan denna inflammation också medföra en kroniskt nedsatt lungfunktion. Det finns olika typer av astma. Den allergiska astman är vanligare under barndomen och hos pojkar, medan den icke-allergiska astman är vanligare hos vuxna och kvinnor.

Det finns idag flera metoder för att undersöka och diagnosticera astma. Att mäta kvävemonoxid i utandningsluft (FeNO) som mått på inflammation i luftvägsslemhinnan, är en relativt ny metod. I studier har man sett att FeNO är förhöjt hos personer med astma och allergier. Förhöjda FeNO-värden kan också förekomma utan luftvägsbesvär och har visat sig innebära en ökad risk för senare utveckling av astma, luftvägssymtom och näsbesvär.

Spirometri är idag den vanligaste metoden för att mäta lungfunktion. För att mäta en persons känslighet i luftvägsslemhinnan kan provokationer göras med luftvägsirriterande ämnen, till exempel metakolin. Genom att en patient andas in metakolin i ökande doser och genomgår upprepade lungfunktionsmätningar kan graden av luftvägskänslighet bedömas.

Det är också viktigt att utvärdera hur personer med astma upplever sin allmänna hälsa, sin sjukdom och dess inverkan på vardagen. Enkla test för att utvärdera dessa upplevelser är ”Astma kontroll test” och ”Självskattad hälsa”.

Syfte och metod

Det övergripande syftet med den här avhandlingen har varit att studera utvecklingen av allergiska besvär och luftvägssymtom, från tidiga tonår till tidig vuxen ålder, och att undersöka vilka riskfaktorer som kan förknippas med uppkomsten av dessa besvär.

För att möjliggöra denna studie användes insamlad data från en kohort av skolbarn, som vid starten 1998–1999 gick i årskurs 7 (12–15 år), i nio slumpvis utvalda skolor i Uppsala kommun. Totalt deltog 959 ungdomar i denna baslinjestudie. Mätningar av lungfunktion och FeNO genomfördes och ungdomarna besvarade ett formulär med frågor angående upplevda besvär från luftvägar och näsa, samt allergiska symptom mot katt, hund och pollen. För att följa utvecklingen av symptom skickades sedan liknande frågeformulär ut till alla deltagare, fyra år (vid 16–19 års ålder) och sexton år (vid 28–31 års ålder) efter undersökningen i tidiga tonår.

Resultat

I Studie I deltog alla de 921 ungdomar som hade besvarat frågeformuläret även vid 16–19 års ålder. Målet med studien var att undersöka om förhöjt FeNO, mätt vid baslinjeundersökningen, hade inneburit en ökad risk för att utveckla nya allergiska symtom. Resultaten från studien visade att ungdomar som vid uppföljningen hade utvecklat allergiska symptom mot katt och hund, men inte mot pollen, hade mycket högre FeNO vid baslinjeundersökningen, jämfört med ungdomar som aldrig hade rapporterat upplevda allergiska besvär mot katt eller hund.

För att få fördjupade kunskaper om förhöjt FeNO och hur det påverkar de drabbade, genomfördes Studie II. Ungdomar som vid baslinjestudien uppvisade eller rapporterade misstänkta besvär erbjöds ett uppföljande besök på Akademiska barnsjukhuset i Uppsala. Bland annat bedömdes om en ungdom hade astma eller inte, utifrån uppgjorda kliniska kriterier. Nitton ungdomar med förhöjt FeNO, som vid bedömningen hade konstaterats inte ha astma, kallades till sjukhuset för att mäta luftvägskänslighet och tecken på olika typer av inflammation i blod och luftvägsslem. Dessutom togs blodprov för att mäta IgE-sensibilisering mot olika luftburna allergen. Andra ungdomar i kohorten (28 stycken) med lågt FeNO och utan luftvägs- eller allergisymptom, så kallade friska kontroller, undersöktes på samma sätt som jämförelse. Resultaten från denna studie visade att ungdomarna med högt FeNO mycket oftare var IgE-sensibiliserade, hade ökad luftvägskänslighet och i högre grad hade tecken på allergisk inflammation i blod, jämfört med de friska kontrollerna. För att studera långtidseffekten av detta förhöjda FeNO analyserades data från uppföljningarna, efter fyra och sexton år, gällande aktuell förekomst av rapporterade besvär. Ungdomarna med förhöjt FeNO rapporterade vid bägge tillfällen markant ökad förekomst av allergiska besvär och luftvägssymtom, jämfört med kontrollgruppen.

I Studie III ingick alla ungdomar som besvarade enkäten vid 16–19 års ålder, och som vid baslinjeundersökningen inte hade rapporterat att de hade haft symtom i form av pipande eller väsande andning under det senaste året, det vill säga 795 ungdomar. I studien framkom att det fanns könsskillnader. Riskfaktorerna för att fyra år senare ha utvecklat dessa luftvägsbesvär var olika för

pojkar och flickor. Fetma, näsbesvär och rökning vid tidpunkten för uppföljningen var de faktorer som ökade risken hos flickor medan rapporterad pollenallergi, astma i familjen samt sänkt lungfunktion var riskfaktorerna hos pojkar. I Studie IV ingick de 491 personer som hade deltagit i alla tre undersökningarna. Det hade skett en betydande ökning av både rapporterade allergiska besvär och luftvägssymtom under dessa sexton år. Åter visade resultaten på könsskillnader och att kvinnorna hade utvecklat astma samt pipande och väsande andning i högre grad än männen. Riskfaktorer i tidiga tonår för att utveckla astma som ung kvinna var försämrad lungfunktion, näsbesvär och näsbesvär i familjen. Motsvarande riskfaktorer för unga män var förhöjt FeNO, näsbesvär och astma i familjen. Gällande utveckling av allergiska besvär sågs en betydande ökning men inga könsskillnader. Kvinnor med astma hade lägre självskattad hälsa än kvinnor utan astma, men detta resultat sågs inte hos män.

Slutsatser

Utvecklingen av luftvägsbesvär, men inte av allergiska besvär, följer olika mönster hos kvinnor och män under tonårstiden och fram till tidig vuxen ålder, och påverkas av delvis olika riskfaktorer. De faktorer som var relaterade till uppkomst av astma hos de unga vuxna kvinnorna var sådana som till stor del ses vid icke-allergisk astma, medan faktorer kopplade till uppkomst av allergisk astma i högre grad sågs hos de unga vuxna männen. För att kunna optimera vården av och välbefinnandet hos astmatiker måste dessa könsskillnader tas i beaktande.

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Paper I

ORIGINAL ARTICLE

Elevated Exhaled Nitric Oxide in Adolescents Is Associated With Incident Allergic Symptoms: A Prospective Cohort Study

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Abstract

Background: Fractional exhaled nitric oxide (FeNO) is a marker of type-2 inflammation in the airways. Elevated FeNO may precede the development of allergic disease. The aim of the present study was to investigate the association between elevated FeNO and the development of allergic symptoms.
Methods: A total of 959 adolescents from the general population and their parents completed a standardized questionnaire. Lung function and FeNO were assessed at baseline. Four years later, 921 of these individuals (96%) completed the same version of the baseline questionnaire.
Results: Adolescents with self-reported incident allergic symptoms to cat (n=50) or dog (n=33) had higher baseline FeNO (*P*<.001) than those without allergic symptoms to cat and dog at both time points (n=776 and n=838, respectively). Adolescents with incident allergic symptoms to pollen did not have elevated baseline FeNO. The adjusted odds ratio (aOR [95%CI]) for incident allergic symptoms to cat was 4.2 (2.2-8.0) times higher if FeNO was >75th percentile (vs <75th percentile) at baseline. This was consistent after exclusion of individuals with reported asthma, wheeze, or rhinitis at baseline (8.6 [3.0-24.1]).
Conclusion: Elevated FeNO in adolescents was associated with an increased risk of developing allergic symptoms to cat and dog allergens, but not to pollen allergens, after 4 years.

Key words: Adolescents. Breath test. Epidemiology. Hypersensitivity. Incidence. Nitric oxide.

Resumen

Introducción: La fracción de óxido nítrico exhalado (FeNO) es un marcador de inflamación de tipo 2 en las vías respiratorias y un valor de FeNO elevado puede preceder al desarrollo de enfermedad alérgica. El objetivo del presente estudio fue investigar la asociación entre FeNO elevado y el desarrollo posterior de síntomas alérgicos.
Métodos: Un total de 959 adolescentes, procedentes de población general, respondieron, junto con sus padres, a un cuestionario estandarizado, realizaron una prueba de función pulmonar y una medición de FeNO en una visita basal. Cuatro años después, 921 de estos sujetos (96%) completaron, la misma versión, en gran medida, del cuestionario de referencia.
Resultados: Los adolescentes con síntomas alérgicos incidentes autoinformados por gato (n = 50) o perro (n = 33) tenían mayor FeNO inicial (p <0,001) que los sujetos sin síntomas alérgicos por estos alérgenos, en cualquier momento del estudio (n = 776 y n = 838, respectivamente). Por el contrario, los adolescentes con síntomas alérgicos incidentes por polen no presentaban un FeNO inicial elevado. La razón de riesgo ajustada [aOR (intervalo de confianza del 95%)] para síntomas alérgicos incidentes por gato fue 4,2 (2,2, 8,0) veces mayor si el FeNO fue mayor que percentil 75 de la muestra (vs. menor del percentil 75) al inicio del estudio. Este resultado se mantuvo también después de la exclusión de los sujetos con asma, sibilancias o rinitis notificados al inicio del estudio [aOR (IC 95%) 8,6 (3,0, 24,1)].
Conclusiones: El FeNO elevado en adolescentes se relacionó con un mayor riesgo de desarrollar en los cuatro años siguientes síntomas alérgicos inducidos por gatos y perros, pero no por los alérgenos del polen.

Palabras clave: Adolescentes. Pruebas en aire exhalado. Epidemiología. Hipersensibilidad. Incidencia. Óxido nítrico.

Introduction

In the early 1990s, fractional exhaled nitric oxide (FeNO) was reported to be elevated in asthma [1]. Several studies have reported correlations between FeNO and blood and sputum eosinophils, as well as the degree of airway hyperresponsiveness [2,3]. Recent studies indicate that FeNO is more representative of type-2 inflammation, which is specifically related to the expression of interleukin (IL) 4 and IL-13 in the bronchial mucosa, than general eosinophilic inflammation [4]. FeNO is a marker of short-term changes in type-2 inflammation of the airways, but is also associated with long-term changes in IgE-antibody concentrations [5]. Furthermore, measurement of FeNO is considered a useful tool for identifying the atopic phenotype among asthmatics [4], as well as corticosteroid-sensitive inflammation in the airways [6].

Allergic sensitization is associated with increased FeNO in both the general population and asthma patients [7,8]. Moreover, exhaled NO correlates with the degree of IgE-mediated sensitization, in terms of both the number of positive skin prick test (SPT) results [9,10] and IgE antibody concentrations [11,12]. We previously reported the association between upper airway symptoms such as rhinitis, rhinoconjunctivitis, and asthma and increased exhaled NO in the present cohort at baseline [13].

Increased FeNO has been reported in both children and adults with no confirmed respiratory symptoms and is related to an increased risk of developing wheeze; thus, it may indicate subclinical inflammation or "early asthma" [13-15]. We previously reported that the adolescents with elevated NO levels in the present cohort had an increased likelihood of new-onset rhinitis within a 4-year follow-up period [16].

To our knowledge, no studies have assessed the utility of exhaled NO in predicting the incidence of allergic symptoms to airborne allergens. The aim of this study was to evaluate the predictive value of FeNO for self-reported incident allergic symptoms to cat, dog, or pollen within a 4-year time frame in a large population-based cohort of adolescents.

Materials and Methods

General Design

Baseline data were collected during 1998-1999 from 959 individuals aged 12-15 years attending 9 schools selected at random in Uppsala, Sweden. The study, *Screening Project Asthma in Schools* (SPAIS I), has previously been described in detail [13]. The pupils completed a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) [17,18], and lung function and FeNO were measured. Parents answered additional written questions about their children concerning hypersensitivity to cat, dog, or pollen allergens, diagnosis of asthma, asthma medication, atopic disease in childhood, family history of asthma and rhinitis, family smoking, and environmental issues. If FeNO and FEV_1 were assessed between March and September, then the measurements were considered to have been taken during the pollen season; otherwise they were not. The follow-up study, SPAIS II, with an identical ISAAC questionnaire and identical questions concerning hypersensitivity to cat, dog, and pollen allergens, diagnosis of asthma, asthma medication, environmental issues, family smoking, and an additional question concerning own smoking was performed 4 years later (2002-2003). In this case, the adolescents completed the questionnaires themselves.

Questionnaires and Definitions

At baseline, allergic symptoms were defined as the individual's hypersensitivity to cat, dog, or pollen allergens, as noticed and reported by the parents. For negative answers, there was an additional question regarding parental suspicion of hypersensitivity to cat, dog, or pollen allergens. Allergic symptoms at follow-up were defined as above but reported by the participant and only at the level of the question "*Have you noticed hypersensitivity to cat, dog, or pollen*?". Incident allergic symptoms were reported at follow-up but not at baseline.

Asthma was defined as ever having had parent- or self-reported asthma plus having received corticosteroids for treatment of asthma or having wheeze and whistling in the chest (ISAAC) or having a respiratory infection that caused wheeze and whistling in the chest during the previous year (ISAAC). Wheeze was defined as having had wheeze or whistling in the chest at any time in the previous year (ISAAC). Rhinitis was defined as having had sneezing, nasal congestion, or rhinorrhea during the previous 12 months, without having a cold (ISAAC).

Asthma and rhinitis in the family were assessed using a questionnaire, with separate questions for the mother, father, and siblings. Family smoking, the individual's own current smoking habits during follow-up, and exposure to cat and dog allergens at home were also assessed using a questionnaire.

Exhaled NO Measurements

Measurements of FeNO were performed using the Aerocrine NO system (Aerocrine AB), including the CLD 77 AM chemiluminescence analyzer (Eco Physics AG), as previously described [13] and in accordance with the recommendations of the European Respiratory Society [19]. Before measurement, each participant's mouth was washed with 25 mL of 10% sodium bicarbonate for 20 seconds. Three exhalations of 10 seconds each were performed, and an average value was calculated. A recent study has shown high reproducibility of FeNO measurements and no need for further repeated measurements during the same session [20]. FeNO was measured at 0.1 L/s. To help interpret the FeNO values in this paper, one may transform the $FeNO_{0.1}$ values to obtain a rough estimate of $FeNO_{0.05}$ by multiplying them by a coefficient of approximately 1.6 [21].

Pulmonary Function

Pulmonary function was assessed in accordance with the criteria of the American Thoracic Society using a Spirolab spirometer (Medical International Research). The lower limit of normal, Z-scores, and percentiles for FEV_1 were calculated for each individual in the study population based on the Excel macro for The Global Lung Function Initiative (GLI) [22] reference values.

Skin Prick Test

SPTs were performed in a subsample (n=374) at baseline, as previously described [9]. This subsample consisted of all volunteering participants from 2 of the schools, as well as adolescents from all the other schools who had reported asthma or symptoms suggestive of asthma or had $FeNO_{0.1}$ values ≥15 ppb or FEV_1 % predicted <80%, and attended a clinical follow-up visit within 2 months from the baseline examination at school. The most common airborne allergens in the area (cat, dog, birch pollen, and timothy pollen) were tested (Soluprick, ALK), together with *Dermatophagoides pteronyssinus*, which is uncommon in this part of Sweden. Since only 3.3% had positive SPT results for *D pteronyssinus*, these data were not included in further analyses. A positive SPT result was defined as a mean wheal diameter of at least 3 mm after 15 minutes [23].

Statistics

Statistical analyses were performed using STATA 1C 14 (StataCorp). Comparisons at the group level were performed using the *t* test for normally distributed continuous variables or χ^2 tests for categorical variables. FeNO was log-transformed to achieve a normal distribution before the *t* tests. Multiple logistic regression analyses were performed with incident allergic symptoms as dependent variables, FeNO, and relevant confounders identified as significant ($P<.05$) in the univariate analyses were independent variables. Height, which is the best determinant of FeNO in healthy schoolchildren [24], and FeNO measurement during the pollen season were additionally used as independent variables. Furthermore, a model was created where a FeNO value above arbitrary levels (50th percentile, 75th percentile, and 90th percentile) was used as an independent variable of incident allergic symptoms to cat and dog, respectively, after adjustments for confounders identified in previously described univariate analyses. A P value <.05 was considered statistically significant.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University, Sweden (registration numbers 243/1998, 499/2001). Informed consent was obtained from the children and their legal guardians.

Results

A total of 921 of the 959 participants (96%) completed the questionnaire in SPAIS II. There were 38 (4%) nonresponders, who differed only with regard to having higher FEV_1 % predicted and having reported less wheeze at baseline than those who participated in SPAIS II (Supplementary Table 1). Questions concerning smoking were only addressed to the mother, father, and older siblings in SPAIS I, although in SPAIS II there was an additional question concerning current self-reported smoking, whose frequency was found to be 8.9%.

The prevalence of wheeze, but not asthma, rhinitis, and allergic symptoms to cat, dog, and pollen allergens, increased between baseline and the end of follow-up (Table 1).

Table 1. Individuals' Characteristics at Baseline (SPAIS I) and After Follow-up (SPAIS II)[a]

	SPAIS I (n=921)	SPAIS II (n=921)	*P* Value
Male sex, %	49.5		
Age, y	13.6 (0.41)		
FeNO0.1, ppb	4.78 (4.47-5.10)		
FEV_1, % predicted	94.8 (10.8)		
Height, cm	162.4 (8.1)		
Weight, kg	52.8 (10.5)		
Asthma, %	8.7	7.9	.19
Wheeze, %	13.7	16.4	.04
Rhinitis, %	25.3	31.2	.001
Allergic symptoms to cat, %	10.3	14.7	<.001
Allergic symptoms to dog, %	5.4	7.6	.003
Allergic symptoms to pollen, %	17.5	23.7	<.001
Family members smoking, %	32.5	31.8	.59

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95%CI.

FeNO measured during the pollen season (March to September) was significantly higher (P=.03) than FeNO measured between October and February.

Incident Allergic Symptoms to Cat

Participants with self-reported incident allergic symptoms to cat had significantly higher FeNO values at baseline than those without parent- or self-reported symptoms to cat at both assessments. When individuals with parent-reported suspected hypersensitivity to cat at baseline were excluded (n=14), this result remained significant ($P<.001$). The group with self-reported incident allergic symptoms to cat had higher FEV_1 % predicted, more frequent parent- and self-reported asthma, wheeze, rhinitis, and allergic symptoms to dog and pollen allergens at baseline. Furthermore, in the incident group, fewer individuals had undergone FeNO measurements during the pollen season than individuals who never reported any allergic symptoms to cat (Table 2). During follow-up, 30% of participants with self-reported incident allergic symptoms to cat also self-reported allergic symptoms to dog and 60% to pollen. However, the number of individuals with parent- and self-reported evidence of having a cat at home decreased over the 4 years in this group (from 26% to 18%). At follow-up, but not at baseline, there was a significant difference in having a cat at home (P=.04), with a lower prevalence in the incident group than among individuals who never developed allergic symptoms to cat.

Incident Allergic Symptoms to Dog

Participants with self-reported incident allergic symptoms to dog had significantly higher FeNO values and more

Table 2. Individuals' Characteristics at Baseline in Relation to Incident Allergic Symptoms to Cat (SPAIS II)[a]

	No Allergic Symptoms to Cat at Baseline or After Follow-up (n=776)	Incident Allergic Symptoms to Cat (n=50)	*P* Value
Male sex, %	49.7	42	.29
Age, y	13.6 (0.41)	13.6 (0.39)	.42
$FeNO_{0.1}$, ppb	4.17 (3.90-4.46)	6.89 (4.93-9.62)	< .001
FEV_1, % predicted	94.7 (10.6)	98.0 (11.5)	.03
Height, cm	162.5 (8.1)	160.2 (6.6)	.05
Weight, kg	52.6 (10.3)	52.4 (8.8)	.89
Asthma, %	3.7	14.0	.001
Wheeze, %	8.5	20.0	.006
Rhinitis, %	18	52.0	<.001
Allergic symptoms to dog, %	0.4	8.0	< .001
Allergic symptoms to pollen, %	10.6	30.0	<.001
Exposure to cat, year 1, %	21.3	10.0	.06
Exposure to cat, SPAIS I, %	30.2	26.0	.53
Exposure to cat, SPAIS II, %	31.7	18.0	.04
FeNO measurements inside pollen season, %	36.6	22.0	.04
Ever asthma (mother), %	9.1	14.0	.26
Ever asthma (father), %	8.1	12.0	.34
Ever asthma (siblings), %	13.5	12.0	.76
Ever allergic rhinitis (mother), %	24.4	34.0	.13
Ever allergic rhinitis (father), %	22.7	22.0	.91
Ever allergic rhinitis (siblings), %	19.1	20.0	.87

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95% confidence interval.

Table 3. Individuals' Characteristics at Baseline in Relation to Incident Allergic Symptoms to Dog (SPAIS II)[a]

	No Allergic Symptoms to Dog at Baseline or After Follow-up (n=838)	Incident Allergic Symptoms to Dog (n=33)	*P* Value
Male sex, %	49.6	45.5	.64
Age, y	13.6 (0.41)	13.6 (0.38)	.96
$FeNO_{0.1}$, ppb	4.37 (4.10-4.67)	9.60 (7.26-12.69)	<.001
FEV_1, % predicted	94.9 (10.6)	94.7 (10.2)	.91
Height, cm	162.3 (8.1)	162.8 (8.1)	.68
Weight, kg	52.5 (10.4)	55.3 (10.0)	.14
Asthma, %	5	24.2	<.001
Wheeze, %	10.1	39.4	<.001
Rhinitis, %	21.7	60.6	<.001
Allergic symptoms to cat, %	4.7	39.4	<.001
Allergic symptoms to pollen, %	12.9	51.5	<.001
Exposure to dog, year 1, %	14.7	18.2	.58
Exposure to dog, SPAIS I, %	21.4	24.2	.69
Exposure to dog, SPAIS II, %	22.8	21.2	.83
FeNO measurements inside pollen season, %	35.7	39.4	.66
Ever asthma, (mother), %	9.4	21.2	.03
Ever asthma (father), %	8.5	18.2	.05
Ever asthma (siblings), %	13.8	27.3	.03
Ever allergic rhinitis (mother), %	25.4	45.5	.01
Ever allergic rhinitis (father), %	24.1	27.3	.68
Ever allergic rhinitis (siblings), %	19.9	27.3	.30

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.
[a]All results are presented as % or mean (SD) or geometric mean and 95% confidence interval.

frequent parental- and self-reported asthma, wheeze, rhinitis, and allergic symptoms to cat and pollen at baseline than those without allergic symptoms to dog at both time points. After excluding individuals with parent-reported suspected hypersensitivity to dog at baseline (n=5), there was still a significant difference in FeNO (*P*<.001). A family history of asthma was more common in the incident group, as was rhinitis reported by the mother (Table 3). Among participants with self-reported incident allergic symptoms to dog, 76% self-reported allergic symptoms to cat and 55% to pollen in SPAIS II. There was no difference in the number of individuals with parent- and self-reported evidence of having a dog at home, at baseline or follow-up.

Incident Allergic Symptoms to Pollen

Participants who self-reported incident allergic symptoms to pollen more often had parent- and self-reported asthma, wheeze, rhinitis, allergic symptoms to cat, and allergic rhinitis

among siblings at baseline than individuals without parent- and self-reported allergic pollen symptoms at both time points. There was no significant difference regarding FeNO, for parent-and self-reported incident hypersensitivity to pollen (P=.08) (Supplementary Table 2) or when individuals with suspected hypersensitivity to pollen at baseline were excluded (P=.11). Among participants who self-reported incident allergic symptoms to pollen at SPAIS II, 29% also self-reported allergic symptoms to cat and 6% to dog.

Multivariate Analysis

Multiple logistic regression analysis revealed that elevated FeNO at baseline was independently related to self-reported incident allergic symptoms to cat (P<.001), after adjustments were made for possible confounders (see Material and Methods). Similarly, elevated FeNO values were independently related to self-reported incident allergic symptoms to dog (P<.048).

Analyses based on arbitrary FeNO cut-offs showed that having a FeNO value above the 50th, 75th, or 90th percentile was related to incident allergic symptoms to cat after the same adjustments as above. Except for FeNO >50th percentile, which only tended towards significant, this association held for individuals without parent- and self-reported asthma, wheeze, or rhinitis at baseline (18 individuals with incident self-reported allergic symptoms to cat remained in these analyses) (Table 4).

Similarly, having a FeNO above the 50th, 75th, or 90th percentile was related to self-reported incident allergic symptoms to dog after adjusting for the confounders described above. However, no significant associations were found when looking only at individuals without parent- and self-reported asthma, wheeze, or rhinitis at baseline (9 individuals with incident allergic symptoms to dog remained in these analyses) (Table 5).

SPT results from baseline were available for 374 participants. A positive SPT result for cat was recorded in 69 individuals, 45 of whom had parent-reported allergic symptoms to cat and 24 had not. A positive SPT result for dog at baseline was identified in 46 individuals, 25 of whom had parent-reported allergic symptoms to dog and 21 had not. In the group of participants who had developed allergic symptoms to cat in SPAIS II (n=50), 25 underwent SPT at baseline; of these, 14 had a positive SPT result and 11 had a negative SPT result for cat. Using the same logistic regression model as above for the subgroup with SPT results available (not excluding individuals with asthma, wheeze, or rhinitis at baseline) and adding adjustment for sensitization to cat, we found significant associations for FeNO >50th (aOR, 6.2 [1.2-33.3]) and > 75th percentile (aOR, 4.1 [1.2-13.9]), but not for FeNO >90th percentile. SPT results were also available for dog in 330 participants. Of the 33 individuals with self-reported incident allergic symptoms to dog, 22 underwent SPT at baseline; of these, 8 had a positive result and 14 had a negative result for dog. Adding the adjustment for sensitization to dog in a similar logistic regression model as that described above revealed no significant association with FeNO at baseline.

Table 4. Adjusted[a] OR for Self-reported Incident Allergic Symptoms to Cat

aOR (95%CI) for Incident Allergic Symptoms to Cat	All Individuals Without Allergic Symptoms to Cat at Baseline (n = 826)	All Individuals Without Allergic Symptoms to Cat and No Asthma, Wheeze, or Rhinitis at Baseline (n=610)
$FeNO_{0.1}$ > 50th percentile[b]	3.0 (1.5-6.1)	2.9 (1.0-8.4)
$FeNO_{0.1}$ > 75th percentile[b]	4.2 (2.2-8.0)	8.6 (3.0-24.1)
$FeNO_{0.1}$ > 90th percentile[b]	4.0 (1.9-8.6)	10.9 (3.6-33.0)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; OR, odds ratio.
[a]Adjusted for FEV_1 % predicted, height, asthma, wheeze, rhinitis, allergic symptoms to dog and pollen, FeNO measurement during the pollen season, at baseline; and cat exposure at SPAIS II.
[b]In the case of incident allergic symptoms to cat, levels are 4.6 ppb for the 50th percentile, 7.3 ppb for the 75th percentile, and 12.1 ppb for the 90th percentile.

Table 5. Adjusted[a] OR for Self-reported Incident Allergic Symptoms to Dog

aOR (95%CI) for Incident Allergic Symptoms to Dog	All Individuals Without Allergic Symptoms to Dog at Baseline (n = 871)	All Individuals Without Allergic Symptoms to Dog and No Asthma, Wheeze, or Rhinitis at Baseline (n=621)
FeNO >50th percentile[b]	2.8 (1.1-7.5)	2.3 (0.5-10.2)
FeNO >75th percentile[b]	3.3 (1.5-7.6)	4.4 (1.0-20.1)
FeNO >90th percentile[b]	3.2 (1.4-7.6)	1.1 (0.1-12.6)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s.
[a]Adjusted for asthma, wheeze, rhinitis, allergic symptoms to cat and pollen, maternal asthma and rhinitis, height and FeNO measurement during the pollen season, at baseline.
[b]In the case of incident allergic symptoms to dog, levels are 4.7 ppb for the 50th percentile, 7.6 ppb for the 75th percentile, and 13 ppb for the 90th percentile.

Discussion

The main finding of this population-based, longitudinal study of schoolchildren is that increased levels of exhaled NO predicted the onset of self-reported allergic symptoms to cat and dog within 4 years, despite the fact that the number of individuals reporting having a cat at home had decreased and remained unchanged for dogs. However, no association

between elevated FeNO at baseline and self-reported incident allergic symptoms to pollen was found in the same timeframe.

The group with self-reported incident allergic symptoms to cat or dog had elevated FeNO at baseline, and this probably signals subclinical T_H2-driven inflammation of the airways that precedes the development of such allergic symptoms. FeNO was higher in the group with self-reported incident allergic symptoms to dog than in the group with such symptoms to cat, thus indicating a higher degree of airway inflammation [9]. Less than one third of the participants who self-reported incident allergic symptoms to cat also reported allergic symptoms to dog, while more than three quarters of those with self-reported incident allergic symptoms to dog also reported allergic symptoms to cat. Thus, it seems that cat is a more common initial sensitizer than dog, and that dog-sensitized individuals are sensitized to more allergens. This may explain the higher NO values, as the level of FeNO is related to the degree of IgE-mediated sensitization [11,12], as well as exposure to allergens.

In support of the above, we previously reported, for a subgroup with SPT results in this cohort, that dog-sensitized individuals have higher levels of FeNO than cat-sensitized individuals [9]. In the case of participants with incident self-reported allergic symptoms to dog or cat, FeNO at baseline was between the levels for nonsensitized individuals and those for individuals sensitized to dog or cat. This supports the view that FeNO is a marker of subclinical airway inflammation that precedes the development of allergic symptoms and even confirmed sensitization. Other studies have shown that low-grade IgE-mediated sensitization (IgE antibody concentrations <0.35 kU_A/L) may precede symptoms [25]. Such low-grade IgE-mediated sensitization cannot be detected through SPTs.

A family history of asthma and allergy is a known risk factor for developing asthma and allergic symptoms. In our study, a family history was seen more frequently among participants who developed allergic symptoms to dogs than among those who developed allergic symptoms to cats. This may be because cat is a stronger sensitizing allergen that is not dependent on family history of atopic disease to break tolerance, whereas sensitization to dog may require a family predisposition. This reasoning is further supported by the findings described above. Moreover, in line with a previous study [26], our results showed a trend towards a lower risk of developing allergic symptoms to cat for individuals who had been exposed to cat during the first year of life. However, no such effect was found for having a dog in the home during the first year of life.

Adolescents with self-reported incident allergic symptoms to cat or dog had parent- and self-reported asthma, wheeze, and rhinitis to a larger extent at baseline; all of these conditions are related to elevated FeNO [1,16,15]. Therefore, it could be argued that the presence of these conditions was related to both elevated FeNO at baseline and self-reported incident allergic symptoms. However, we were able to confirm the main findings after adjustments for asthma, wheeze, or rhinitis at baseline. Furthermore, this association was found even after exclusion of individuals with parent- and self-reported asthma, wheeze, or rhinitis at baseline, at least for incident allergic symptoms to cat.

Elevated FeNO at baseline was not associated with self-reported incident allergic symptoms to pollen, which is in line with findings from other studies. Together with asthma, sensitization to perennial but not seasonal allergens is the most important determinant for FeNO [9,27]. The baseline FeNO measurements were performed during the school year, from the beginning of September to the end of May and included the birch pollen period, but not the grass or mugwort pollen periods. Participants whose FeNO measurements were taken between March and September had significantly higher values than those whose values were measured between October and February. These findings were adjusted for in the logistic regression models and did not affect the relationship between FeNO and incident self-reported allergic symptoms to cat or dog.

A major strength of the current longitudinal study of schoolchildren is the very high participation rate during follow-up (96%). Another strength is the use of well-validated questions from the ISAAC questionnaire and, to a large extent, the same additional questions at both time points. However, questionnaire data are limited because they depend on how individual participants interpret the questions and how they assess possible experienced symptoms. Furthermore, some additional bias might have been introduced by the fact that parents reported their children's allergic symptoms at baseline, whereas the participants themselves reported their allergic symptoms after follow-up. There is a risk of report bias, as the parents may not remember their child's past medical history and may not perceive the child's symptoms appropriately. While the adolescents were judged to be too young to answer that part of the questionnaire in SPAIS I, 4 years later, when they were aged 16-19 years, they were more appropriate responders than their parents. However, we find bias unlikely, as baseline FeNO is an objective measure and the participants did not have information on FeNO available during follow-up. Furthermore, sensitivity at baseline was increased by asking the parents if they suspected hypersensitivity in their child, thus making the omission of hypersensitivity less likely.

Another limitation of the study may be that we only had SPT results for a subpopulation. However, our study focused on allergic symptoms and not on IgE-mediated sensitization, and the SPT results were only used to validate the specificity of the questions regarding parent- and self-reported allergic symptoms. Furthermore, the available data showed a poor relationship between a positive SPT result and parent-reported ongoing allergic symptoms at baseline, as well as incident self-reported symptoms. Moreover, we could confirm that elevated FeNO was associated with incident allergic symptoms to cat, even after adjusting for a positive SPT result for cat. Given our generally cold and dry climate, *D pteronyssinus* is not a major sensitizer or inducer of allergic symptoms in this part of Sweden. Consequently, we chose not to study these allergic symptoms any further, thus preventing us from generalizing our results to other parts of Europe or the world, where mite is a major cause of allergic symptoms.

This study was performed 20 years ago with a questionnaire-based follow-up 4 years later. The data reported on the prevalence of allergic diseases differ from those reported today, although the aim of this study was mainly to evaluate

the relationship between FeNO and the development of allergic symptoms in a 4-year time frame.

We are aware that by using $FeNO_{100}$ and presenting the results using percentiles, our data are not entirely typical of clinical practice. More studies are needed to establish useful reference values for FeNO. Nevertheless, this study highlights elevated FeNO as a risk factor for the development of perennial allergies.

Conclusions

Our results showed that increased levels of exhaled NO in adolescents aged 12-15 years precede incident self-reported allergic symptoms to cat and dog within 4 years. These results were consistent for cat when individuals with any kind of respiratory symptoms at baseline were excluded. Therefore, elevated FeNO seems to indicate an increased risk of perennial allergies.

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Conflicts of Interest

KA has received research support from Aerocrine AB. The other authors declare that they have no conflicts of interest.

Previous Presentation

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E-table 1. Demographic characteristics of the SPAIS II population and those lost to follow-up

	Followed up in SPAIS II (n = 921)	Lost to follow-up in SPAIS II (n = 38)	p value
Male sex (%)	49.5	55.3	0.49
Age (years)	13.6 ± 0.41	13.7 ± 0.53	0.35
$FeNO_{0.1}$ (ppb)	4.78 (4.47, 5.10)	3.67 (2.30, 5.84)	0.12
FEV_1 (% predicted)	94.8 ± 10.8	100.0 ± 8.4	0.004
Height (cm)	162.4 ± 8.1	163.7 ± 9.8	0.34
Weight (kg)	52.8 ± 10.5	53.5 ± 9.6	0.67
Asthma (%)	8.7	7.9	0.87
Wheeze (%)	13.7	2.6	0.049
Rhinitis (%)	25.3	26.3	0.89
Allergic symptoms to cat (%)	10.3	10.5	0.97
Allergic symptoms to dog (%)	5.4	2.6	0.45
Allergic symptoms to pollen (%)	17.5	18.4	0.88

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in 1 second; ppb, parts per billion.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

E-table 2. Characteristics of subjects at baseline in relation to incident allergic symptoms to pollen at SPAIS II

	No allergic symptoms to pollen at baseline or follow-up (n = 675)	Incident allergic symptoms to pollen (n = 85)	p value
Male sex (%)	49	47.1	0.73
Age (years)	13.6 ± 0.40	13.7 ± 0.46	0.20
$FeNO_{0.1}$ (ppb)	4.26 (3.96, 4.60)	5.23 (4.18, 6.54)	0.08
FEV_1 (% predicted)	94.8 ± 10.8	94.6 ± 11.3	0.89
Height (cm)	162.4 ± 8.1	162.3 ± 8.2	0.91
Weight (kg)	52.4 ± 10.2	54.3 ± 11.7	0.11
Asthma (%)	3.6	10.6	0.03
Wheeze (%)	8.7	18.8	0.003
Rhinitis (%)	12.4	41.2	< 0.001
Allergic symptoms to cat (%)	3.1	30.3	< 0.001
Allergic symptoms to dog (%)	1.6	3.5	0.22
Cat exposure year 1 (%)	23	14.1	0.06
Dog exposure year 1 (%)	15.3	15.3	0.99
Ever asthma (mother) (%)	8.9	12.9	0.23
Ever asthma (father) (%)	7.7	12.9	0.10
Ever asthma (siblings) (%)	13.2	12.9	0.95
FeNO measurements inside pollen season (%)	36.6	29.4	0.19
Ever allergic rhinitis (mother) (%)	23.3	28.2	0.31
Ever allergic rhinitis (father) (%)	22.7	25.9	0.51
Ever allergic rhinitis (siblings) (%)	16.7	30.6	0.002

Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiatory volume in one second; ppb, parts per billion.

All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

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Concurrence of elevated FeNO and airway hyperresponsiveness in nonasthmatic adolescents

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Abstract

Objectives: The aim of this study was to investigate airway responsiveness and eosinophil and neutrophil inflammatory markers in clinically confirmed nonasthmatic adolescents with elevated fractional exhaled nitric oxide (FeNO), a marker of type-2 inflammation in the airways.

Methodology: A total of 959 subjects from a general population, aged 12 to 15 years, answered a standardised questionnaire and underwent FeNO measurements at a screening visit at school. Adolescents without asthma, who had elevated FeNO ($FeNO_{100}$ > 15 ppb) (n = 19), and control subjects, with low FeNO ($FeNO_{100}$ < 5 ppb) and without reported symptoms of asthma or allergy (n = 28), participated in a follow-up study where $FeNO_{50}$, airway responsiveness to methacholine (PD_{20}), blood eosinophil counts, and serum neutrophil lipocalin (HNL) and myeloperoxidase (MPO) levels were measured. Questionnaire follow-ups were performed 4 and 16 years later.

Results: Airway responsiveness (PD_{20}: 6.94 [1.87, 11.39] vs 11.42 [6.33, 59.4] μmol; $P < .05$) and blood eosinophil counts (0.31 [0.20, 0.44] vs 0.13 [0.1, 0.22] 10^9/L; $P < .001$) (geometric mean [95% CI]) were higher among cases than controls. A significant correlation between blood eosinophils and FeNO was found (rho = 0.41; $P = .005$). In contrast, serum HNL and MPO were lower in cases than controls ($P < .05$ both), and there was a negative correlation between HNL and FeNO ($r = -0.31$; $P = .04$). At both follow-ups, a higher proportion of subjects reported allergic symptoms compared with baseline ($P = .02$, $P = .01$).

Conclusions: Elevated FeNO in nonasthmatic adolescents was associated with airway hyperresponsiveness, elevated blood eosinophil counts, and lower systemic activation of neutrophils.

KEYWORDS
adolescents, breath test, eosinophilia, nitric oxide, respiratory hypersensitivity

1 | INTRODUCTION

Airway inflammation may be present in all degrees of asthma severity, with both eosinophils and neutrophils contributing to the pathology. The fractional exhaled nitric oxide (FeNO) is elevated primarily in allergic asthma.[1] It is a marker of type-2 inflammation in the airways and several studies have reported a correlation between FeNO and the degree of airway hyperresponsiveness (AHR).[2,3]

Increased FeNO has been reported in children from the general population who have atopy[2] and is related to an increased risk of developing asthma, wheeze, and rhinitis; it may, therefore, indicate subclinical inflammation or "early asthma".[4–7]

A methacholine challenge test is most commonly used to identify AHR and may be used to confirm an asthma diagnosis. Known factors associated with increased responsiveness to inhaled methacholine are female gender, decreased lung function, higher age, atopy, respiratory infections, smoking, and chronic bronchitis.[8,9]

The aim of our study was to examine the relationship between elevated FeNO and blood and sputum markers of granulocyte activation, as well as AHR, in a group of adolescents with elevated FeNO and where, for the first time, a diagnosis of asthma had been excluded after a thorough clinical examination. A second aim was to examine the long-term effects of elevated FeNO on self-reported respiratory and allergic symptoms, 4 and 16 years after the baseline examination.

2 | MATERIAL AND METHODS

2.1 | Study subjects

The study, Screening Project Asthma in Schools (SPAIS), has been described in detail previously[5] (Figure 1). Baseline screening data were collected in 1998–1999 and encompassed 959 subjects, aged 12 to 15 years, from nine randomized schools in Uppsala, Sweden. The subjects answered a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC),[10,11] parents answered additional questions concerning their children's asthma and allergic symptoms to cat, dog, and pollen, and the subjects underwent lung function and FeNO measurements at their schools. Adolescents with $FeNO_{100}$ between 15 and 20 ppb were asked to undergo an additional FeNO measurement during the same month that the examinations were performed at school, to confirm the FeNO result.

The following four criteria were then used:

1. Parent-reported asthma,
2. self-reported symptoms indicative of asthma,
3. elevated $FeNO_{100}$ defined as either ≥15 ppb on two separate occasions or >20 ppb at one examination,
4. FEV_1 ≤ 80% predicted (Knudson et al[12]).

Adolescents who fulfilled at least one of the criteria, based on screening data, were invited to a clinical reinvestigation at the

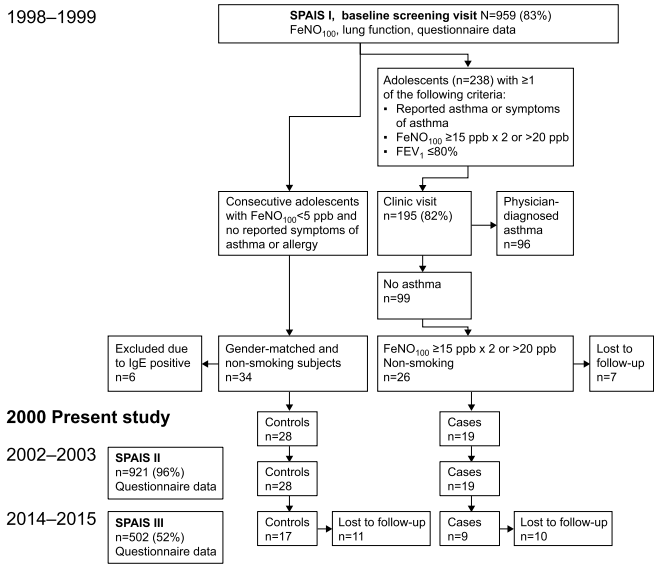

FIGURE 1 Study design

Uppsala University Children's Hospital, within 2 months of the screening at school, for assessment of clinical asthma by a senior pediatric allergologist. Of the 238 adolescents invited, 195 subjects (82%) participated in clinical reinvestigations. A clinical diagnosis of asthma was made for 96 of the 195 investigated subjects, after thorough interviews held by the allergologist and considering results from skin prick tests against cat, dog, birch pollen, timothy pollen, and *Dermatophagoides pteronyssinus*, performed at the reinvestigations (Soluprick, ALK, Horsholm, Denmark). Furthermore, both the following criteria had to be fulfilled, in the preceding 12 months, to obtain a diagnosis of asthma: (a) repeated episodes of wheeze and/ or chest tightness upon exercise, in the absence of respiratory tract infection, and (a) repeated episodes of wheeze and/or chest tightness in conjunction with respiratory tract infection or allergen exposure (furred animals, pollen). The reinvestigations were performed to acquire more objective data, beyond self-reported symptoms of asthma and wheeze.

Out of the 99 subjects who were found to be nonasthmatic in the clinical assessment, all adolescents with elevated FeNO (as defined above) at the screening examination at school were invited to a follow-up study, including two clinic visits, in April to November 2000. Randomly selected subjects, who had participated in the screening study, had $FeNO_{100}$ values ≤5 ppb and reported no symptoms indicative of asthma or allergy in the questionnaires were selected as controls. Subjects eligible as controls were randomized in regard to gender and approached for participation, in the resulting order, until at least 26 participants had been included. Further, neither cases nor controls could have a known pulmonary disease, be on corticosteroid or ß2-agonist treatment, be current smokers, or have had signs of respiratory infection within 2 weeks before participation in either of the two study visits. Six of the eligible cases were lost to follow-up and one was excluded after visit one because of initiation of asthma medication. These seven subjects did not differ in baseline FeNO compared with cases included in the study ($P = .84$). Cases with reported allergic symptoms to pollen, according to screening data, were invited to perform the study visits outside the pollen seasons. At baseline, two of the cases had self-reported wheeze and five had ever wheeze, whereas none had parental-reported asthma or $FEV_1 < 80\%$.

Two follow-up studies (SPAIS II and SPAIS III) of the entire cohort (N = 959), with slightly abbreviated versions of the original questionnaire, were performed 4 and 16 years after the baseline examination (2002-2003 and 2014-2015), with the subjects completing the questionnaires themselves.

2.2 | Study design

At clinic visit one, height, weight, $FeNO_{50}$, and lung function were measured, and blood and induced sputum were sampled. At the second clinic visit, 16 days (median) after visit one, $FeNO_{50}$ measurements and methacholine challenge tests were carried out. At both clinic visits, the FeNO measurements were performed before any measurements of lung function, including sputum induction procedures, and methacholine challenge tests. Thirty-four controls participated in the two clinic visits but six were excluded before data analyses due to confirmed IgE sensitization (≥0.35 kU_A/L) to mite, dog, birch, or timothy, in spite of no parental-reported allergic symptoms to dog or pollen at the screening examination. The excluded controls' airway responsiveness to methacholine did not differ from that of the 28 controls who remained in the analyses ($P = .3$).

2.3 | Questionnaires and definitions

Ever wheeze was self-reported and defined as having had wheezing or whistling in the chest at any time in the past and wheeze was defined as above, but with symptoms during the previous year. Rhinitis was self-reported and defined as having had sneezing, nasal congestion, or rhinorrhoea during the previous 12 months, without having had a cold. Allergic symptoms were defined as the subject's hypersensitivity to cat, dog, or pollen, reported by a parent at baseline, and by the subject at the follow-ups. Asthma and rhinitis in the family were questionnaire-assessed, with separate questions regarding mother, father, and siblings.

2.4 | Exhaled NO measurements

Online measurements of FeNO and monitoring of flow and pressure were performed using the Aerocrine NO system (Aerocrine AB, Stockholm, Sweden), including the CLD 77 AM chemiluminiscence analyzer (Eco Physics AG, Dürnten, Switzerland). Before the measurements, each subject washed their mouth with 25 mL of 10% sodium bicarbonate for 20 seconds.[13] At the screening visit, FeNO was measured at 100 mL/s; it was measured at both 100 mL/s and 50 mL/s at the first clinic visit, but only at 50 mL/s at the second. At each session, three correctly performed exhalations at the requested flow rates were recorded. The NO measurements were carried out in accordance with the existing recommendations of the European Respiratory Society,[14] except the mouthwash procedure. To facilitate interpretation of the $FeNO_{100}$ values from baseline, one way to obtain a rough estimate of $FeNO_{50}$ is to multiply them with a coefficient of approximately 1.6.[15]

2.5 | Measurements of pulmonary function

Pulmonary function measurements were performed in accordance with the criteria of the American Thoracic Society, using a Spirolab spirometer (Medical International Research, Rome, Italy). Forced expiratory volume in 1 second (FEV_1) was measured and Knudson's reference values were used.[12] Methacholine challenge tests were performed applying a dosimetric method, using an automatic inhalation-synchronized jet nebulizer (Spira Elektro 2, Spira, Hämeenlinna, Finland) and controlled tidal breathing[16] (protocol see E-Table 1). The FEV_1 was measured 3 minutes after each dose of methacholine and the challenge test was terminated when the subject had completed all inhalations, or

when FEV_1 had fallen by 20% or more from the baseline value. The dose of methacholine that caused 20% reduction of FEV_1 (PD_{20}) was calculated and expressed in μmol,[17] and twice the highest dose was arbitrarily used as the PD_{20} for subjects who completed all steps in the protocol without a 20% reduction in FEV_1.[18]

2.6 | Sputum induction and processing

Sputum was induced by inhalations of hypertonic saline using an Omron U1 ultrasonic nebulizer, in principle following a protocol described by Iredale et al.[19] Collected sputum was weighed and treated with Sputolysin® (dithiothrietol-DTT) for 15 minutes and then with N-Cetyl-N, N, N-trimethyl-ammonium bromide for 1 hour, both at room temperature, in accordance with Metso et al.[20] After centrifugation, the supernatant was collected and kept at −20°C until analysis was performed.

2.7 | Blood, serum, and sputum analyses

Eosinophilic cationic protein (ECP), human neutrophil lipokalin (HNL), myeloperoxidase (MPO), and IgE antibodies were analyzed in serum. IgE antibodies to mite, cat, dog, birch, and timothy were measured in the ImmunoCAP system (Pharmacia Diagnostics, Uppsala, Sweden). Measurements of eosinophils and neutrophils in blood were performed by the Clinical Chemistry Laboratory at the Uppsala University Hospital using an automated cell counter, and analyses of released ECP, HNL, and MPO in serum and of total ECP and HNL in sputum were performed using in-house radioimmunoassays.

2.8 | Statistics

Statistical analyses were performed using STATA 1C 14 (StataCorp, College Station, Texas). Comparisons at the group level were done using *t* tests for normally distributed continuous variables—FeNO was log-transformed to achieve normal distribution before *t* tests—or using chi-squared tests for categorical variables. The significance of the differences in serum and sputum ECP, HNL, serum MPO, and cell counts between the groups was examined using the Mann-Whitney nonparametric *U* test. Correlations between FeNO and PD_{20}, on one hand, and the aforementioned biomarkers, on the other hand, were determined using Spearman's rank correlation. A *P* value <.05 was considered statistically significant.

2.9 | Ethics

The study was approved by the Ethics Committee of the Medical Faculty of Uppsala University, Sweden (registration numbers 243/1998, 014/2000, 499/2001, 440/2013) and informed consent was obtained from the participants and a parent of each participant.

3 | RESULTS

In the present study, there were 19 cases among 26 eligible subjects (73%) and 28 controls who completed the two study visits.

There were no differences in demographic data between the two groups (Table 1). Two-thirds of the subjects in each group were male. Significantly more of the cases reported allergic symptoms, ever wheeze (Table 2), and rhinitis in the last 12 months but there were no statistical differences between the two groups in the screening

TABLE 1 Demographic characteristics of the study population and measurements performed at the baseline screening visit

	Cases (n = 19)	Controls (n = 28)	*P* value
Male, %	63.2	67.9	.74
Age, y	13.47 ± 0.40	13.47 ± 0.48	.99
Weight[a], g	62.72 ± 10.45	57.70 ± 10.08	.11
Height[a], cm	171.42 ± 9.72	169.07 ± 10.06	.43
$FeNO_{100}$, ppb	27.78 (22.34, 34.55)	2.79 (2.37, 3.27)	<.001
Wheeze, %	10.5	0	.08
Rhinitis, %	31.6	0	<.01
Family smoking, %	31.6	39.3	.59
Ever asthma (reported for mother), %	10.5	3.6	.34
Ever asthma (reported for father), %	0	7.1	.23
Ever asthma (reported for siblings), %	21.1	14.3	.55
Ever allergic rhinitis (reported for mother), %	36.8	17.9	.14
Ever allergic rhinitis (reported for father), %	0	10.7	.14
Ever allergic rhinitis (reported for siblings), %	36.8	21.4	.25

Note: All results presented as %, or mean ± standard deviation, or geometric mean and 95% confidence interval.
Abbreviations: $FeNO_{100}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; ppb, parts perper billion.
[a]Weight and height measurements at clinic visit 1.

TABLE 2 Subjects' characteristics at the baseline visit, (SPAIS I) and at follow-ups (SPAIS II and SPAIS III)

	SPAIS I cases (n = 19)/ controls (n = 28)	*P* value	SPAIS II Cases (n = 19) /Controls (n = 28)	*P* value	SPAIS III cases (n = 9)/ controls (n = 17)	*P* value
Male, %	63.2/67.9	.74	63.2/67.9	.74	55.6/64.7	.65
Ever wheeze, %	26.3/0	.004	47.4/17.9	.03	55.6/11.8	.02
Allergic symptoms, %	21.1/0	.02	63.2/0	<.001	77.8/5.9	<.001

Note: All results presented as %.

questionnaire data concerning reported wheeze in the past year and family asthma, rhinitis, or smoking (Table 1). The questionnaire in SPAIS II was completed by 921 subjects in the cohort (96%) and by all 47 subjects in this present study. In SPAIS III, 502 (52%) subjects participated and 26 (55.3%) of the subjects in this study completed the questionnaire. The proportion of cases who reported allergic symptoms increased from 21.1% to 63.2% between SPAIS I and II ($P = .02$) and from 21.1% to 77.8% between SPAIS I and SPAIS III ($P = .01$). Self-reported ever wheeze was significantly higher among cases than controls at all assessments and the proportion of cases who reported these symptoms increased from 26.3% to 55.6% between SPAIS I and SPAIS III. However, this increase was not significant ($P = .21$). There was no significant change in the controls regarding reported allergic symptoms or ever wheeze during the same period (Table 2).

3.1 | Respiratory measurements

The cases had significantly higher $FeNO_{50}$ values at both visits ($P < .001$ for both), whereas there was no difference concerning lung function values between the two groups at any visits (Table 3). In the case group, 21.1% managed to complete all ten steps in the methacholine challenge test, compared with 42.9% in the control group ($P = .13$). The cases had significantly lower PD_{20} values than the controls (Table 3).

3.2 | Analyses of serum, blood, and sputum

Most of the cases were IgE-sensitized (79%), most commonly to cat and dog (Table 3). We measured serum ECP, HNL, MPO, and counted blood eosinophils and neutrophils in all 47 subjects. Blood eosinophil counts were significantly higher among cases, and serum ECP showed a statistical trend to be higher in that group. HNL and MPO were higher in the controls, whereas blood neutrophil counts did not differ between the two groups (Table 3). All subjects took part in the sputum induction procedure and 89.5% of the cases and 89.3% of the controls succeeded in producing sputum. We did not find any significant differences in sputum ECP or HNL between the two groups (Table 3).

TABLE 3 Inflammatory markers, PD_{20}, cell counts, IgE, and lung function in cases and controls

	Cases (n = 19)	Controls (n = 28)	*P* value
$FeNO_{50}$ visit 1, ppb	30.98 (24.79, 38.72)	5.87 (4.73, 7.30)	<.001
$FeNO_{50}$ visit 2, ppb	30.56 (22.94, 40.72)	6.11 (4.84, 7.72)	<.001
FEV_1 visit 1 (% predicted)	102.21 ± 12.33	101.71 ± 14.43	.90
FEV_1 visit 2 (% predicted)	99.05 ± 10.25	98.54 ± 12.38	.88
PD_{20}, μmol	6.94 (1.87, 11.39)	11.42 (6.33, 59.4)	<.05
HNL sputum, μg/L	1 025 (591, 2 096)	1 167 (649, 2 613)	.81
ECP serum, μg/L	13.3 (9.1, 18.3)	8.6 (6.2, 13.7)	.05
HNL serum, μg/L	55.3 (47, 60.6)	60.75 (49.8, 76.8)	<.05
MPO serum, μg/L	219 (195, 272)	334 (228, 410)	<.05
Eosinophils blood, 10^9/L	0.31 (0.20, 0.44)	0.13 (0.1, 0.22)	<.001
Neutrophils blood, 10^9/L	3.28 ± 1.04	3.33 ± 1.58	.90
IgE sensitization to mite, %	21.1	0	NR
IgE sensitization to cat, %	52.6	0	NR
IgE sensitization to dog, %	57.9	0	NR
IgE sensitization to birch, %	26.3	0	NR
IgE sensitization to timothy, %	36.8	0	NR

Note: All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval or median and interquartile range.
Abbreviations: $FeNO_{50}$, fractional exhaled nitric oxide measured at 50 mL/s; FEV_1, forced expiratory volume in 1 s; NR, not relevant; ppb, parts perper billion; PD_{20}, cumulative dose methacholine causing a fall of 20% in FEV1.

TABLE 4 Spearman correlations between FeNO and PD_{20} and other inflammatory markers in the whole population (n = 47)

$FeNO_{50}$	rho values	*P* values	PD_{20}	rho values	*P* values
ECP sputum	0.20	.21	ECP sputum	0.05	.77
HNL sputum	0.03	.87	HNL sputum	0.05	.75
ECP serum	0.24	.10	ECP serum	−0.04	.79
HNL serum	−0.31	<.05	HNL serum	−0.02	.91
MPO serum	−0.25	.12	MPO serum	0.05	.74
Eosinophils blood	0.41	<.01	Eosinophils blood	−0.14	.33
Neutrophils blood	0.06	.68	Neutrophils blood	−0.008	.60

Abbreviations: ECP, eosinophilic cationic protein; $FeNO_{50}$, fractional exhaled nitric oxide measured at 50 mL/s; HNL, human neutrophil lipokalin; MPO, myeloperoxidase; PD_{20}, cumulative dose methacholine causing a fall of 20% in FEV_1.

3.3 | Correlations between FeNO and PD_{20} and other inflammatory biomarkers

There was a significant positive correlation between FeNO and blood eosinophil count and a negative correlation between FeNO and HNL in serum, whereas no other correlations between inflammatory markers were found (Table 4). There was no significant correlation between PD_{20} and FeNO (rho = −0.21, *P* = .17), nor between PD_{20} and any of the measured inflammatory biomarkers (Table 4).

4 | DISCUSSION

One of the main findings of this population-based follow-up study of adolescents with elevated FeNO levels and no clinical asthma was that they were significantly more reactive to methacholine than controls. Furthermore, a majority were IgE-sensitized and had higher blood eosinophil counts than controls. Interestingly, cases had significantly lower serum levels of both HNL and MPO, markers of neutrophil activity, compared with controls. Data from the questionnaires at SPAIS II and SPAIS III showed that the long-term effect of elevated FeNO at baseline for this specific group of cases was a significant increase in reported allergic symptoms. Furthermore, the cases reported significantly more ever wheeze, compared with the controls, at all three-time points.

Allergic sensitization relates to increased FeNO in both population-based studies and asthma patients[1,21] and may explain our high number of sensitized subjects. Together with asthma, sensitization to perennial allergens is the most important determinant of FeNO,[22,23] and this is in concordance with our result that the cases were most commonly sensitized to dog and cat. Furthermore, FeNO represents local inflammation in the airways and relates closely to airway hyperresponsiveness in asthmatics[3,24] or may precede development of airway hyperresponsiveness. This is in line with our result, where the cases with elevated FeNO had significantly lower PD_{20} than the controls. Our cases were at an early stage of airway disease; their elevated FeNO values were signs of airway inflammation, but their airway hyperresponsiveness was not advanced enough to result in a positive correlation between FeNO and PD_{20} and the other inflammatory biomarkers measured. A study from Israel investigated the association between AHR and blood eosinophils counts among asymptomatic flight academy candidates and found that subjects with a positive methacholine challenge test had significantly higher levels of blood eosinophil counts than subjects with a negative test, which is in line with our results. Still, that study could not demonstrate a significant correlation at the individual level between PD_{20} and blood eosinophil counts.[25] One previous study has shown a close relationship between mannitol reactivity and FeNO in random adolescents and young adults.[26] However, in that study, asthma was not excluded by clinical examination. In a Danish pediatric study, FeNO and airway responsiveness, measured as hyperreactivity to cold air, were associated regardless of asthma symptoms, suggesting a continuous subclinical to clinical process underlying asthma.[27]

In allergic asthma, airway inflammation is driven by the activation of, for example, mast cells, eosinophils and T-helper type-2 (Th2) lymphocytes, which produce the typical cytokines IL-4, IL-5, and IL-13. In the present study, there was a significant correlation between eosinophil counts in blood—a sign of systemic inflammation—and FeNO. A possible limitation of blood cell counts is that they do not provide much information on the extent to which the cells are involved in local processes or on the activity of the cells.[28] ECP, a protein released when eosinophils are activated, may better reflect the degree of eosinophilic inflammation in the airways and is closely related to recent exacerbations.[29,30] This may explain our result that the cases had significantly higher eosinophil counts in blood but their serum ECP only showed a statistical trend to be higher than in the controls, as their airway inflammation had not yet developed into symptomatic asthma. Measurements of ECP in sputum also showed a tendency to be higher in the cases than in the controls, which is in line with another study that showed a correlation between sputum ECP and FeNO, in children with asthma.[30]

There were no differences in blood neutrophil counts between the groups but the cases had significantly lower serum levels of both HNL and MPO, markers of neutrophil activity. Atopic disease is generally considered to be associated with a shift in the immune response from a Th1 to a Th2 profile. For example, studies have shown that serum IL-4 was elevated and interferon (IFN)-γ was decreased in asthmatic children as compared with controls,[31] and adults with persistent atopic asthma had a reduced allergen-induced

IFN-γ response while subjects with resolved asthma did not.[32] IFN-γ is a cardinal Th1 cytokine and is important in both innate and acquired immune responses. Specifically, IFN-γ seems to prime neutrophils in vivo, leading to an upregulated release of granule proteins upon stimulation.[33] Furthermore, there was a significant negative correlation between FeNO and HNL. Taken together, our results may support the Th1/Th2 dichotomy, with suppressed IFN-γ levels and, consequently, lower neutrophil activity in the preasthma group.

Neutrophil activity markers in sputum have been studied previously and Metso et al[20] showed elevated sputum HNL in subjects with chronic obstructive lung disease or respiratory infections compared with both healthy controls and subjects with steroid-naïve asthma, whereas no differences were noted between the asthmatics and controls. In line with this, we found no differences in sputum HNL levels between our cases and controls. However, it should be kept in mind that we measured total sputum HNL, in accordance with Metso et al[20] and this method will not detect different degrees of neutrophil priming in contrast to serum measurements.[28]

A strength with this clinical follow-up study may be that it used a large population-based material (N = 959), encompassing a follow-up period of 16 years, with high-participation rates at SPAIS II (96%) and SPAIS III (52%). Another strength is the use of the well-validated ISAAC questionnaire and identical additional questions at all three-time points. A further strength of the present study may be the homogenous group of participants: they were all of the same age, matched by gender, nonsmokers, and exhibited normal lung function values. There are known subject-related factors that affect FeNO levels and methacholine responsiveness and we have, already at inclusion of participants, controlled for some of them.

In spite of the fact that a large material was screened, we were only able to identify a small group of subjects of interest (n = 26), that is, those without asthma and having elevated FeNO. The loss to follow-up of seven subjects could probably be explained by the demanding study protocol, with two study visits including both a methacholine challenge test and a sputum induction procedure. The low number of study subjects also limited the possibilities to perform multivariate statistics. At SPAIS II, all 47 subjects completed the questionnaire but at SPAIS III only 26 (55%) subjects participated, which made the group of interest smaller (n = 9). Another limitation may be that we did not exclude the two cases with self-reported wheeze in the past year or the five cases who reported ever wheeze at baseline. Instead, an experienced allergologist evaluated the subjects' self-reported symptoms and determined if the subjects had asthma or not based on clinical experience, skin prick test results, and if the subjects fulfilled the two specific criteria that were set.

To confirm a diagnosis of asthma, such clinical examination is probably more sensitive than using strict lung function criteria with reversibility in this age group. In a British study of 630 children from the general population in the same age group (13-16 years),[34] 56 subjects had current asthma. Results from multivariable logistic regression models showed that FEV_1/FVC ($P = .008$) and FeNO ($P < .0001$), but not bronchodilator reversibility ($P = .97$), were independently associated with asthma, illustrating the poor sensitivity of reversibility for asthma in this age group.

Another potential limitation is that subjects were selected based on $FeNO_{100}$, the recommended method at the time of the study (ERS),[14] and these results in absolute values (ppb) are lower than the values currently assessed ($FeNO_{50}$). For example, the inclusion criterion for controls of having a $FeNO_{100} < 5$ ppb would translate to $FeNO_{50} < 8$ ppb, approximately.[15] Furthermore, the current reference values of the Global Lung Function Initiative[35] are preferred when performing lung function measurements but due to difficulties in adapting them to the protocol we used for the methacholine challenge test, a decision was made to keep the original lung function values, as used by Knudson.

It is important to detect risk factors for the development of respiratory and allergic disease at an early stage, as these diseases constitute a major burden for society today. Elevated FeNO, a marker of type-2 inflammation in the airways, is considered to be an early sign of airway disease and may imply an increased risk for the development of clinical asthma.[4,6] We believe that atopy is a part of the type-2 inflammatory pathway, which may or may not result in asthma. In the follow-up studies we performed, we could confirm development of allergic symptoms but not development of asthma symptoms, in the group with elevated FeNO and no diagnosis of asthma at baseline. Future follow-ups may reveal the development of respiratory symptoms at higher ages. A recent study provided strong support for the use of FeNO measurements to guide decisions on prescribing inhaled corticosteroids to patients with nonspecific respiratory symptoms but no confirmed asthma.[36] Screening of school children at certain ages with FeNO measurements, for early detection of those at risk of developing allergic asthma, and a proper clinical follow-up of subjects with elevated FeNO, might be beneficial to population health.

In conclusion, elevated levels of exhaled nitric oxide in nonasthmatic adolescents were associated with higher levels of blood eosinophil counts, airway hyperresponsiveness, and reduced neutrophil activity. A majority of the cases were sensitized and their reported allergic symptoms continued to increase over the 16-year follow-up period, which confirms the well-established association between FeNO and atopy.

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CONFLICTS OF INTEREST

KA has received research support from Aerocrine AB and LN from AstraZeneca. The other authors have reported no conflicts of interest.

AUTHOR CONTRIBUTIONS

LN and KA designed the SPAIS study. Data were collected, statistical analyses were performed, and the first draft of the manuscript was prepared by PK-S. PV contributed with analytical expertize. AM and KA were involved in discussions about the results and, together with the other authors, including CJ, approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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E-table 1. Methacholine challenge test protocol

Dose number	Methacholine concentraition (mg/mL)	Number of inhalations	FEV_1 was determined 3 min after each dose.	Cumulative dose of methacholine (μmol)
1	Physiological NaCl	10		
2	2.5	1		0.06
3	2.5	2		0.18
4	2.5	4		0.42
5	2.5	8		0.90
6	25.0	2		2.1
7	25.0	4		4.5
8	25.0	6		8.1
9	25.0	12		15.3
10	25.0	24		29.7

Abbreviations: FEV_1, forced expiratory volume in one second

Paper III

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REGULAR ARTICLE

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

Different baseline characteristics are associated with incident wheeze in female and male adolescents

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Abstract

Aim: To investigate the independent relationships between baseline characteristics and incident wheeze in adolescents, with particular regard to gender.

Methods: Adolescents (N = 959), aged 12-15 years, answered a standardised respiratory questionnaire and underwent height and weight measurements at baseline. Four years later, 96% of the subjects completed a similar questionnaire. The present study included the adolescents without self-reported wheeze at baseline (n = 795; 394 girls).

Results: The proportion of adolescents with obesity was higher among subjects with incident wheeze than among subjects who never reported wheeze: 19.1% vs 8.3%. When stratifying for gender, this difference was only found in girls. In stepwise logistic regression models (odds ratios [95% confidence interval]), obesity (2.84 [1.17-6.86]) and rhinitis (3.04 [1.53-6.03]) at baseline and current smoking (2.60 [1.16-5.82]) at follow-up were associated with incident wheeze in girls. For boys, FEV_1 <−1.65 standard deviation (3.20 [1.04-9.79]), family asthma (3.16 [1.46-6.86]) and seasonal allergic symptoms (5.61 [2.56-12.27]) at baseline were independently associated with incident wheeze.

Conclusion: Data stratified by gender showed that obesity in girls and an atopic constitution in boys were independently associated with increased risk of developing wheeze within four years.

KEYWORDS

adolescents, gender, incidence, obesity, wheeze

1 | INTRODUCTION

Asthma, with wheeze as one of the main symptoms, is the most common chronic illness of childhood. Obesity in children and adolescents is a priority health concern globally and the occurrence of wheeze and asthma increased with body mass index (BMI) in a study of children aged 2-18 years.[1] Furthermore, obesity appeared to increase the risk of asthma symptoms more than overweight.[2] However, there are indications that there is a gender difference in relation to the association between obesity and asthma symptoms.

Abbreviations: BMI, body mass index; FeNO, fraction of exhaled nitric oxide; FEV_1, forced expiratory volume in one second; IgE, immunoglobulin E; ISAAC, international study of asthma and allergies in childhood; SD, standard deviation; SPAIS, screening project asthma in schools.

Andrei Malinovschi and Kjell Alving contributed equally.

In a Norwegian study of 2883 participants, aged 17-19 years, more females reported asthma and wheeze in association with overweight, compared with males.[3]

Several hypotheses have been proposed to explain the mechanisms through which obesity is associated with asthma symptoms and wheeze. A recent study showed that obesity was associated with airway dysanapsis.[4] The authors suggested that obesity was primarily related to anatomical and developmental aberrations, leading to airway obstruction unrelated to airway inflammation or bronchospasm. Objective measures of allergic disease in relation to increased weight have been studied previously, but no associations were reported between BMI and the fraction of exhaled nitric oxide (FeNO), airway hyperresponsiveness, skin prick test results, sputum and blood eosinophil counts or total IgE.[5-7]

The aim of this longitudinal study was to investigate the relationship between baseline characteristics and the development of wheeze in a population-based cohort of adolescents, with particular emphasis on obesity and possible gender differences regarding risk factors associated with wheeze.

Key notes

- Allergy and obesity are increasing health problems among adolescents but little is known about the relative importance of these conditions for the development of respiratory symptoms.
- We found that obesity in girls and an atopic constitution in boys were independently associated with increased risk of developing wheeze within four years.
- Further studies are needed to fully understand the relationship between weight and respiratory symptoms, especially with regard to sex differences.

2 | PATIENTS AND METHODS

2.1 | General design

Baseline data were collected 1998-1999 and included 959 subjects, aged 12-15 years, from nine randomised schools in Uppsala, Sweden. The study, Screening Project Asthma in Schools (SPAIS), has been described in detail previously.[8] At baseline, the subjects answered a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC),[9] and one or both of their parents answered additional questions about their children, concerning allergic symptoms, asthma diagnosis, asthma medication, family history of asthma, family rhinitis and family smoking. Further, all subjects underwent lung function, FeNO, height and weight measurements at their schools. The girls were asked if they had reached menarche.

A follow-up study, SPAIS II, was performed four years later (2002-2003) with an identical ISAAC questionnaire, and additional questions, including the subject's current smoking habits at follow-up. The adolescents completed this questionnaire themselves.

2.2 | Questionnaires and definitions

Wheeze was defined as having had wheezing or whistling in the chest at any time in the previous year. Wheeze was self-reported by the adolescents in both the baseline study and SPAIS II. Incident wheeze refers to reporting symptoms at follow-up but not at baseline. Asthma was defined as ever having had parental- or self-reported asthma, in combination with having used corticosteroid asthma treatment, having wheezing or whistling in the chest or having a respiratory infection that caused wheezing or whistling in the chest in the previous year.[10] Rhinitis was self-reported and defined as having had sneezing, nasal congestion or rhinorrhoea in the previous 12 months, without having a cold. Atopic dermatitis was defined as an itchy rash which was coming and going for at least six months, in the last 12 months. Allergic symptoms were defined as the subject's hypersensitivity to perennial allergens, including cat and dog or seasonal allergens, including pollen. These symptoms were reported by the parent at baseline and by the participant at follow-up. Asthma, rhinitis and smoking habits in the family, involving symptoms reported by mothers, fathers and siblings, were questionnaire-assessed as well as the subject's current smoking habits at follow-up.

2.3 | Exhaled NO

Measurements of FeNO were performed with the Aerocrine NO system (Aerocrine AB), including the CLD 77 AM chemiluminescence analyser (Eco Physics AG), as previously described.[8] The measurements were performed in accordance with recommendations from the European Respiratory Society.[11] Before a measurement, the subject's mouth was washed with 25 mL of 10% sodium bicarbonate for 20 seconds. Three exhalations of 10 seconds each were performed, and an average value was calculated. FeNO was measured at 0.1 L/s. To help interpret the $FeNO_{0.1}$ values in this paper, and to obtain a rough estimate of $FeNO_{0.05}$, which is the standard flow-rate today, is to multiply them with a coefficient of approximately 1.6.[12]

2.4 | Pulmonary function

Pulmonary function measurements were performed in accordance with criteria from the American Thoracic Society,[13] using a Spirolab spirometer (Medical International Research). Lower limit of normal and percentiles for forced expiratory volume in 1 second (FEV_1) were calculated using the Excel (Microsoft Corporation) macro for the Global Lung Function Initiative (GLI) reference values.[14] The lower limit of normal was defined as $FEV_1 < -1.65$ standard deviation (SD). Consequently, a reduced FEV_1 was defined as <−1.65 SD.

2.5 | Statistical analyses

Statistical analyses were performed using STATA IC 14 (StataCorp). Comparisons at the group level were made using *t* tests for continuous variables or using chi-squared tests for categorical variables. McNemar's test was used to assess within-subject changes of categorical variables across two time points. FeNO values were log-transformed to achieve normal distribution, and then, *t* tests were performed on log-transformed FeNO. Multiple logistic regression was performed with incident wheeze as outcome, and all variables identified as significant in the univariate analyses, for either girls or boys, were used as predictors (Tables 3 and 4). Menarche at baseline was an additional predictor for girls and current smoking at SPAIS II for both girls and boys. The used multiple regression model was stepwise, and variables were excluded if no significant association with the outcome was found (*P* values > .05). All subjects with reported asthma at baseline were excluded in these models.

BMI was calculated as weight in kilograms divided by the square height in metres. BMI values were used to group subjects into underweight, normal weight, overweight and obese, based on growth reference data for children aged 5-19 years issued by the World Health Organization.[15]

A *P* value < .05 was considered statistically significant.

2.6 | Ethics

The study was approved by the Ethical Committee of the Medical Faculty of Uppsala University, Sweden (registration numbers 243/1998 and 499/2001), and the study procedures were in accordance with the Declaration of Helsinki. According to an information letter attached to the questionnaire, a completed parent part of the questionnaire was judged as a written informed consent from the parents. The adolescents gave their informed consent by completing the ISAAC part of the questionnaire and by orally agreeing to participate in the study.

3 | RESULTS

In SPAIS II, 921 (96%) subjects completed and returned the questionnaires. In the present study, only adolescents without self-reported wheeze at baseline were included (n = 795). Out of them, 84 subjects (10.6%) had developed self-reported wheeze four years later. The number of participants self-reporting rhinitis and allergic symptoms to perennial and seasonal allergens at follow-up was significantly higher than at the baseline visit, and self-reported atopic dermatitis had decreased (Table 1). The baseline characteristics for boys and girls separately showed that the boys on average were significantly taller and weighed more than the girls. Further, the girls self-reported more atopic dermatitis, family asthma and smoking, than the boys (Table S1). The adolescents who self-reported incident wheeze had higher mean weight and mean BMI and a majority were girls (58.3%). Furthermore, these adolescents significantly more often reported rhinitis, allergic symptoms to both perennial and seasonal allergens, as well as family asthma and allergic rhinitis than the subjects who reported no wheeze at both time points. They were more often current smokers at follow-up than adolescents who never reported wheeze (Table 2).

TABLE 1 Subjects' characteristics at baseline and follow-up, and measurements performed at the baseline visit

	Baseline (n = 795)	Follow-up (n = 795)	*P* value
Female gender, n (%)	394 (49.6)		
Age (years)	13.6 ± 0.40		
$FeNO_{0.1}$ (ppb)	4.35 (4.06, 4.66)		
FEV_1 (% predicted)	94.9 ± 10.5		
FEV_1 (<−1.65 SD), n (%)	60 (7.5)		
Height (cm)	162.6 ± 8.1		
Weight (kg)	52.7 ± 10.4		
Asthma, n (%)	19 (2.4)	25 (3.1)	.13
Wheeze, n (%)	0 (0)	84 (10.6)	NR
Rhinitis, n (%)	158 (19.9)	214 (26.9)	<.001
Atopic dermatitis, n (%)	149 (18.7)	124 (15.6)	<.05
Perennial allergic symptoms, n (%)	48 (6.0)	86 (10.8)	<.001
Seasonal allergic symptoms, n (%)	110 (13.8)	158 (19.9)	<.001
Family smoking (%)	260 (32.7)	255 (32.1)	.62

Note: All results presented as number, n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.
Abbreviations: $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; n, number; NR, not relevant; ppb, parts per billion; SD, standard deviation.

When analysing the data stratified for gender, girls with incident wheeze were on average taller, weighed more and had higher mean BMI. Further, they had more often parental- and self-reported asthma, rhinitis and perennial allergic symptoms than girls without wheeze at both time points (Table 3). Furthermore, there were more girls classified as obese among those with incident wheeze than among those without (Table S2; Figure 1A). It was significantly more common among girls who had incident wheeze to have reached menarche at baseline than among girls who never reported symptoms of wheeze. The boys with incident wheeze did not exhibit higher BMI (Table 4), and there was no difference in the proportion of boys who were obese with regard to incident wheeze (Table S2; Figure 1B). Instead, boys with incident wheeze had higher FeNO, and more often reduced FEV_1, reported rhinitis, allergic symptoms to both perennial

and seasonal allergens, family asthma and family rhinitis, at baseline, than boys who never reported any symptoms of wheeze (Table 4). At follow-up, self-reported current smoking was more common among girls than boys (Table S1). However, a higher prevalence of current smoking, in subjects with incident wheeze compared with subjects without symptoms of wheeze, was found among both girls (Table 3) and boys (Table 4). There was no difference between the subjects with incident wheeze and those without wheeze at both time points with regard to baseline mean FEV_1, irrespective of gender. However, in the incident wheeze group, there were an increased number of boys with reduced FEV_1 at baseline, compared with boys who never reported any symptoms of wheeze.

In multiple logistic regression analyses, obesity was independently related to self-reported incident wheeze in girls after adjustments for confounders (see Statistical analyses) (Table 5). The adjusted odds ratio [aOR (95% confidence interval)] for incident wheeze was 2.84 (1.17-6.86) times higher for girls with obesity compared with those of normal weight at baseline. These analyses were performed after exclusion of the three girls with reported asthma, but no reported wheeze at baseline. Further, incident wheeze in girls was independently and positively associated with reported rhinitis at baseline and current smoking at follow-up (Table 5). Corresponding logistic regression analyses in boys showed that allergic symptoms to seasonal allergens, family asthma and reduced FEV_1 at baseline were independently associated with incident wheeze in boys (Table 5). These analyses were performed when the one boy with reported asthma but no wheeze at baseline was excluded.

4 | DISCUSSION

The main finding of this longitudinal, population-based study of schoolchildren was that increased BMI, especially obesity, was related to an increased risk of development of wheeze within a four-year time frame. Interestingly, when subjects were stratified by gender, this relationship was only significant in females, while an atopic constitution was associated with incident wheeze in males.

4.1 | Previous studies

A few studies have investigated whether the asthma-obesity association differs between genders and the results have been mixed, which may be explained by different study designs and ages of study populations.[3,6,16] The relationship between age and asthma symptoms depends on gender, with boys being more affected in childhood and girls more affected in adolescence and adulthood.[17] The observation that the incidence of asthma tends

TABLE 2 Characteristics of subjects at baseline, except current smoking, in relation to incident wheeze

	No wheeze at baseline or follow-up (n = 711)	Incident wheeze (n = 84)	*P* value
Female gender, n (%)	345 (48.5)	49 (58.3)	.09
Age (year)	13.6 ± 0.40	13.6 ± 0.42	.89
$FeNO_{0.1}$ (ppb)	4.30 (4.01, 4.61)	4.83 (3.70, 6.31)	.31
FEV_1 (% predicted)	95.1 ± 10.5	94.8 ± 10.6	.83
FEV_1 (<−1.65 SD), n (%)	50 (7.0)	10 (11.9)	.10
Height (cm)	162.4 ± 8.15	163.1 ± 7.14	.43
Weight (kg)	52.3 ± 10.2	55.4 ± 12.1	.01
BMI (kg/m^2)	19.8 ± 2.99	20.7 ± 3.79	<.01
Asthma, n (%)	15 (2.1)	4 (4.8)	.13
Rhinitis, n (%)	127 (17.9)	31 (36.9)	<.001
Atopic dermatitis, n (%)	130 (18.3)	19 (22.6)	.34
Perennial allergic symptoms, n (%)	34 (4.8)	14 (16.7)	<.001
Seasonal allergic symptoms, n (%)	86 (12.1)	24 (28.6)	<.001
Current smoking, n (%)	54 (7.6)	17 (20.2)	.001
Family asthma, n (%)	164 (23.1)	33 (39.3)	.001
Family smoking (%)	232 (32.6)	28 (33.3)	.90
Family allergic rhinitis, n (%)	348 (48.9)	51 (60.7)	<.05

Note: All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; n, number; ppb, parts per billion; SD, standard deviation.

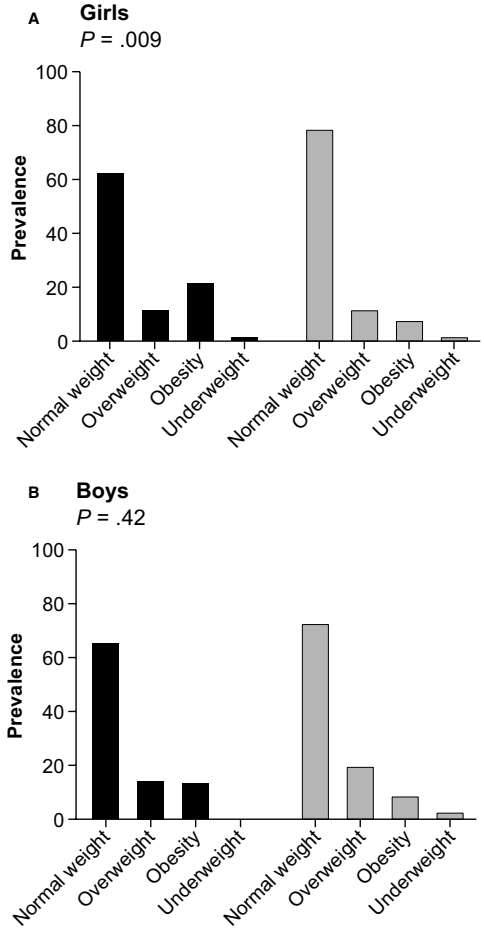

FIGURE 1 A, Prevalence of BMI groups in girls with incident wheeze (black bars) and with no reported wheeze (grey bars). B, Prevalence of BMI groups in boys with incident wheeze (black bars) and with no reported wheeze (grey bars)

to decrease after menopause has suggested a role of female sexual hormones in the incidence and persistence of asthma symptoms in women.[18] The adolescents in our study were at an age when a majority of the girls had reached menarche already at baseline. More girls with incident wheeze, especially obese girls, had started to menstruate at baseline, compared with girls who never reported wheeze. Evidence from several epidemiological studies has indicated a relationship between increased BMI and earlier puberty in girls.[19,20]

In a Swedish study of adolescents from the general population, increased BMI was reported to be more common among 15-year-old boys than girls.[21] However, this result was not confirmed in our study. Girls reporting more incident wheeze than boys are in line with a previous cross-sectional Norwegian study of adolescents of corresponding ages, 17-19 years. In that study, 33.5% of the girls and 22.1% of the boys reported current wheeze.[3] Furthermore, an independent association between reported current wheeze and overweight, compared with normal weight, was reported in girls, but not boys, in the same study.

Obesity is known to be associated with chronic low-grade systemic inflammation, characterised by, for example, blood leucocytosis and increased serum levels of C-reactive protein, but negatively associated with sputum eosinophils and FeNO.[22,23] Accordingly, in a Swedish birth cohort study where the subjects were followed up to 16 years no association was found between BMI and FeNO. However, a relationship between higher BMI and increased numbers of circulating neutrophils was found in girls, although not in boys.[24] Further, asthma in obese children has previously been reported to be mainly non-eosinophilic in girls but not boys,[25] and obesity has been associated with neutrophilic airway inflammation in female but not male adults.[23] The adolescents with incident wheeze, both girls and boys, reported more symptoms of rhinitis and parental-reported allergic symptoms to perennial—and for boys also seasonal allergens—at baseline, as compared with subjects who never reported symptoms of wheeze. This association between current wheeze and rhinitis in adolescents has been found previously,[3] and results from our study showed that reported rhinitis was independently associated with incident wheeze in girls. The boys with incident wheeze also reported more family asthma and allergic rhinitis, and seemed to have a family predisposition for atopic disease, which may explain the development of asthma symptoms. Atopic disease is most commonly related to type 2 inflammation, and the boys with incident wheeze exhibited higher FeNO and more often reduced FEV_1, at baseline, compared to subject who never reported any symptoms of wheeze. Several studies have reported a relationship between airway inflammation and reduced lung function.[26] The girls with incident wheeze did not have increased FeNO or reduced lung function at baseline. This strengthens the view that obesity is associated with another phenotype of airway disease, with chronic low-grade systemic inflammation more related to non-type 2 inflammation.[22] In addition to reporting more respiratory and rhinitis symptoms, the adolescents with incident wheeze were also more commonly current smokers at follow-up compared with those who never reported wheeze and this relationship was especially strong in girls. In a German follow-up study of 2936 adolescents, aged 16-18 years, who had completed an ISAAC questionnaire at two time points, active smoking was associated with increased incidence of wheeze within a five-year time frame.[27] In our study, the main risk factors for later development of wheeze in girls were obesity and rhinitis at baseline, and current smoking at follow-up. Besides symptoms of rhinitis, it seems that factors associated with life style are more associated with development of wheeze in girls than in boys.

TABLE 3 Characteristics of female subjects at baseline, except current smoking, in relation to incident wheeze

	No wheeze at baseline or follow-up (n = 345)	Incident wheeze (n = 49)	*P* value
Age (year)	13.6 ± 0.41	13.6 ± 0.42	.66
$FeNO_{0.1}$ (ppb)	4.08 (3.71, 4.49)	3.96 (2.64, 9.96)	.85
FEV_1 (% predicted)	94.8 ± 10.5	96.0 ± 10.5	.44
FEV_1 (<−1.65 SD), n (%)	30 (8.7)	5 (10.4)	.70
Height (cm)	160.3 ± 6.41	162.4 ± 6.26	<.05
Weight (kg)	51.1 ± 9.06	55.2 ± 12.3	<.01
BMI (kg/m^2)	19.9 ± 3.06	20.9 ± 4.06	<.05
Menarche, n (%)	247 (71.6)	43 (87.8)	<.05
Asthma, n (%)	4 (1.2)	3 (6.1)	.01
Rhinitis, n (%)	59 (17.1)	18 (36.7)	<.01
Atopic dermatitis, n (%)	75 (21.7)	11 (22.4)	.91
Perennial allergic symptoms, n (%)	16 (4.6)	7 (14.3)	<.01
Seasonal allergic symptoms, n (%)	39 (11.3)	9 (18.4)	.16
Current smoking, n (%)	36 (10.4)	12 (24.5)	<.01
Family asthma, n (%)	91 (26.4)	19 (38.8)	.07
Family smoking, n (%)	126 (36.5)	18 (36.7)	.98
Family allergic rhinitis, n (%)	169 (49.0)	30 (61.2)	.11

Note: All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; n, number; ppb, parts per billion; SD, standard deviation.

TABLE 4 Characteristics of male subjects at baseline, except current smoking, in relation to incident wheeze

	No wheeze at baseline or follow-up (n = 366)	Incident wheeze (n = 35)	*P* value
Age (year)	13.6 ± 0.39	13.6 ± 0.43	.95
$FeNO_{0.1}$ (ppb)	4.52 (4.08, 5.00)	6.38 (4.76, 8.55)	<.05
FEV_1 (% predicted)	95.3 ± 10.6	93.1 ± 10.5	.24
FEV_1 (<−1.65 SD), n (%)	20 (5.5)	5 (14.3)	<.05
Height (cm)	164.4 ± 9.07	164.1 ± 8.19	.84
Weight (kg)	53.5 ± 10.9	55.9 ± 11.9	.22
BMI (kg/m^2)	19.7 ± 2.92	20.5 ± 3.43	.09
Asthma, n (%)	11 (3.0)	1 (2.9)	.96
Rhinitis, n (%)	68 (18.6)	13 (37.1)	<.01
Atopic dermatitis, n (%)	55 (15.0)	8 (22.9)	.22
Perennial allergic symptoms, n (%)	18 (4.9)	7 (20.0)	<.001
Seasonal allergic symptoms, n (%)	47 (12.8)	15 (42.9)	<.001
Current smoking, n (%)	18 (4.9)	5 (14.3)	<.05
Family asthma, n (%)	73 (20.0)	14 (40.0)	<.01
Family smoking, n (%)	106 (29.0)	10 (28.6)	.96
Family allergic rhinitis, n (%)	179 (48.9)	21 (60.0)	.21

Note: All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; n, number; ppb, parts per billion; SD, standard deviation.

TABLE 5 Independent risk factors (recorded at baseline, except current smoking) for incident wheeze in adolescent females and males

	aOR	95% CI	*P* value
Females			
Normal weight	1.0	1.0	1.0
Obesity vs normal weight	2.84	1.17-6.86	.02
Rhinitis	3.04	1.53-6.03	.001
Current smoking	2.60	1.16-5.82	.02
Males			
Seasonal allergic symptoms	5.61	2.56-12.27	<.001
FEV_1 (<−1.65 SD)	3.20	1.04-9.79	.04
Family asthma	3.16	1.46-6.86	.004

Note: aOR: variables adjusted for in all models: BMI groups, rhinitis, perennial and seasonal allergic symptoms, family asthma, $FeNO_{0.1}$ and FEV_1 < −1.65 SD, at baseline and current smoking at follow-up. Additional variable in females: menarche at baseline. All subjects with asthma at baseline were excluded. Pseudo R^2 was 0.07 for female model and 0.13 for male model.

Abbreviations: aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 s; SD, standard deviation.

4.2 | Methodological considerations

A major strength of the current population-based, longitudinal study of schoolchildren was the very high participation rate (96%) in the follow-up. Another strength was the use of the well-validated ISAAC questionnaire and identical additional questions at both time points. However, questionnaire data have the weakness of depending on how subjects interpret the questions and on how they experience any symptoms. Self-reported symptoms of wheeze can be caused by other conditions than asthma, for example laryngeal obstructions or psychosocial factors.

Some additional bias might have been introduced by parents responding to some of the questions at baseline, whereas the participants themselves completed the entire questionnaire at follow-up. Symptoms of rhinitis showed a strong association with development of wheeze in girls. As data on treatment of rhinitis were missing in this study, we have not been able to evaluate the effect of treatment of rhinitis in relation to development of asthma and wheeze, which was a limitation.

Data on weight and height at baseline were obtained through direct physical measurements, using the same equipment for all participants, which was more reliable than use of self-reported values. Unfortunately, we have no reported data on height and weight at follow-up. Thus, we could not investigate if changes in BMI also related to incident wheeze. The lack of data on physical activity levels was also a limitation, as symptoms of wheeze may depend on the degree of physical activity.2

5 | CONCLUSIONS

The results from this longitudinal study of adolescents showed that there were different risk factors for girls and boys concerning the development of wheeze. The main risk factors for girls were obesity, together with rhinitis and current smoking, while the main risk factors for boys were reduced FEV_1, seasonal allergic symptoms and a family history of asthma.

Further studies are needed to fully understand the relationship between BMI and wheezing disorders, and this study emphasises the importance of examining boys and girls separately.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

KA has received research support from Aerocrine AB and LN from AstraZeneca. The other authors reported no conflicts of interest in relation to this work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Table S1. Characteristics of female and male subjects with no wheeze at baseline, except current smoking (n = 795).

	Females (n = 394)	Males (n = 401)	p value
Age (year)	13.6 ± 0.39	13.6 ± 0.41	0.89
$FeNO_{0.1}$ (ppb)	4.06 (3.69, 4.48)	4.65 (4.22, 5.12)	0.08
FEV_1 (% predicted)	94.9 ± 10.5	95.1 ± 10.6	0.79
FEV_1 (< -1.65 SD), n (%)	35 (8.9)	25 (6.2)	0.16
Height (cm)	160.5 ± 6.43	164.4 ± 8.98	< 0.001
Weight (kg)	51.6 ± 10.0	53.7 ± 11.0	< 0.01
BMI (kg/m^2)	21.0 ± 3.21	19.7 ± 2.97	0.27
Asthma, n (%)	7 (1.8)	12 (3.0)	0.26
Rhinitis, n (%)	77 (19.5)	81 (20.2)	0.82
Atopic dermatitis, n (%)	86 (21.8)	63 (15.7)	< 0.05
Perennial allergic symptoms, n (%)	23 (5.8)	25 (6.2)	0.81
Seasonal allergic symptoms, n (%)	48 (12.2)	62 (15.5)	0.18
Current smoking	48 (12.2)	23 (5.7)	0.001
Family asthma, n (%)	110 (27.9)	87 (21.7)	< 0.05
Family smoking, n (%)	144 (36.5)	116 (28.9)	< 0.05
Family allergic rhinitis, n (%)	199 (50.5)	200 (49.9)	0.86

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in 1 second; number, n; ppb, parts per billion; SD, standard deviation.

All results presented as n, %, mean ± standard deviation, or geometric mean and 95% confidence interval.

Table S2. Baseline BMI groups for all subjects and boys and girls separate, in relation to incident wheeze at SPAIS II

	Normal weight, n (%)	Overweight, n (%)	Obese, n (%)	Underweight, n (%)	p value
Incident wheeze n=84	54 (64.3)	13 (15.5)	16 (19.1)	1 (1.2)	
No reported wheeze n=711	540 (76.0)	95 (13.4)	59 (8.3)	17 (2.4)	0.01[#]
Incident wheeze in boys n=35	23 (65.7)	7 (20.0)	5 (14.3)	0 (0)	
No reported wheeze in boys n=366	268 (73.2)	54 (14.8)	33 (9.0)	11 (3.0)	0.42[#]
Incidence wheeze in girls n=49	31 (63.3)	6 (12.2)	11 (22.4)	1 (2.0)	
No reported wheeze in girls n=345	272 (78.8)	41 (11.9)	26 (7.5)	6 (1.7)	0.009[#]

Abbreviations: BMI, body mass index; n, number.

chi-2 test.

All results presented as n (%)

Paper IV

Incidence of asthma between adolescence and adulthood: early risk factors and gender differences

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Abstract

Background: Several studies have shown gender di□erences in the prevalence of asthma at various ages. The aim was to investigate the development of respiratory symptoms between adolescence and adulthood in relation to baseline risk factors and gender, and the effect on self-rated health.

Methods: In the study Screening project asthma in schools, adolescents aged 12–15 years answered a standardised respiratory questionnaire (ISAAC) and underwent measurements of fractional exhaled nitric oxide (FeNO) and lung function (FEV_1) at baseline. Two follow-ups with similar questionnaires were performed after four and 16 years, with 491 subjects participating in all three examinations.

Results: The prevalence rates of asthma and wheeze were unchanged after four years, but had increased after 16 years; the increase was significant for females only. A more continuous increase in allergic symptoms showed no gender difference. The adjusted odds ratio [aOR (95% confidence interval)] for the development of asthma was 4.11 (1.27, 13.24) times higher in females with reduced FEV_1 and 1.13 (1.06, 1.20) times higher in males with higher FeNO at baseline. Females, but not males, with asthma, rated their health as poor to a higher extent than individuals without asthma at the last follow-up, 20.0% vs. 7.7% ($p < 0.01$).

Conclusions: An increased prevalence of respiratory symptoms was seen primarily between late adolescence and young adulthood, and was significant for females but not males. To optimise health and wellbeing, gender differences in asthma development, associated risk factors, and treatment of respiratory symptoms, must be considered.

Introduction

A number of studies have shown gender di□erences in the prevalence of wheeze and asthma and a relationship to age, with boys being more affected in childhood and girls more affected in adolescence and adulthood (1-3). Results from a population-based longitudinal study of children investigated at age 11.1, 13.6, and 16.3 years concluded that a gender shift in the prevalence of asthma, from male to female dominance, had occurred at 16.3 years (4). However, no association was found between pubertal stages and asthma prevalence. Others have suggested a role of female sexual hormones in the incidence and persistence of asthma symptoms in women, an argument strengthened by the fact that the incidence of asthma tends to decrease after menopause (5).

Age of incidence of asthma was examined in a retrospective analysis of the European Respiratory Health Survey (6), including subjects from the general population, aged 20–44 years. The study confirmed a gender reversal with more females than males with incident asthma after puberty. In a follow-up study with case-control design, forced expiratory volume in one second (FEV_1) at adult age was a significant factor explaining the different patterns of asthma incidence in females and males, but there was no confirmed effect of smoking on the development of asthma. The gender shift in asthma incidence and prevalence during puberty has recently been confirmed in large meta-analyses, and the shift seems to be stronger for non-atopic asthma (7). Thus, several studies have shown a gender shift in asthma prevalence during puberty but, as far as we know, there is no study yet that has identified any gender differences with regard to objective risk factors in early adolescence. Moreover, the majority of previous longitudinal prospective studies looking at the development of respiratory symptoms have focused on either children or adults. Thus, studies following up individuals from childhood to adulthood are scarce.

The Screening project asthma in schools (SPAIS) is a cohort of 959 schoolchildren followed from early adolescence to early adulthood, between 1998 and 2015 (8). In the baseline study, the pupils underwent measurements of fraction of exhaled nitric oxide (FeNO), dynamic spirometry, and completed the International study of Asthma and Allergies in Childhood (ISAAC) questionnaire (9,

10) at their schools. Questionnaire follow-ups were performed four and 16 years later. We have previously reported, based on the baseline study, that FeNO, but not FEV_1, related to self-reported allergic and asthma symptoms (8). Moreover, we have shown that higher FeNO at baseline predicted new-onset rhinitis (11) and new-onset allergic symptoms to cat and dog (12) at the four-year follow-up of SPAIS. Furthermore, obesity at baseline, and current smoking, were related to an increased risk of developing wheeze in females, while an atopic constitution was associated with incident wheeze in males in late adolescence (13). In the present study, participants also provided data on self-rated health (SRH) at the 16-years-follow-up. SRH is a validated general health status indicator and refers to how a subject responds to the question "How do you rate your general health status?" (14).

The aim of the this longitudinal study was to investigate the development of respiratory symptoms, from early adolescence to young adulthood, with a total follow-up time of 16 years, in relation to baseline risk factors, gender differences, and allergic symptoms. Further, we wanted to investigate how respiratory symptoms were related to SRH in young adulthood.

Material and methods

Study subjects

The *Screening project asthma in schools* (SPAIS) study has been described in detail previously (8). Baseline screening data were collected in 1998–1999 from 959 subjects, aged 12–15 years, at nine randomised schools in Uppsala, Sweden. The subjects answered a questionnaire from ISAAC, while parents answered additional questions concerning their child's hypersensitivity to cat, dog or pollen, asthma diagnosis, asthma medication, atopic disease in childhood, family history of asthma and rhinitis, family smoking, and environmental issues. Further, all subjects underwent lung function, FeNO, height, and weight measurements at their schools.

Two follow-up studies (SPAIS II and SPAIS III), with slightly abbreviated versions of the original questionnaire, were performed four and 16 years after the baseline examination (2002−2003 and 2014–2015). At SPAIS II, 921 subjects (96.0%) participated, and at SPAIS III, 502 subjects (52.3%) participated. At both follow-ups, the subjects completed the questionnaires themselves (Figure 1).

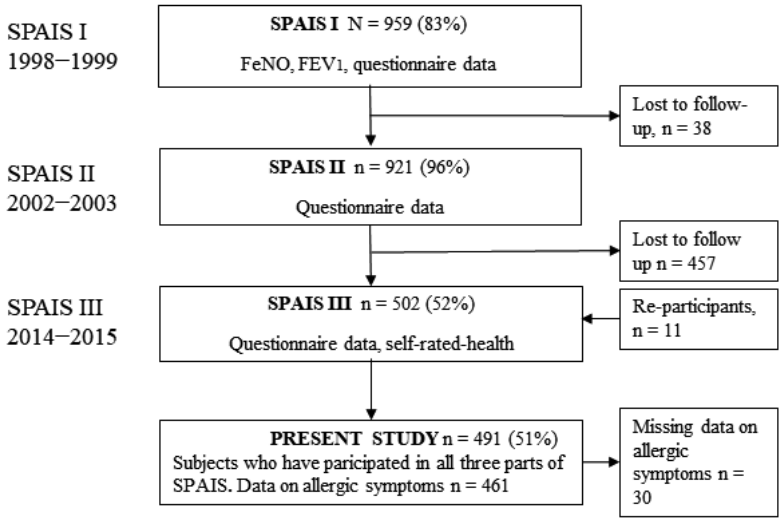

Figure 1. Flow chart of the SPAIS studies.

Questionnaires and definitions

Asthma was defined as ever having had parent- or self-reported asthma and having used corticosteroid asthma treatment or having wheezing or whistling in the chest or having a respiratory infection that caused wheezing or whistling in the chest in the preceding year (12). At SPAIS III, asthma was defined as above, but instead of only corticosteroid asthma medication, any asthma medication in the preceding year was included in the definition. Wheeze was defined as having had wheezing or whistling in the chest at any time in the preceding year. Rhinitis was defined as having had sneezing, nasal congestion, or rhinorrhoea during the preceding year, without having a cold.

At baseline, allergic symptoms were defined as the subject's hypersensitivity to cat, dog, or pollen, noticed and reported by the parents. Allergic symptoms at SPAIS II were defined as above, but reported by the participant. At SPAIS III, allergic symptoms were defined as the subject's experience of ever having had allergic symptoms to cat, dog, or pollen. Due to a missing page in the paper questionnaire, data on allergic symptoms were missing for 30 subjects.

Incidence of symptoms refers to no reported symptoms at baseline but at SPAIS III. Persistent symptoms were those reported at both SPAIS I and III and remission was defined as symptoms reported at baseline but not at follow-up.

Asthma, rhinitis, and smoking habits in the family, involving symptoms reported by mothers, fathers, and siblings, were questionnaire-assessed. The subject's current smoking habits at follow-up was defined as smoking at least one cigarette a day during the preceding six months.

At SPAIS III, the SRH question "How do you rate your general health status?" was included in the questionnaire. There were five response alternatives: "very good", "quite good", "neither good nor poor", "quite poor", and "very poor".

Exhaled NO

Measurements of FeNO were performed with the Aerocrine NO system (Aerocrine AB, Sweden), including the CLD 77 AM chemiluminescence analyser (Eco Physics AG, Dürnten, Switzerland), as previously described (8), and in accordance with the prevailing recommendations from the European Respiratory Society (15). Before measurement, each subject's mouth was washed with 25 ml of 10% sodium bicarbonate for 20 s. Three exhalations were performed during 10 s each and an average value was calculated. FeNO was measured at 0.1 L/s. To facilitate interpretation of the $FeNO_{0.1}$ values from baseline, one way to obtain a rough estimate of $FeNO_{0.05}$ is to multiply them with a coefficient of approximately 1.6 (16).

Pulmonary function

Pulmonary function measurements were performed in accordance with the criteria from the American Thoracic Society, using a Spirolab spirometer (Medical International Research, Rome, Italy). Lower limit of normal and percentiles for forced expiratory volume in 1 second (FEV_1) were calculated using the Excel (Microsoft Corporation, Redmond, WA, USA) macro for the Global Lung Function Initiative reference values (17). The lower limit of normal was defined as $FEV_1 < -1.65$ standard deviations (SDs) and is referred to as reduced FEV_1 in the text.

Statistical analyses

Statistical analyses were performed using STATA IC 14 (StataCorp, College Station, Texas, USA). Comparisons at the group level were made using t-tests for normally distributed continuous variables or using chi-squared tests for categorical variables. McNemar's test was used to assess within-subject changes of categorical variables across two timepoints. FeNO was log-transformed to achieve normal distribution and t-tests were performed on log-transformed FeNO. BMI was calculated as weight in kilograms divided by height in metres squared. SRH was dichotomised into good (response alternatives: very good and quite good) and poor (response alternatives: neither good nor poor, quite poor, and very poor) (18).

Multiple logistic regressions were performed with incident asthma as outcome and all variables identified as significant in the univariate analyses, for either girls or boys, used as predictors. The used multiple regression model was stepwise and variables were excluded if no significant association with the outcome was found. A p value < 0.05 was considered statistically significant.

Ethics

The study was approved by the Ethical Committee of the Medical Faculty of Uppsala University, Sweden (registration numbers 243/1998, 499/2001), and the Regional Ethical Review Board in Uppsala, Sweden (registration number 440/2013). The study procedures were in accordance with the Declaration of Helsinki (19). As described in an information letter appended to the questionnaire at SPAIS I, a completed parental part of the questionnaire was seen as written informed consent from the parents. The adolescents gave their informed consent by completing the ISAAC part of the questionnaire and by verbally agreeing to participate in the study. At SPAIS II and III, a completed and returned questionnaire was seen as written informed consent from the participants, in accordance with an information letter appended to the questionnaire.

Results

Participants' characteristics

A total of 502 (52.3%) subjects completed the questionnaire at SPAIS III. In the present study, only subjects who participated in all three parts of SPAIS were included, n = 491 (51.2%). There were 468 (48.8%) non-responders who differed only with regard to being slightly older, more often being males, and more commonly reporting smoking in the family at baseline (Table 1). The prevalence of asthma, wheeze, rhinitis, and allergic symptoms to cat, dog, and pollen, had all increased significantly between baseline and follow-up at SPAIS III (Figure 2).

Table 1. Baseline characteristics in the study population and those lost to follow up, N = 959.

	SPAIS III n = 491 (51.2%)	Lost to follow-up n = 468 (48.8%)	p value
Sex (male), n (%)	218 (44.4)	259 (55.3)	0.001
Age (years)	13.6 ± 0.40	13.7 ± 0.42	0.002
$FeNO_{0.1}$ (ppb)	4.68 (4.27, 5.12)	4.78 (4.35, 5.25)	0.76
FEV_1 (% predicted)	94.86 ± 10.35	95.18 ± 11.19	0.65
FEV_1 (< -1.65 SD), n (%)	37 (7.5)	42 (9.1)	0.40
BMI (kg/m^2)	19.78 ± 2.96	20.06 ± 3.21	0.16
Height (cm)	162.1 ± 8.04	162.8 ± 8.25	0.20
Wheeze, n (%)	68 (13.9)	59 (12.6)	0.57
Asthma, n (%)	41 (8.4)	42 (9.0)	0.73
Rhinitis, n (%)	122 (24.9)	121 (25.9)	0.72
Family smoking, n (%)	137 (27.9)	176 (37.6)	0.001
Allergic symptoms to cat, n (%)	49 (10.0)	50 (10.7)	0.72
Allergic symptoms to dog, n (%)	31 (6.3)	20 (4.3)	0.16
Allergic symptoms to pollen, n (%)	91 (18.5)	77 (16.5)	0.40

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; ppb, parts per billion; SD, standard deviation.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

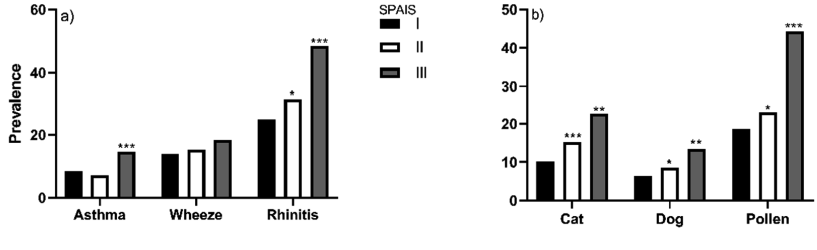

Figure 2. Prevalence (%) of a) respiratory symptoms and b) allergic symptoms reported at SPAIS I–III.

*** $p < 0.001$, ** $p = 0.01$, * $p < 0.05$, significant increase in reported symptoms

Incidence of allergic and respiratory symptoms

At SPAIS III, the prevalence of asthma and symptoms of wheeze had increased significantly for females, but not for males (Table 2). Remission was low for both sexes. The prevalence of allergic symptoms to cat had increased significantly over sixteen years, with no gender differences, and remission was low.

When examining the incidence of symptoms stepwise between SPAIS I, II, and III, we found that the incidence of asthma was very low at SPAIS II, and that the overall prevalence of asthma tended to be reduced between SPAIS I and SPAIS II (Figure 2a, E-table 1). In contrast, overall asthma prevalence increased between SPAIS II and III, with a strongly significant change in females and a trend in males (E-table 2). Concerning the prevalence of wheeze, there was a continuous increase from SPAIS I to III, but with no significant changes between the three study timepoints (E-tables 1 and 2). The prevalence of allergic symptoms to cat increased significantly during both time periods, in both males and females, and remission was low (Figure 2b, E-tables 1 and 2).

Table 2. Development of reported symptoms between SPAIS I (age 12-15 years) and III (age 28-31 years).

	Prevalence of symptoms at SPAIS I	Remission of symptoms between SPAIS I and III	Persistence of symptoms between SPAIS I and III	Incidence of symptoms between SPAIS I and III	Prevalence of symptoms at SPAIS III	*p value
Wheeze, n (%)						
All subjects, n = 491	68 (13.8)	39 (7.9)	29 (5.9)	61 (12.4)	90 (18.3)	0.03
Females, n = 273	40 (14.7)	21 (7.7)	19 (7.0)	40 (14.7)	59 (21.6)	0.02
Males, n = 218	28 (12.8)	18 (8.3)	10 (4.6)	21 (9.6)	31 (14.2)	0.63
Asthma, n (%)						
All subjects, n = 491	40 (8.1)	16 (3.3)	24 (4.9)	48 (9.8)	72 (14.7)	< 0.001
Females, n = 273	22 (8.1)	7 (2.6)	15 (5.5)	35 (12.8)	50 (18.3)	< 0.001
Males, n = 218	18 (8.3)	9 (4.1)	9 (4.1)	13 (6.0)	22 (10.1)	0.39
Cat symptoms, n (%)						
All subjects, n = 461	47 (10.2)	3 (0.7)	44 (9.5)	60 (13.0)	104 (22.6)	< 0.001
Females, n = 258	27 (10.5)	3 (1.2)	24 (9.3)	33 (12.8)	57 (22.1)	< 0.001
Males, n = 203	20 (9.9)	0 (0)	20 (9.9)	27 (13.3)	47 (23.2)	< 0.001

* p values compare prevalence rates at SPAIS I and III (McNemar's test)

Gender differences

At baseline (SPAIS I), the only significant gender differences were that males were taller and females tended to report more family asthma (E-table 3). A total of 72.5% of the girls had reached menarche. Current smoking at SPAIS III was reported by 53 subjects, or 11.5% of the females and 10.4% of the males (p=0.69). When examining gender differences in the prevalence of reported respiratory symptoms at all three timepoints, there were no significant differences at SPAIS I and II, but females tended to report more wheeze at SPAIS II (E-table 4). At SPAIS III, females reported more asthma than males (Figure 3a) as well as reporting more wheeze (E-table 4), whereas the prevalence rates of rhinitis and allergic symptoms to cat or dog (Figure 3b, E-table 4) did not show any gender differences at any timepoint.

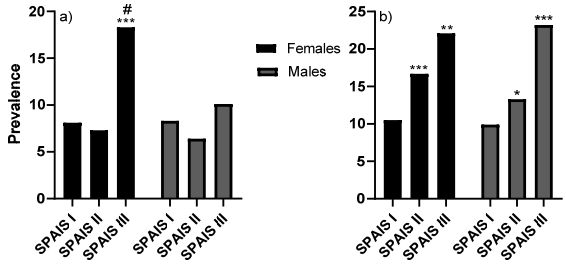

Figure 3. Prevalence (%) of a) asthma and b) allergic symptoms to cat in female and male subjects reported at SPAIS I–III. *** $p < 0.001$, ** $p = 0.01$, * $p < 0.05$, significant increase in reported symptoms. # $p = 0.01$, significant gender difference in reported symptoms.

Risk factors in early adolescence

As the development of respiratory symptoms showed clear gender differences, gender-specific analysis of baseline risk factors for incident asthma was undertaken. Females with incident asthma more often had reduced FEV_1 and reported more rhinitis, allergic symptoms to cat, and family asthma at baseline than females with no reported asthma at SPAIS I or SPAIS III (Table 3). Males who developed asthma had higher FeNO and more reported wheeze, rhinitis, and allergic symptoms to both cat and dog at

baseline than males who did not report asthma symptoms at any timepoint. Generally, males with incident asthma reported more allergic symptoms at baseline than females. At SPAIS III, 46.9% of the females and 53.9% of the males with incident asthma reported allergic symptoms to cat and 68.8% of the females and 61.5% of the males reported allergic symptoms to pollen. Furthermore, 66.7% of subjects with persistent asthma between SPAIS I and III reported allergic symptoms to cat at SPAIS III, compared with 31.3% of those with asthma remission during the same period. The corresponding percentages for allergic symptoms to pollen were 91.7% and 50%, respectively. There were more female than male cat and/or dog owners among the subjects who had developed asthma at SPAIS III, but pet ownership did not differ from that of subjects without asthma symptoms for either sex.

Table 3. Characteristics of subjects at baseline, except current smoking and pets at home*, in relation to incidence of asthma at SPAIS III (age 28-31 years).

	Girls			Boys		
	No asthma at baseline or at SPAIS III (n = 216)	Incident asthma at SPAIS III (n = 35)	p value	No asthma at baseline or at SPAIS III (n = 186)	Incident asthma at SPAIS III (n = 13)	p value
$FeNO_{0.1}$ (ppb)	4.46 (3.99, 4.99)	3.37 (2.12, 5.35)	0.09	4.39 (3.79, 5.10)	9.69 (4.58, 20.48)	0.009
FEV_1 (% predicted)	95.02 ± 8.98	92.88 ± 9.60	0.20	95.67 ± 11.08	93.36 ± 12.18	0.47
FEV_1 (< -1.65 SD) (%)	5.1	14.3	0.04	7.0	15.4	0.27
BMI ($kg/m^{2)}$	19.66 ± 2.94	20.12 ± 3.20	0.39	19.80 ± 2.99	21.18 ± 2.73	0.11
Height (cm)	160.7 ± 6.51	161.0 ± 5.43	0.83	163.6 ± 9.52	164.4 ± 10.6	0.76
Wheeze (%)	7.4	17.1	0.06	5.4	30.8	0.001
Rhinitis (%)	18.5	42.9	0.001	17.2	53.9	0.001
Allergic symptoms to cat (%)	4.2	17.1	0.003	4.8	30.8	< 0.001
Allergic symptoms to dog (%)	1.4	5.7	0.09	3.2	15.4	0.03
Allergic symptoms to pollen (%)	12.0	17.1	0.40	16.1	23.1	0.52
Family asthma (%)	29.6	42.9	0.12	20.4	69.2	< 0.001
Family rhinitis (%)	51.9	74.3	0.01	45.7	69.2	0.21
Family smoking (%)	31.9	25.7	0.46	25.3	30.8	0.66
Current smoking (%)	11.3	11.8	0.93	11.6	8.3	0.73
Cat at home (%)	17.8	22.9	0.47	15.1	8.3	0.52
Dog at home (%)	12.2	17.1	0.42	9.2	7.7	0.85

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; ppb, parts per billion; SD, standard deviation.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval. * self-reported at SPAIS III.

In multiple logistic regression analyses, stratified for gender and after adjustments for confounders (see Statistical analyses), reduced FEV_1, reported rhinitis, and family rhinitis at baseline were related to incident asthma in females. In contrast, higher FeNO, reported rhinitis, and family asthma at baseline were related to incident asthma in males (Table 4). There were no effects on the results when data on current smoking at SPAIS III, a non-significant variable for both sexes (Table 3), were added into the female model. However, in the male model, reduced FEV_1, wheeze, and allergic symptoms to cat at baseline became related exposures, and rhinitis at baseline ceased being a related exposure.

Table 4. Independent baseline risk factors for incident asthma at SPAIS III (age 28-31 years).

Risk factors	Incident asthma Females (aOR [95% CI])	Incident asthma Males (aOR [95% CI])
FEV_1 < -1.65 SD	4.11 (1.27, 13.24)	4.51 (0.59, 34.69)
$FeNO_{0.1}$	0.98* (0.92, 1.05)	1.13* (1.06, 1.20)
Rhinitis	3.34 (1.54, 7.25)	7.39 (1.78, 30.78)
Family asthma	1.47 (0.66, 3.25)	12.74 (2.88, 56.31)
Family rhinitis	2.89 (1.25, 6.68)	0.73 (0.14, 3.72)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; $FeNO_{0.1}$, fraction of exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; SD, standard deviation.
aOR: Variables adjusted for in incident asthma models: $FeNO_{0.1}$, FEV_1 < -1.65 SD, rhinitis, wheeze, allergic symptoms to cat and dog, family asthma and rhinitis, at baseline.
*per 1ppb increase in FeNO.

Self-rated health in young adulthood

The proportion of females with current asthma who rated their health as poor was significantly higher than for females with no asthma at SPAIS III, 20.0% vs. 7.7% (p = 0.008). Similar findings were seen for females for reported symptoms of wheeze and allergic symptoms to cat at SPAIS III: 20.3% vs. 7.0% (p = 0.003) and 21.1% vs. 7.0% (p = 0.002), respectively. These differences in poor SRH were not seen for males concerning any of these reported symptoms. However, when comparing the proportion of males and females with poor SRH in the group with reported asthma at SPAIS III, no significant gender difference was observed (p = 0.86).

Discussion

The main findings of this population-based cohort study on the development of respiratory symptoms, from early adolescence to early adulthood, were that the incidence of respiratory and allergic

symptoms had increased significantly between these life stages. When stratifying for gender, the incidence of both asthma and wheeze had increased significantly in females, but not in males, while the incidence of allergic symptoms increased significantly in both sexes. Objective measurements at baseline revealed that reduced FEV_1 in females and higher FeNO in males were independent risk factors for having developed asthma sixteen years later. Females with current asthma in young adulthood rated their health lower than females without asthma, whereas this was not found for males.

Previous studies

The prevalence of asthma, wheeze, and rhinitis in young adulthood in our study was in line with results of a Swedish cross-sectional study for subjects of similar age, 22–40 years (20). That study showed that 35.1% of the subjects were IgE-sensitised to pollen, 23.4% to cat, and 22.7% to dog. In our study, with self-reported allergic symptoms, the prevalence rates were similar, except that fewer reported allergic symptoms to dog, 13.4%. A Finnish population-based cross-sectional respiratory questionnaire study of more than 4,000 subjects showed that the incidence of asthma peaked in young boys (0–9 years) and in middle-aged women (40–49 years) (21). These results were confirmed by a recent study, encompassing six population-based birth cohorts, where a male predominance in prevalence was seen before puberty, as was a "sex shift" towards females after puberty, which was strongest in subjects who had asthma and rhinitis concurrently (22).

Studies have reported that female gender, allergic sensitisation, asthma severity, and family history of asthma are inversely related to asthma remission (23, 24). Furthermore, allergic sensitisation is a risk factor for persistent asthma (23, 24). In accordance, two thirds of the subjects with asthma at SPAIS III reported allergic symptoms to cat, compared with less than a third of the subjects with asthma remission at SPAIS III.

Results from a review study concerning impact of gender on asthma during childhood and adolescence concluded that asthma after childhood was more severe in females than in males, and was underdiagnosed and undertreated in female adolescents (1). This is in line with our results showing that the prevalence of wheeze was higher in females than males at all three study timepoints, indicating that some of the wheeze reported by females could be due to untreated asthma. At SPAIS II,

incident wheeze in girls was related to higher baseline BMI (obesity), reported rhinitis, and current smoking (13). At the same timepoint, baseline risk factors for the development of wheeze in boys were allergic symptoms to pollen, a family history of asthma, and reduced FEV_1. Thus, it seems that factors associated with lifestyle were more strongly associated with development of wheeze in girls, whereas factors associated with an atopic constitution were related to this development in boys. These lifestyle risk factors may have been more important during adolescence, but in a long-term perspective, rhinitis, family rhinitis and reduced FEV_1 at baseline were the risk factors for incident asthma in females. In males with incident asthma at SPAIS III, risk factors related to an atopic constitution, including type-2 airway inflammation (higher FeNO), remained, and this pattern was further strengthened after adjusting also for current smoking. Thus, incident asthma in young subjects seems to be more closely related to atopy in males than in females. The greater susceptibility of females to asthma may instead be explained by their smaller airway calibre (6). Another explanation may be irreversible airflow obstruction, developed in early childhood during periods of bronchial obstruction, and with symptom recurrence in adult life, even after long periods of clinical remission (25). Accordingly, periods of remission were characterised by reduced FEV_1 but no reported asthma symptoms in early adolescence, and the reduction in lung function was independently related to asthma development 16 years later. Furthermore, looking at girls with incident wheeze in the four-year follow-up of SPAIS, a higher proportion had started to menstruate at baseline, compared with girls who never reported wheeze (13). These results are in line with the view that female sexual hormones might contribute to the development of respiratory disease.

In a Swedish population-based study, poor SRH was associated with asthma and the association was at least as strong as that with poor asthma-related quality of life, with SRH having the advantage of being easier to apply (18). In another Swedish study, adolescent girls with asthma rated their quality of life with the DISABKIDS HRQoL asthma module, which focuses on specific physical and emotional aspects of asthma (26). Their ratings were lower than those of boys with asthma (27). These results are in line with our results that females with current asthma at SPAIS III rated their health lower than females without asthma, while this was not seen for males.

Methodological considerations

A major strength of the current population-based, longitudinal study of schoolchildren was the long follow-up period of sixteen years. Another strength was the availability of objective functional and inflammatory measurements at baseline as well as the use of the well-validated ISAAC questionnaire and similar additional questions at all three timepoints. A selection bias might be argued due to the response rate of 51.2% in the present study. However, this is similar to response rates in other cross-sectional epidemiological studies (21, 28) and longitudinal studies with similar follow-up times (29). Moreover, non-responders did not differ significantly in any baseline characteristics when compared with responders, with the exception of a slightly higher representation of males and family smoking history, a finding in line with other studies (30).

A limitation of the study may be missing data on allergic sensitisation and that only self-reported data on allergic symptoms were available. Another limitation may be the use of 100 mL/s as exhalation flow rate for FeNO measurement, the standard flow rate at the time of SPAIS I. However, though the FeNO values cannot be fully extrapolated to current clinical practice, we believe that the validity of the findings of an association between FeNO and incident asthma in males is not impaired.

Conclusions

The results from this longitudinal study, between early adolescence and young adulthood, confirmed previous findings concerning higher incidence of respiratory symptoms, both asthma and wheeze, in females than males. There was also a significant increase of allergic symptoms to cat, dog, and pollen over time, but without gender differences. Reduced FEV_1 at baseline was related to incident asthma symptoms in adult females, whereas higher FeNO at baseline related to incident asthma symptoms in adult males. Our data suggest that it may be necessary to detect risk factors for the development of respiratory diseases at an early stage to optimise health and wellbeing, and that gender differences in asthma development, associated risk factors, and treatment of respiratory symptoms, must be considered.

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Conflicts of interests

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E-table 1. Development of reported symptoms between SPAIS I (age 12-15 years) and II (age 16-19 years).

	Prevalence of symptoms at SPAIS I	Remission of symptoms between SPAIS I and II	Persistence of symptoms between SPAIS I and II	Incidence of symptoms between SPAIS I and II	Prevalence of symptoms at SPAIS II	*p value
Wheeze, n (%)						
All subjects, n = 491	68 (13.8)	35 (7.1)	33 (6.7)	42 (8.6)	75 (15.3)	0.43
Females, n = 273	40 (14.7)	20 (7.3)	20 (7.3)	29 (10.6)	49 (17.9)	0.20
Males, n = 218	28 (12.8)	15 (6.9)	13 (6.0)	13 (6.0)	26 (11.9)	0.71
Asthma, n (%)						
All subjects, n = 491	40 (8.1)	9 (1.8)	31 (6.3)	3 (0.6)	34 (7.1)	0.08
Females, n= 273	22 (8.1)	3 (1.1)	19 (7.0)	1 (0.4)	20 (7.3)	0.32
Males, n = 218	18 (8.3)	6 (2.8)	12 (5.5)	2 (0.9)	14 (6.4)	0.16
Cat symptoms, n (%)						
All subjects, n = 461	47 (10.2)	2 (0.4)	45 (9.8)	25 (5.4)	70 (15.2)	< 0.001
Females, n = 258	27 (10.5)	0 (0.0)	27 (10.5)	16 (6.2)	43 (16.7)	< 0.001
Males, n = 203	20 (9.9)	2 (1.0)	18 (8.9)	9 (4.4)	27 (13.3)	0.03

* p values compare prevalence rates at SPAIS I and II (McNemar's test).

E-table 2. Development of reported symptoms between SPAIS II (age 16-19 years) and III (age 28-31 years).

	Prevalence of symptoms at SPAIS II	Remission of symptoms between SPAIS II and III	Persistence of symptoms between SPAIS II and III	Incidence of symptoms between SPAIS II and III	Prevalence of symptoms at SPAIS III	*p value
Wheeze, n (%)						
All subjects, n = 491	75 (15.3)	37 (7.5)	38 (7.7)	52 (10.6)	90 (18.3)	0.11
Females, n = 273	49 (17.9)	22 (8.1)	27 (9.9)	32 (11.7)	59 (21.6)	0.17
Males, n = 218	26 (11.9)	15 (6.9)	11 (5.0)	20 (9.2)	31 (14.2)	0.40
Asthma, n (%)						
All subjects, n = 491	34 (7.1)	11 (2.2)	23 (4.7)	49 (10.0)	72 (14.7)	< 0.001
Females, n = 273	20 (7.3)	5 (1.8)	15 (5.5)	35 (12.8)	50 (18.3)	< 0.001
Males, n = 218	14 (6.4)	6 (2.8)	8 (3.7)	14 (6.4)	22 (10.1)	0.07
Cat symptoms, n (%)						
All subjects, n = 461	70 (15.2)	10 (2.2)	60 (13.0)	44 (9.5)	104 (22.6)	< 0.001
Females, n = 258	43 (16.7)	9 (3.5)	34 (13.2)	23 (8.9)	57 (22.1)	0.01
Males, n = 203	27 (13.3)	1 (0.5)	26 (12.8)	21 (10.3)	47 (23.2)	< 0.001

* p values compare prevalence rates at SPAIS II and III (McNemar's test).

E-table 3. Gender differences in relation to baseline characteristics at SPAIS I (age 12-15 years) and data reported at SPAIS III (age 28-31 years).

	SPAIS I Male n = 218	 Female n = 273	p value	SPAIS III Male n = 218	 Female n = 273	p value
$FeNO_{0.1}$ (ppb)	4.78 (4.13, 5.54)	4.60 (4.10, 5.15)	0.68	NR	NR	
FEV_1 (% predicted)	95.06 ± 11.19	94.69 ± 9.65	0.70	NR	NR	
FEV_1 (< -1.65 SD), (%)	8.3	7.0	0.59	NR	NR	
Age (years)	13.59 ± 0.39	13.55 ± 0.41	0.29	NR	NR	
BMI (kg/m^2)	19.83 ± 2.96	19.74 ± 2.97	0.74	NR	NR	
Height (cm)	163.6 ± 9.49	160.9 ± 6.43	< 0.001	NR	NR	
Menarche, (%)	NR	72.5	NR	NR	NR	
Family asthma, (%)	25.7	33.7	0.06	NR	NR	
Family rhinitis, (%)	53.7	56.0	0.60	NR	NR	
Family smoking, (%)	24.8	30.4	0.17	NR	NR	
Current smoking, (%)	NR	NR		10.4	11.5	0.69
Cat at home, (%)	28.4	25.6	0.49	13.4	17.7	0.19
Dog at home, (%)	21.1	18.7	0.50	9.3	13.0	0.20

Abbreviations: BMI, body mass index; $FeNO_{0.1}$, fractional exhaled nitric oxide measured at 100 mL/s; FEV_1, forced expiratory volume in one second; NR, not relevant; ppb, parts per billion; SD, standard deviation.
All results presented as % or mean ± standard deviation or geometric mean and 95% confidence interval.

E-table 4. Gender differences in relation to prevalence of reported symptoms at SPAIS I (age 12-15 years), II (age 16-19 years), and III (age 28-31 years).

	SPAIS I Male n = 218	Female n = 273	p value	SPAIS II Male n = 218	Female n = 273	p value	SPAIS III Male n = 218	Female n = 273	p value
Wheeze, n (%)	28 (12.8)	40 (14.7)	0.56	26 (11.9)	49 (17.9)	0.07	31 (14.2)	59 (21.6)	0.04
Rhinitis, n (%)	52 (23.9)	70 (25.6)	0.65	64 (29.6)	91 (33.3)	0.35	97 (44.5)	141 (51.7)	0.12
	n = 203	n = 258		n = 203	n = 258		n = 203	n = 258	
Allergic symptoms to dog, n (%)	12 (5.9)	17 (6.6)	0.77	17 (8.4)	22 (8.5)	0.95	24 (11.8)	38 (14.7)	0.36
Allergic symptoms to pollen, n (%)	42 (20.7)	44 (17.1)	0.32	46 (22.7)	60 (23.3)	0.88	86 (42.4)	118 (45.7)	0.47