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Asthma: Respiratory Symptoms, Atopy and Bronchial Hyperresponsiveness in Young Adults in Estonia and Sweden

BY

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Abstract

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Morbidity of asthma has increased over the world. The reasons for this increase have remained unclear. Studies in children have reported considerable East-West difference in the prevalence of atopy and respiratory allergies.

The aim of this thesis was to compare the prevalence and risk factors of respiratory symptoms, atopic sensitisation and bronchial hyperresponsiveness (BHR) in young adults in Estonia and Sweden.

Following the protocol of the European Community Respiratory Health Survey (ECRHS), two random population samples, 3000 from Tartu, Estonia, and 3600 from Uppsala, Sweden were investigated with postal questionnaires. Random sub samples and subjects with asthma-like complaints were subsequently interviewed, BHR was tested and serum samples analysed for total and specific IgE and eosinophil cationic protein (ECP). In a separate study two methacholine challenge methods, using either Spira Elektro2 or Mefar MB3 as dosimeters, were compared on 28 mild to moderate asthma patients.

Symptoms of asthma and hay fever were less common in Estonia than in Sweden, while respiratory symptoms in general were more common in Estonia. The prevalence of BHR was high and the prevalence of atopy and the levels of serum ECP were low in Tartu. The differences between the two centres in the prevalence of atopy and allergic rhinitis diminished with age, indicating a probable cohort effect. Current smoking was a dominant risk factor BHR and for all respiratory symptoms, except attacks of asthma, both in Tartu and Uppsala. There was some difference between risk factors for BHR and atopy between Tartu and Uppsala, mostly of social and environmental origin. The low prevalence of hay fever and asthma in Tartu seemed to be partly explained by a lack of awareness of atopy and allergic diseases in the Estonian society. The estimated cumulative dose causing a 20% fall in FEV_1 was smaller and the decline of FEV_1 /log(dose) curve steeper, using the Spira, compared to Mefar protocol.

Key words: asthma, atopic sensitisation, prevalence, epidemiology, ECRHS.

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Kitkus Irmi
Katkus Armi
Murdis maha Mustukene

"Kalevipoeg", kolmas lugu

List of papers

This thesis is based on the following papers, referred in the text by Roman numbers I-V:

I. **The prevalence of asthmatic respiratory symptoms among adults in an Estonian and Swedish university town**. Rain Jõgi, Christer Janson, Eythor Björnsson, Gunnar Boman, Bengt Björkstén. Allergy 1996; 51:331-336.

II. **Atopy and allergic disorders among adults in Tartu, Estonia compared to Uppsala, Sweden**. Rain Jõgi, Christer Janson, Eythor Björnsson, Gunnar Boman, Bengt Björkstén. Clin Exp Allergy 1998; 28:1072-1081.

III. **Bronchial hyperresponsiveness in two populations with different prevalences of atopy**. Rain Jõgi, Bengt Björkstén, Gunnar Boman, Christer Janson. Submitted.

IV. **Serum eosinophil cationic protein (S-ECP) in a population with low prevalence of atopy**. Rain Jõgi, Bengt Björkstén, Gunnar Boman, Christer Janson. Submitted.

V. **Comparison of two methacholine challenge methods using Spira-2 or Mefar dosimeter**. Rain Jõgi, Christer Janson, Hans Hedenström. Clin Phys 1999; 4:300-303.

List of abbreviations

AMP	Adenosine monophosphate
BCG	Attenuated bovine tuberculosis vaccine bacillus Calmette-Guérin
B-Eos	Eosinophil granulocyte in peripheral blood
BHR	Bronchial hyperresponsiveness
BMI	Body mass index
CI	Confidence interval
DDD	Defined daily dose
DNA	Deoxyribonucleic acid
ECP	Eosinophil cationic protein
ECRHS	European Community Respiratory Health Survey
ETS	Environmental tobacco smoke
FEV_1	Forced expiratory flow in first second
FEV_1%	Forced expiratory flow in first second as percentage of the predicted value
FVC	Forced vital capacity
GNP	Gross national product
IgE	Immunoglobuline E
ISAAC	International Study of Asthma and Allergy in Childhood
IUATLD	International Union Against Tuberculosis and Lung Disease
NO_2	Nitrogen dioxide
OR	Odds ratio
PD_{20}	Provocative dose causing 20% decline if FEV_1
PGE_2	Prostaglandin E_2
RHINE	Respiratory Health in Northern Europe
SD	Standard deviation
SO_2	Sulphur dioxide
SPT	Skin prick test
Th_x	Lymphocyte class T $helper_x$
VOC	Volatile organic compound

CONTENTS

INTRODUCTION

Asthma has been recognised by clinicians for more than two millenniums. Probably not much has changed from that time in the clinical picture of the disease, as the first descriptions of asthma resemble to that what doctors would also nowadays consider classical asthma. The first epidemiological studies of asthma date back to the first half of the XXth century. Since then a global increase in asthma prevalence has been revealed. While the reasons for this increase remain unclear, there can be no doubt that asthma is now a major health problem worldwide. Clustered in families, asthma has a genetic predisposition. It is unlikely, however, that the genetic makeup of stable populations can change significantly within one century, so the probable cause of the asthma epidemic must lie in the environment.

Asthma epidemiological studies are currently in the phase of that of cancer and coronary heart disease at the mid of the XXth century when international comparisons of the prevalence and incidence of those diseases revealed the major risk factors and formed the bases of the further research. The major obstacles in asthma epidemiology are the lack of a definition of asthma, the transient nature of the principal symptom, and the absence of simple, sensitive, and specific markers for the condition.

BACKGROUND

Definition of asthma

Many pulmonary diseases can be defined by their causative agent e.g., tuberculosis, or by defined pathology e.g., squamous cell carcinoma, or by characteristic clinical presentation. For asthma, the primary cause remains unknown, pathology is rarely available at the time of diagnosis and the clinical presentation can be variable. Hence, to date, no universally acceptable definition has been formulated [1]. The term "asthma" encompasses a disparate group of disorders which produce similar clinical effects – that is, variable airflow obstruction - and this has formed the basis of the definition of asthma. Perhaps the most concise and useful description of asthma is: a clinical syndrome of "variable airflow obstruction."

Development of understanding the mechanisms of asthma, and desire to give a definition that would satisfy clinicians, physiologist, genetics, immunologists, molecular biologists, pharmacologists, epidemiologists as well as pathologists have added new components to the definition. Current consensus definition of asthma has captured the history of development of understanding of the mechanisms of asthma in last decades and states:

Asthma is a chronic inflammatory disorder of the airways, in which many cells play a role, in particular mast cells, eosinophils, and T-lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, chest tightness, and cough, particularly at night and/or in the early morning. Theses symptoms are usually associated with widespread but variable airflow limitation that is at least partially reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli [2].

This descriptive definition of asthma, however, can not be translated into practical terms for epidemiological studies. Furthermore, despite the definition accepted for asthma, a diagnosis of asthma in clinical practice is made on the basis of combined information from history, physical examination, and respiratory function tests, often over a period of time. Time-consuming clinical testing is of little value in epidemiological setting because of low response rates and possible noncomparability of methods across countries and regions. For these reasons, comparisons of asthma prevalence are increasingly being based on a simple comparison of symptom prevalence in a questionnaire survey of a large number of people, followed by more intensive testing

of the underlying immunological and physiological components that often characterise clinical asthma, like bronchial hyperresponsiveness (BHR) and atopy, in a sub sample.

Outcome variables in asthma epidemiology

Variable obstruction

Methods used in clinical diagnosing of asthma are difficult to apply in epidemiological settings, especially in cross sectional studies. The main reason for that is the variable nature of asthma. Verification of reversible airway obstruction, which is considered critical in establishing a clear clinical diagnosing of asthma [2,3], would therefore underestimate the true prevalence, as reversible airway obstruction might not be revealed at time of testing.

Self reported asthma

Questionnaire based self reported asthma is often used in epidemiological studies. It has usually high specificity but low sensitivity to the actual disease, as all the cases of asthma are never diagnosed in the population [4]. This leads to an underestimation of the true prevalence of asthma. Further problems arise, when different populations with differences in labelling of asthma and diagnostic practice are compared, as the differences in diagnostic practice may be as great in magnitude as the real differences in asthma morbidity. A definition of high specificity is however important, when risk factors are estimated [5].

Symptoms of asthma

A number of symptoms, including wheezing, chest tightness, breathlessness, and coughing with or without sputum, are recognized by physicians as indicative of asthma. Symptom recording, based on standard postal and interview questionnaires, have also widely been used in epidemiological studies and can overcome some of the problems with diagnostic labelling. Symptom recording has, however, its own potential problems, arising from subjective symptoms recognition and recall, depending on variety of psychological, social and cultural characteristics, including healthcare practices, and also on the translation of the questionnaire. Those problems are evidently enhanced, when populations with different background regarding those factors are compared. To give an example, the term “wheeze” that has been used most often in epidemiological studies for the identification of asthma, can not be easily translated into many languages, furthermore, the specificity of the symptom to asthma may differ in populations with different prevalence of other conditions that can cause wheeze, like chronic bronchitis or COPD. High international consistency has, however, been shown in answers to different questions in multi-

lingual studies, indicating that international comparisons are not affected by errors due to cross-cultural variations in the reporting of symptoms [6].

To overcome cultural and language differences, video questionnaires have been introduced to asthma epidemiology studies. So far they have been used in children, and have shown good repeatability and slightly lower accuracy in detecting asthma [7], but general good correlation with the results of the written questionnaires [8].

Bronchial hyperresponsiveness (BHR)

The main objective using BHR in asthma epidemiology studies is, to avoid problems of subjective symptom recall that may occur with symptom questionnaires. The results of BHR testing clearly depend on which agent or stimulus has been used for provocation [9]. The best standardised methods are today available for direct stimuli, like histamine and methacholine that cause air-flow limitation by a direct action on the effector cells involved in the air-glow limitation, such as airway smooth-muscle cells, bronchial vascular endothelial cells and mucus-producing cells [10]. Comparisons between different delivery systems [11-13] and agents used [14] have shown good correlation, so different methods using either histamine or methacholine, have been considered roughly equivalent [9]. As choosing a threshold is always arbitrary in defining if the subject is bronchially hyperreactive or not, and to overcome the data loss in dichotomous analyses of the data, slope measures have been introduced that allow to get data for analyses from all participants in the study [11]. Agents that act as specific constrictors in asthma through indirect stimuli via cellular activation or neural stimuli, such as inhaled adenosine monophosphate (AMP) are more closely associated with eosinophilic airway inflammation in asthma, but have so far been used sparsely in community studies [15,16].

BHR testing was introduced to the asthma epidemiology studies at the beginning of the 1980s, based on early studies, where high sensitivity of BHR for diagnosed asthma was shown on selected groups of patients compared to healthy controls [17,18]. Subsequent, population based validations, comparing both symptom questionnaires and BHR, with asthma defined on the basis of a clinical assessment by a physician, however, did not confirm those results [4,19,20]. Thus, while the sensitivity of questionnaires for physician diagnosis of asthma was 80% in adults and 61% in children, and the specificity 97% and 94%, respectively, the sensitivity of BHR for physician diagnosed asthma was only 39% for adults and 54% for children [19]. The problems of the validity of BHR testing in asthma prevalence studies can not be overcome by combining BHR with symptoms in defining asthma, which has also been suggested [3,18]. A definition of asthma re-

quiring both a positive questionnaire response and BHR was highly specific but not sensitive for adults (37%) or children (47%) [19].

The subject of validity of BHR testing in asthma prevalent studies has recently been summarised by Pearce *et al*. [21], concluding that current evidence suggests that BHR testing has no greater validity (and may even have lesser) than symptom questionnaires for measuring the difference in asthma prevalence between populations with the same language and similar symptom recognition and reporting, but it may provide more comparable information when comparing populations which do not share these characteristics. BHR testing in epidemiological studies can, besides potentially lowering the response rate, introduce selection bias, as severe obstruction is usually considered a contraindication for provocation test and the most severe cases will therefore not be tested.

Thus, BHR testing can not provide validation of the existence of differences in the prevalence of asthma between populations but can, however, be useful in terms of interpreting the findings of symptom prevalence questionnaire, showing to what extent the differences can be explained by BHR.

Other outcome variables

Although asthma and atopy are strongly associated, they also occur independently of each other [22]. Thus, in asthma epidemiology studies, atopy is not a surrogate measure for asthma. It is both an associated condition (which is of interest in itself), and a risk factor for developing asthma. In the latter context, atopy can also be considered as an intermediate step in the causal pathways leading from allergen exposure to asthma – i.e., it may be an intermediate factor and a modifier of the effects of other exposures [23]. Atopy is also strongly associated with BHR, but the two conditions often occur independently of each other.

The number of eosinophil granulocytes in peripheral blood (B-Eos) is traditionally the most common method in assessing inflammation in epidemiological research [24-28]. More recently, measurements of eosinophil degranulation products, such as eosinophil cationic protein (ECP) in peripheral blood has been introduced as a method to assess and monitor inflammation in asthmatic patients [29-31]. There is, however, limited experience in using these kinds of inflammatory markers in asthma epidemiological research [32,33].

Increase in prevalence

Data from epidemiological studies conducted in several countries worldwide have revealed that the prevalence of allergic conditions, including allergic rhinitis, asthma and eczema, have increased from the 1940s/50s to the 1990s [34-37]. Although the increase of asthma prevalence may, in part, be explained by altered diagnostic criteria and increased awareness of the disease, both in the general population and among physicians, these studies together indicate that the increased prevalence of asthma during the last decades is real. Trends of increasing prevalence of asthma have also been shown in Nordic countries [38,39]. Data on asthma prevalence in Estonia are limited to studies conducted in the 1990s and it might be too early to look for changes in prevalence.

Regional differences in prevalence

International and regional asthma prevalence comparisons are required as a key step in ascertaining the causes of asthma [40]. The key issue in prevalence comparisons is, that the information is obtained in a comparable manner across all participating centres and countries, and that problems of translation of questionnaires and other problems of noncomparability of information are minimised. Two large scale epidemiological studies that allow comparison across several countries fulfil these criteria: The European Community Health Survey (ECRHS) [41,42] among adults and the International Study of Asthma and Allergy in Childhood (ISAAC) [43] in children.

The database of the ECRHS includes information from approximately 140,000 individuals from 22 countries. Based on analyses of the whole ECRHS data set, geographical variations in different outcome variables, i.e. diagnosed asthma, respiratory symptoms, atopic sensitisation and bronchial responsiveness have been described [44-47]. An eight-fold variation in the prevalence of wheeze, a four-fold variation in the prevalence of nasal allergy [44] and a six-fold variation in the prevalence of current asthma [44,47] was found. The prevalence of all symptoms varied widely. Although these were generally lower in northern, central and southern Europe and higher in the British Isles, New Zealand, Australia and the United States, there were wide variations even within some countries [48]. Analyses of bronchial responsiveness showed an eight-fold variation in BHR (PD20 $\leq$ 1 mg). The geographical distribution of bronchial responsiveness (ECRHS slope) and BHR fitted well with that for symptoms and asthma [45]. A wide geographical variation was also found when investigating the prevalence of atopic sensitisation (specific IgE) [46]. A high prevalence was found in Australia, New Zealand, United Kingdom and United States, while the prevalence was low in Iceland, Greece, Norway, Italy and parts of Spain. A five-fold

variation was found in the geometric mean of total IgE but there was no correlation between total IgE and atopic sensitisation [46].

The ISAAC design comprises three phases. Phase One used simple core written questionnaires for two age groups, and was completed in 156 collaborating centres in 56 countries and a total of 721.601 children participated. In the 13-14 years age group 155 centres from 56 countries participated, of which 99 centres also completed a video questionnaire. For the 6-7 years age group there were 91 collaborating centres in 38 countries. The study has demonstrated a large variation in the prevalence of asthma symptoms in children throughout the world. The prevalence of wheeze in the last 12 months ranged from 2-32% in the older age group and from 4-32% in the younger age group and was particularly high in English speaking countries and Latin America. A video questionnaire, completed in the older age group in 99 centres (42 countries), showed a similar pattern [8,49]. The prevalence of atopy-related disorders was higher in Scandinavia than in Estonia, Latvia and Poland, which in turn had a higher prevalence than five other countries of eastern Europe with a culture less similar to western Europe [50]. The prevalence data from the ISAAC study were compared to the data from the ECRHS [51]. The prevalence estimates in the ECRHS were consistently lower than the prevalence rates in the 13-14 years age group in the ISAAC study. There was, however, generally a good correlation between the prevalence of wheeze and asthma in the ECRHS and the corresponding prevalence in the ISAAC study.

Within country comparisons of urban and rural populations have shown lower prevalence of asthma in the latter, both in western [52] and in developing countries [53]. In some cases the differences have been striking [54]. Furthermore, comparing the lifetime prevalence of physician diagnosed asthma in Finnish first-year university students aged 18-24 according to childhood residence, revealed a significantly lower prevalence in those students who grew up in farm environments compared to those who grew up in urban or rural areas but not in a farm. No difference in asthma prevalence was found when students from urban and from rural non-farm environment were compared [55].

Prevalence studies have revealed three distinctive patterns: first, asthma is more prevalent in English-speaking countries; second, asthma is more common in Western countries than in developing countries and the difference is larger in later birth cohorts; third, the prevalence of asthma is lower in rural than in urban regions, especially among those, who have grown up in

farm environment. These results also indicate that the current recognised risk factors for the development of asthma probably cannot fully account neither for the worldwide increase in prevalence nor for the international variations in asthma prevalence. For example, the global pattern of asthma prevalence is consistent with the considerable body of evidence that air pollution is not a major risk factor for the development of asthma. Regions such as China and Eastern Europe, where some of the highest levels of traditional air pollution such as particulate matter and SO_2 are found, generally have lower asthma prevalence than the countries of Western Europe and North America, Australia, and New Zealand, which have lower levels of pollution.

East-West difference in asthma prevalence

Western lifestyle has been accused for the increase in the prevalence of asthma and allergies. Prosperity in West European countries with market economy might have changed the environment to the extent, where the spread of possible disease modifiers have reached to the extent where it is not possible to evaluate their impact without comparing them with reference populations. Comparisons of populations that are culturally and geographically similar but differ on the level of "westernisation" could give better possibility to extract risk factors from the "westernisation" than comparing populations that differ in many other aspects. The end of the Soviet era in Eastern Europe has given a unique possibility to evaluate the health effects of "westernisation".

The firs east-west comparison was carried out shortly after the reunification of Germany among 10-year old schoolchildren living in Leipzig, former East, and Munich, former West Germany [56]. The study found lower prevalence of hay fever in the eastern part of Germany. The prevalence of asthma, defined as either doctor diagnosed asthma ever or recurrent wheezy bronchitis did not differ significantly between the two populations, while the doctor diagnosed chronic bronchitis was about twice as high in Leipzig. The prevalence of wheeze was similar and there was no difference in the prevalence of BHR measured by cold air challenge.

In a subsequent study, the same population in Munich was compared to a bigger random sample from Leipzig and Dresden. Atopic sensitisation was estimated by skin prick testing, current asthma, and life long incidence of asthma was reported, and definition of BHR was slightly changed. In this study the prevalence of asthma, hay fever, atopic sensitisation and BHR was lower in Leipzig and Dresden, while the prevalence of respiratory symptoms and chronic bronchitis was higher in Leipzig and Dresden than in Munich [57].

Other prevalence comparisons in the beginning of the 1990s between previously socialist countries in Eastern Europe and industrialised countries with market economy in Western Europe, have constantly shown lower prevalence of atopic sensitisation and hay fever, both among children [52,57-61] and adults [62,63]. The difference in sensitisation between the centres has been found to decrease with increasing age [63,64], indicating a cohort effect, i.e. the causative environmental factors would be operating mainly in early childhood. There is at least one presented exception, where no east-west difference in the prevalence of atopic sensitisation was revealed in the study conducted in the year 1991. In this study five- to seven-year-old children from Eastern and Western Germany were tested by a multipuncture device [60]. The groups were, however, relatively small (Eastern Germany was represented only by 287 subjects) and there were big differences in the prevalence of atopy between centres from the western part of Germany.

The rate for diagnosed asthma has also tended to be higher in western than in eastern populations [48,57,61,62,65], whereas less information is available on BHR and the results have been contradictory. Thus, the comparisons of eastern and western Germany have shown a higher prevalence of BHR in the West, both among children, measured either by cold air challenge [57] or hypertonic saline [65] and among adults, measured by methacholine challenge [62]. The prevalence of BHR, as measured by methacholine challenge, was however high among Estonian children [66].

The reasons for the East-West differences in atopy and related respiratory diseases has remained mostly unexplained. In most cases neither personal nor environmental risk factors could provide satisfactory explanations for the area differences in symptoms, BHR [62] or atopic sensitisation [57,63], as the differences in prevalence have remained statistically significant after allowing for confounding factors. In some reports the observed prevalence differences in atopy and respiratory allergies have partly been related to factors like "wood or coal heating" [61,62] or "reported family history of atopy" [61], that can also serve as surrogate measures for some environmental factors not measured. The published data do not support the view that the differences in sensitisation are caused by differences in the exposure to specific allergens [67]. The east-west difference in the prevalence of respiratory symptoms and chronic bronchitis has been explained by higher prevalence of smoking and ambient air pollution with particulate matter and SO_2 [56,68].

Recent studies have shown some converging in the described differences. Thus, the prevalence of atopic sensitisation and hay fever had increased in Leipzig after four years of unification [69]

and no difference in the prevalence of atopic sensitisation and hay fever among 9-11 year old children could be revealed between Leipzig and Munich [65]. Similar trends have shown among adults. By the ECRHS study group in Germany a second stage 1 questionnaire was sent out to a new random population sample 3-4 years after the previous one. The result of this study showed that the prevalence rates of wheezing, asthma attacks, asthma medication and allergic rhinitis were stable in Hamburg but increasing in Erfurt and approaching those of Hamburg [70]. In a subsequent follow up of the German stage 2 sample the prevalence of BHR was found to have increased in subjects living in Erfurt, while BHR remained unchanged in the group of subjects from Hamburg [71]. In Estonia two cross-sectional studies, the first in 1992-1993 [58] and the second in 1996-1997 [72], among 10-year-old children did not show significant increase in 12-month prevalence of wheeze, self reported asthma or atopic sensitisation.

Factors that influence asthma

The prime consideration in asthma epidemiology studies is usually to obtain exposure information of similar accuracy for the groups being compared. Strictly speaking, "exposure" refers to the presence of a substance in the environment, whereas "dose" refers to the amount of substance that reaches susceptibility targets within the body. As personal dose measurements can often not be obtained, the term exposure has been used in the very general sense, including other attributes or agents that may be risk factors for asthma, like demographic and genetic factors [23]. Due to inherent variable nature of asthma, it is also usually not possible to distinguish, especially in cross sectional studies, if the factor studied is attributable to development of or exacerbation of asthma. However, major problems of morbidity, occurring through exacerbation and prolongation of asthma symptoms, can best be addressed by studying prevalence rather than incidence. Thus, if factors are found to be associated with asthma prevalence, then this is of major interest in itself, irrespective of whether the etiologic mechanism involves increase in asthma incidence or increase in duration.

Age and Sex

The prevalence of wheeze and reported asthma is negatively related to age among adults [73-76]. BHR declines with age in atopic non-smokers and increases with age in smokers [77,78]. Furthermore, many cases of adult-onset asthma can actually be the return of asthma symptoms in subjects who had asthma in childhood.

In childhood males have a higher incidence of asthma than females, but in adolescence this reverses and between the ages 10-50 years females have a higher incidence than males. In later

life the incidence possibly reverses once more [79]. This has also been reflected in prevalence studies [80-82]. In males, higher BHR has been observed in childhood-adolescence age groups and at older ages, while in females a higher level of BHR has been observed during adulthood [83,84]. Those differences can be partly attributable to sex differences in lung growth and airway geometry [82,85], but may also be connected to the sex-related differences in the immune system. Facilitation of ovule implantation and tolerance to the foetus (a semi allograft) are required for a successful pregnancy. A decrease in the rejection of potential allogens is particularly marked during pregnancy, but also occurs in general in women, during the childbearing age, and more particularly during the periovular phase of the menstrual cycle [78,86]. A positive correlation has been found in asthma incidence and hormone replacement therapy in postmenopausal women [87]. Also, premenstrual worsening of asthma symptoms with increase in airway resistance has been reported [88,89].

Gender differences in the effects of environmental factors may be related to personal habits, like tobacco and alcohol consumption, the occupational environment, and the home environment. Women have been found to be more susceptible than men to environmental factors [90-93]. As it is, in general, socially acceptable for women to be breathless of effort and for men to bring up phlegm and snore, this can introduce recall bias and challenge the interpretation of results [86].

Genetic factors

Asthma appears to be multifactorial in origin and influenced by multiple genes and environmental factors. A particular genetic factor may increase susceptibility to the effects of an environmental exposure and may thereby affect one or more aspects of the complex etiological process involved in asthma, but whether this genetic potential is expressed will depend on whether sufficient exposure to the environmental factor occurs. Asthma genetic studies are further complicated by the difficulties in defining the asthma "phenotype" and by the fact that this phenotypic expression may vary with age. Despite the fact that genetic susceptibility to changing environmental exposures may play an important role in the changes in asthma prevalence, the considerable increase in asthma prevalence during the last decades indicates that genetic factors alone are unlikely to account for a substantial proportion of asthma cases. Major susceptibility genes for asthma and atopy have not been determined to date.

Family concordance

People with family history of asthma are more likely to develop asthma themselves, and parental asthma is a stronger predictor of asthma in the offspring than parental atopy [94]. This associa-

tion, however, is not necessarily due to genetic factors, and could merely reflect similar lifestyles and exposures in family members.

Twin concordance

Twin studies have shown the considerable genetic component of asthma. This component most likely consists of genes of additive effect [95]. The probandwise concordance for asthma in monozygotic twins has been 38-52% [96,97], and even this may in part be due to similar environmental exposures, including common intrauterine environment. Twin studies have also indicated that not only the shared, but also the individual specific environmental factors may be important as well [96]. Most of the environmental risk factors are, however, shared by nature. Furthermore, two presumptions of the genetic studies using twin-designs, first, that twins are representative of the general population and second, that the environment for both monozygous and dizygous twins is similar, may not be totally valid. Thus, the shared intrauterine environment may have an adverse effect on the growth and organ maturation of the foetus and higher similarity in environment for monozygous compared to dizygous twins is likely [95].

Segregation and linkage analysis

Segregation analysis tests explicit models of inheritance in families – for example, by observing the frequency of the condition in offspring and siblings and comparing it to the distribution expected on the basis of various models of inheritance. Linkage analysis uses DNA marker data in order to follow the transmission of genetic information between generations in order to determine if a genetic marker is linked to the gene involved in a particular disease. Attention has been particularly focused on chromosomes 5 and 11, both of which may contain genes relevant to asthma and atopy.

Atopic sensitisation

"Atopy" has previously been used as a poorly defined term to refer to allergic conditions that tend to cluster in families, including hay fever, asthma, atopic eczema, and other specific and non-specific allergic states. More recently, atopy has been characterised by the production of circulating IgE in response to common environmental allergens. Although atopy has sometimes been defined as "a genetic disposition" for this IgE response, most definitions focus on the production of IgE irrespective of the mechanism (genetic or environmental) by which it is produced [23]. The commonly accepted hypothesis of the relationship between atopy and asthma is, that exposure of genetically susceptible individuals to allergen leads to the development of sensitisation, and continued exposure leads to clinical asthma through the development of airway

inflammation, BHR and reversible airway obstruction [22]. The nature of the relationship between allergic sensitisation to specific allergens and asthma can either be a direct causative one, with allergen exposure causing asthma in susceptible individuals, or an indirect one, where the genetically determined atopic diathesis causes both asthma and expression of sensitisation [98].

The association between atopy and asthma depends on the population studied. There is a considerable amount of studies showing an association between atopy and asthma [22,62,81,99-101]. In a Finnish twin cohort, 262 twin pairs discordant for incident asthma were analysed. The atopic twin had an increased risk of asthma compared with the non-atopic co-twin (RR 2.91, 95% CI 1.81 to 4.68) [102]. There are, however, negative reports [103], and inverse association between asthma and atopy has been reported [54]. Reviewing the available epidemiological evidence on the association of asthma and atopy, Pearce and co-authors showed that the proportion of asthmatic and non-asthmatic subjects who are skin prick test positive vary considerably between different studies. The population attributable risk varied from 25% to 63% in children and from 8% to 55% in adults. The results were similar if specific or total IgE was used as an outcome. Furthermore, increase of the prevalence of atopic sensitisation without increase in the prevalence of asthma [69], as well as increase in the prevalence of asthma with only minor changes in the prevalence of atopy [104] has been reported. Allergens differ in their potency to cause asthma symptoms, thus pets are more potent than pollen [105].

The association between allergic sensitisation and bronchial responsiveness has been studied on a combined ECRHS data set [106]. Sensitisation to mite, cat and timothy grass explained between 1.4 to 12.7% of the total variation in bronchial responsiveness in the different centres. The variation of bronchial responsiveness was better explained by taking account of all the individual allergens than by classifying the subjects as atopic or non-atopic. Mite sensitisation was the most important allergen in 15 centres, cat in 8, cat and mite equally in 1, timothy in 8 and *Cladosporium* in 2 centres. Total IgE has been showed to associate with BHR independently of specific IgE [106-108]. The attributable risk of atopic sensitisation for BHR was found to be 19% in Sweden and 21% in Spain [105]. Possibility has been proposed that asthmatics become sensitised disproportionately to those allergens that (because of particle size) are deposited in the inflamed/primed lower airway (so asthma causes sensitisation). Bronchial hyperresponsiveness can in turn prevent the deposition of larger particles in the lower airways, thus prevent sensitisation.

Furry pets

Positive correlation has been reported between the community prevalence of cat ownership and sensitisation to cat, as well as respiratory symptoms and physician diagnosed asthma in young adults [109], stressing the importance of cat allergens in developing and exacerbating asthma [105,110,111]. Children exposed to cat during the first year of life were, however, less often SPT positive to cat at 12-13 years and children exposed to pets during the first year of life had a lower frequency of allergic rhinitis at 7-9 years of age and of asthma at 12-13 years [112].

Analyses of the combined ECRHS data have shown a positive association between current cat ownership and specific sensitisation in subjects reporting no respiratory symptoms associated with pet exposure. Subjects with symptoms associated with exposure to pets were deliberately excluded from the analyses to avoid the selection bias in cat ownership. Having a cat in childhood was, however, negatively associated with cat sensitisation in the same analysis in subjects with a family history of atopy, while this association was not found in subjects without such a family history . Based on the analyses of the same database, Svanes *et al.* reported that atopy was negatively associated with having a dog in childhood, but that there was no significant association with having a cat as a child, and atopy [113]. The possibility, that higher allergen exposure had induced tolerance in those having a dog in childhood is unlikely, as having a dog in childhood was protective also against sensitisation to other allergens. The finding may reflect pet avoidance by allergic families, however, there is no reason why dogs, but not cats, should be selectively avoided, as cats are often more potent to cause symptoms [105]. It is therefore more likely, that some lifestyle factors confound the favourable effect of dog keeping, that are not present among cat keepers, or that more potent cat allergen overwhelms the potential protective effect.

Smoking

Smoking is a well recognised and prevalent risk factor for respiratory symptoms [75,100,114,115], as well as BHR [77,84,84,116]. The association between current smoking and asthma is less clear. Association between smoking and asthma has been found cross-sectionally [101], also an association between adult onset asthma [117,118] and severity of asthma [119] has been found. Many cross-sectional [75,120,121], and some longitudinal studies [115] have, however, not found a clear association between smoking and asthma. Furthermore, in a case control study of subjects from 16 countries, the risk of asthma with an onset within the last 3 years before the survey was found to be 43% lower in current smokers than in never smokers [82]. However, in a three-year follow up

study Plaschke *et al* found that onset of asthma was more common in current smokers than in non-smokers, but this effect of smoking was mainly found in non-atopic subjects [122]. Similarly, Sunyer and co-workers found, that smoking was associated with bronchial responsiveness only in subjects without atopy [123].

In the analysis of the combined ECRHS data set Jarvis *et al* reported a higher risk of sensitisation to mite in smokers than in never smokers [124]. The risk of sensitisation to grass and cat was, however, lower in current and ex-smokers than in never smokers. Smoking has been found to be positively associated with total IgE [124-126] though less than 1% of the variation in total IgE could be explained by smoking [124].

Every risk factor that is voluntary, is selection biased to factors like social status, personal habits and awareness. Smoking in this sense is an extreme example. Unhealthy nutrition patterns have been found consistently higher in smokers than in non-smokers. Smokers consume more saturated fat, more alcohol, more cholesterol, less fruit, and fewer vegetables, more fried foods, less fibre, less antioxidant vitamins, more salt. Smokers have less knowledge on how to improve risk and lower intention to change [127]. Thus, part of the association or lack of association found between respiratory symptoms, asthma and smoking, can be attributed to other factors than smoking itself.

Environmental tobacco smoke (ETS)

Passive smoking is widespread and passive smokers are exposed to both side-stream and mainstream tobacco smoke which contains many potent respiratory irritants. A considerable amount of studies have evaluated the effects of ETS on asthma in childhood [128]. There is strong evidence for a causal role of ETS in the development of asthma in children and parental smoking is related to more severe prognosis of asthma at least until school-age [129].

Much less is, however, known on the relationship between ETS and asthma in adulthood. Six studies, one longitudinal [130], four cross-sectional [131-134] and one nested case-reference study [135] have addressed the role of ETS in induction of asthma in adults, and shown the excess risk of asthma from 10 to over 200% in young adults and from 40 to 60% in older age groups due to ETS exposure [128]. ETS has also shown to worsen the existing asthma [136], and impair the lung function [137]. A recent paper, based on the analysis of the whole ECRHS data set showed that passive smoking in the workplace, but not at home, was significantly related to current asthma as well as all respiratory symptoms except attacks of breathlessness at rest [132]. A significant

dose-related association to passive smoking was found for respiratory symptoms. ETS was also related to increased bronchial hyperresponsiveness.

Indoor environment

Importance of indoor environment is stressed by the fact that in developed countries people spend the major part of the time indoors. The data on the indoor environmental risk factors for asthma in adults is, however, limited. Physical characteristics of the indoor environment include temperature and humidity. The latter increases when ventilation is inadequate. Home dampness, reflected as damp stains or visible mould was shown to associate with cough and asthma in a population based study in adults in the Netherlands [138]. The association between asthma, and both dampness and visible mould, was strong in crude analysis but was not statistically significant when two markers were combined and adjusted for active and passive smoking, indoor NO_2 sources and educational level in multiple regression analyses. Self-reported mould growth at home has been associated with asthma in young adults in a selected population attending the smoking cessation programme [131], first year university students [139] and in a case control study of physician diagnosed asthma patients [140]. In the latter study the severity of asthma correlated with actual measures of total dampness in the dwelling. In Uppsala current asthma was more prevalent in subjects living in damp dwellings and particularly so in those living in dwellings with dampness in the floor construction [141]. Dampness in the home during childhood has been associated with increased airway responsiveness in adolescence [142]. In a recent population based case-references study of adult onset asthma, Thorn *et al*. reported increased risk to outcome in subjects who reported visible mould growth (OR 1.4-3.5), visible dampness and mould growth (OR 1.1-3.1) or who had a wood stove in their dwelling [135].

Homes abound in chemical air pollutants. Sources include unvented combustion appliances, smoking, building materials, carpets etc. In 1996 Jarvis and co-workers reported that in the UK, women who used gas cookers had an increased risk of wheeze and other asthma symptoms as well as lower lung function (FEV_1 and FEV_1/FVC) than women not using gas cookers [91]. No such association was found in men. In a subsequent analysis of the combined data set, Jarvis *et al.* reported that there was an overall association between the use of gas cookers and respiratory symptoms in women, but that this association varied considerably between the centres [92]. In general there was a positive association between gas cooking and symptoms in most European centres but a negative association in Australia, New Zealand and the United States. The reason for this across country heterogeneity was not explained but could possibly be related to differences in the nature of the gas in different countries.

Building materials can act as sources of formaldehyde and other volatile organic compounds (VOC). Nocturnal breathlessness has been shown to associate with higher levels of CO_2, formaldehyde and total concentration of VOC [143]. An association between respiratory symptoms and living or working in a newly painted dwelling, and BHR and living in a dwelling with newly painted wood details or kitchen painting has been reported [144]. Björnsson *et al.* have reported higher levels of house dust mite and airborne bacteria in houses of subjects with asthma related symptoms [145]. This association was, however, not related to mite allergy as none of the subjects who lived in houses with measurable house dust mite levels were sensitised to house dust mite.

Outdoor environment

Air pollution is convincingly associated with many signs of asthma aggravation. The adverse effects of SO_2 , ozone, and particulate matter to asthma patients have been proved in many experimental studies [146]. There is less evidence of effects of NO_2 from experimental studies. However, a slight increase in daily admissions to hospital for asthma in adults has been seen in relation to increasing ambient levels of nitrogen dioxide (NO_2) [147]. In adults no consistent effects of long-term exposure to ambient ozone concentrations on the development of asthma and atopy have been demonstrated [148].

If air pollution influences the prevalence of asthma, it has clearly not been the main driving influence over the last decades, as the main increase in asthma prevalence in Western countries has come at a time when air pollution has been considerably decreased, and Eastern Europe that had particularly bad air pollution had a markedly low prevalence of asthma. That, however, concerns mainly particulate matter and SO_2. It has been argued that NO_2, diesel particles of other products associated with traffic pollution may be responsible for the West-East difference in the prevalence of asthma.

Occupational exposures

Asthma is the most common occupational respiratory disorder in industrialised countries. Occupational asthma can be caused by several high and low molecular weight sensitises, of which more than 250 have been identified [149]. The determination of how many cases of asthma may be caused or worsened by occupational exposures is highly dependent on how asthma is defined, what constitutes work-relatedness, and what specific methodology is employed. The proportion of asthma attributed to occupational exposure has been estimated to be 5–10% [150-153]. Asthma

has been associated with high dose exposure to biological, and mineral dust as well as exposure to gases and fumes [151]. A higher risk for asthma has been found among laboratory technicians, painters, plastic workers, cleaners and agricultural workers [152,153].

Socio-economic status

A high prevalence of asthma has been found in affluent Western countries. On the community level a higher prevalence of asthma has been shown in more affluent regions in developing countries [148,154]. Higher paternal social class was also associated with increased asthma prevalence in a British 1970 birth cohort studied at 26 years of age [155], and in children who had wheezed by five years of age, the persistence of symptoms at 16 years of age was independently related to high social status of the mother [156]. Higher educational level was also a risk factor for clinical allergies among adults in East Germany [63]. However, the more educated twin of 262 twin pairs discordant for incident asthma, had a decreased risk of asthma compared to twin sibling with less education [102], and asthma has shown to be more severe in patients from poorer background [157].

Number of siblings, day-care attendance and infections

One temporal association with the increase in atopy in developed countries has been the use of antibiotics, widespread vaccination of children and the fall in exposure to helminths. In his recent review, Weiss concludes that there is no conclusive evidence that parasitic infections protect against asthma development [158]. The association with antibiotic use and asthma is difficult to study as the use of antibiotics is widespread and the predominant reason for prescription is prolonged respiratory symptoms after a viral infection, where a bacterial involvement in the disease is suspected. Misclassification with asthma in this situations is likely, leading to a non-casual positive association between asthma and antibiotic use. There are social groups where conventional treatments, including antibiotics, are deliberately rejected. One study, carried out on a selected group of children at Rudolf Steiner schools, showed 400% increase in asthma prevalence associated with use of antibiotics [159]. There are, however, many other aspects of the anthroposophic lifestyle which may be relevant and antibiotic use in this context may be a marker of non-adherence to the anthroposophic lifestyle. Nevertheless, the effects of antimicrobial therapy on bacterial colonisation of the infant gut should also be regarded.

Several studies have found negative associations of family size, and particularly with the number of older siblings [160,161] with atopy [59,62,161,162], hay fever [55,112], asthma [81,112,155,163] and BHR [164]. In the analysis of the whole ECRHS data set, atopy was negatively associated with

family size, partly attributable to an independent protective effect of a greater number of brothers, but not older siblings. Bedroom sharing was associated with a lower prevalence of atopy, particularly to cat allergen [113]. However, a protective effect of family size and bedroom sharing could only be detected in subjects reporting no parental allergy, indicating that in subjects with a strong genetic predisposition, environmental factors in childhood are possibly of less importance.

Similarly, day-care attendance has been associated with decreased risk of atopy, but that only in children from families with up to three people. The association was higher among children who attended day-care earlier [165] but an opposite association also has been reported [55], where day-care attendance at age 0-2 years was a weak risk factor for allergic rhinitis but not for asthma. Furthermore, children who attend day-care have shown to have increased risk of asthma with early infections as a probable mediator of risk [166]. In Swedish young adults an increased risk of atopic sensitisation was found in subjects who had attended a day-care centre before the age of five years [125], but this association was not found to be significant when analysing the combined ECRHS data set [113]. No association of adult asthma and day-care attendance has been reported.

Strachan, who first found the negative association between hay fever and number of older children in the household, suggested that small family size could reduce infectious diseases "transmitted by unhygienic contact with older siblings", but not respiratory viral infections in infancy, and that this could in turn increase the risk of atopic disease at older ages [160]. This hypothesis has been developed further. The immunological bases of the hypothesis stands on T helper (Th) lymphocyte differentiation in maturation of the immune system. Th_2-like cytokines (interleukin (IL) 4, IL-13, and IL-5) produced in the uterine environment are important to maintain the pregnancy. The continuation of foetal allergen-specific Th_2 responses during infancy is associated with decreased capacity for production of the Th_1 cytokine interferon γ by atopic neonates [167]. According to the hygiene hypothesis, T-cell responses to the microbial and viral infections generate Th_1-like cytokines such as IL-12 and interferon-γ that down-regulate Th_2 responses, helping T-cell immune responses to mature into a balanced phenotype that would be less likely to favour allergen sensitisation [168,169].

There is also more direct evidence that childhood exposure to infections might prevent atopic diseases. Less atopy [170] and asthma [171], in those with history of measles and less atopy in those with hepatitis A [172] has been reported. Furthermore, in a study of Italian male military students

the prevalence of serum markers of microbes transmitted through the oral rout was higher in the non-atopic than in the atopic cadets, while presence of serum markers of the viruses transmitted by other routes was not associated with atopy [173].

Strongly positive tuberculin responses in early life were associated significantly with less asthma in later childhood in Japanese children [174]. The positive tuberculin responses in early life were also associated with a dominance of lymphocyte class T helper (Th1) over Th2 in the blood cytokine profiles at 12 years of age. However, differences in immune responses and natural resistance in different subjects result in differences in handling both infections and potential allergens, and thus differences in the clinical expression of a disease. This difference in host characteristics is reflected also in differences in cytokine levels. The intensity of tuberculin responses in young adults receiving BCG at 14 years of age did not correlate with total serum IgE in the Norwegian population, nor did the positive tuberculin test distinguish the atopics from nonatopics [175]. These two papers together indicate, that it is not the host characteristics in the Th lymphocyte balance, but rather the subclinical exposure to *M.Tuberculosis* or immunisation earlier in life, that can result in decrease in atopy. The latter hypothesis has, however, been rejected by two Swedish studies on children [176,177]. As a proxy of differences in natural exposure to *M. tuberculosis*, WHO derived tuberculosis notification rates were matched to the prevalence of respiratory symptoms within the ISAAC study in ecological analyses. The results showed that increase in notification was negatively correlated with the prevalence of wheeze and asthma in 85 centres from 23 countries studied [178]. A Finnish registry based study has shown reduced occurrence of subsequent asthma in women *with M.Tuberculosis* infection in childhood, the relation was, however, inverse among men [179]. Thus, BCG vaccination once, either in infancy or adolescence, seems not to shift the Th_1-Th_2 balance. There is also no conclusive evidence that treated active tuberculosis before the age of 20 could do that. However, repeated contacts with mycobacteria at a certain age might have an influence on the immune system. This is supported by the results of von Mutius *et al*. An other possibility is, that these relations can be confounded by other aspects of lifestyle.

While some kinds of infections may have a role in the maturation of the immune system in early childhood with consequences that can last to adulthood and determine the subsequent development of respiratory diseases [148,180], viral upper respiratory infections are a common trigger for episodes of bronchospasm and have been associated with 80% of all asthma exacerbations [181,182]. Prospective studies in children, where viral markers have been measured at clinical respi-

ratory infection episodes in early childhood, have shown variable, but declining with age, association between respiratory virus infections in infancy, and frequent wheeze up to at least 13 years of age [183,184]. The extent of the association depends on the severity of the disease episode. The strongest association has been found between respiratory syncytial virus (RSV) bronchiolitis in infancy, severe enough to cause hospitalisation [183]. A positive association with respiratory syncytial virus infection and BHR has been shown among adults [185]. As no population based studies with serological proof and adult asthma as an outcome are available, the causal role of viral infections in asthma has remained unclear. The role of bacterial infections in exacerbating asthma is more controversial. A positive association with serological signs of a current or recent *Chlamydia pneumoniae* infection and reported wheeze has been reported [186] but not confirmed by others [187].

Diet

There are different ways in which food could affect lung health. First, relative deficiencies of certain foods may alter the lung's defence mechanisms, making it more liable to develop disease. Second, food sensitivity may act through immunological or nonimmunological mechanisms to cause bronchoconstriction [188]. Third, changes from locally produced food, like fresh and fermented vegetables, and non sterilised diary products to industrially prepared and processed foods and use of microwave ovens, can change microbial load and influence the gut micro flora.

One of the first observations on the association of general dietal habits and asthma was, that the regional sales of table salt were related to asthma morbidity statistics in Britain. This gave rise to the suggestion that salt intake is relevant for the risk of asthma [189]. Later studies have not proved this and it has been suggested that salt intake can be a marker of generally poor diet [188,189]. Another early observation was, that low prevalence of asthma in Eskimos could be associated with their diet, rich in fish and marine mammals or, with a genetic deviation in fat metabolism in Eskimos, or both [190]. The increase of asthma prevalence has been subsequent to the fall in the consumption of animal fat and oily fish with high content of saturated fat and increase in the amount of margarine and vegetable oils, containing polyunsaturated fat, in the diet of Western countries. It has been suggested, that an increase in the intake of ω-6 fatty acids, such as lineleic acid, and a decrease in the intake of ω-3 fatty acids, such as eicosapentaenoic acid, may have led to an increase in allergic sensitisation, which in turn may account for the increase in the prevalence of asthma. The reason, why a decreased consumption of ω-3 fatty acids may lead to increased prevalence of allergy may be the ability of ω-3 fatty acids to inhibit the

synthesis of arachidonic acid from linoleic acid and prostaglandin E_2 (PGE_2) from arachidonic acid. The ability of PGE_2 to inhibit the productions of interferon γ would in turn lead to the immune modulation towards Th_2 inflammatory reaction to allergens [191]. Association between the intake of polyunsaturated fat and atopy has been shown in children [192,193] and adults [194]. Epidemiological evidence to show the importance of these mechanisms in asthma is scarce. The association with asthma and dietary fat has been revealed in some studies in children [116,195], but not others [192] and not among adults. Thus, among young Norwegian adults, with an overall high fish intake, fish consumption was not a significant predictor of current respiratory symptoms or asthma [196], similarly, no relation between the intake of various fats and asthma was found in the Nurses' Health Study [197]. However, improvement of asthma in children using fish oil capsules vs. capsules with olive oil in a double blind clinical trial in a strictly controlled environment has been shown [198]. It has been suggested that intake of a mild anti-inflammatory agent, like fish oil, might matter more in children, than in adults [199].

Acting as antioxidants, or through influences on immune function, vitamins C and E and beta carotene in the diet may reduce airway inflammation, thereby decreasing the severity of asthma or preventing the expression of asthma in susceptible individuals. Vitamin E intake from diet was inversely associated with adult onset asthma in the prospective Nurses' Health Study. Thus, women in the highest quintile of daily vitamin E intake from diet alone had a 47% lower risk of asthma than women in the lowest quintile [197].

The effect of processed food consumption instead of eating locally produced food and food products and potential effect of the dietal changes on gut microflora and the subsequent development of atopy and asthma has been less studied. However, studies comparing infants in Sweden and Estonia have shown marked differences in the types of faecal bacteria in unselected infants in the two countries, which broadly match the differences seen between atopic and non-atopic infants in each country [200,201]. The differences included a more intensive colonization with lactobacilli and eubacteria in Estonian children, whereas the Swedish infants had increased numbers of clostridia, particularly *C. difficile*, as well as bacterioides and other anaerobes [201].

In Britain and in the USA the rise in asthma has been accompanied by an epidemic increase in the prevalence of obesity, a characteristic related to diet, and often unbalanced diet. An independent association between increase of body mass index (BMI) and prevalence of current

wheeze, chest tightness and breathlessness at night, after controlling for age, sex, occupational exposure, smoking and dietary fish consumption, has been relieved in young Norwegian adults [196]. The prevalence of asthma increased with BMI also among British adults studied at 26 years of age. The association between fatness and asthma was stronger among women [155]. In an Australian study among adults 17-73 years, it was found that severe obesity, defined as a BMI of >35 kg/m^2, was associated with a higher prevalence of wheeze, diagnosed asthma and medication use. Despite the fact that FEV_1 and FVC were significantly reduced in severely obese subjects, there was no increase in airway responsiveness to histamine among them. A low level of physical activity, clearly associated with higher BMI, can be a result of asthmatic individuals avoiding physical exercise. However, asthmatic children have been shown to be physically as active as non-asthmatic children [202]. Furthermore, it has been shown, that weight reduction has both short time and long-time favourable effects on lung function, and symptoms [203].

THE AIMS OF THE STUDY

1. To compare the prevalence of respiratory symptoms, self reported asthma and allergic rhinitis based on postal questionnaire results among adults in Tartu, Estonia and in Uppsala, Sweden in relation to age, gender and smoking history (I).

2. To estimate the prevalence of BHR and atopic sensitisation, among young adults in Estonia and to compare the prevalence of certain risk factors for BHR, atopy, allergic rhinoconjunctivitis, and allergic asthma in Tartu, Estonia and Uppsala, Sweden (II, III).

3. To study the relationship between levels of S-ECP and variables related to asthma and allergy among young adults in Estonia (IV).

4. To compare PD_{20} and log-slope of methacholine challenge methods using either Mefar or Spira as a dosimeter and to calculate the respective transition equations to permit the comparison of the results obtained by the two methods (V).

MATERIAL AND METHODS

Definitions used

Allergic heredity: Asthma, skin allergy of hay fever either in parents or in siblings.

Allergic rhinitis: Positive answer to the question: ”Do you have any nasal allergies, including hay fever?”

Allergy associated symptoms of asthma (pollen, mite or cat asthma): The presence of cough, wheeze, chest tightness or shortness of breath upon contact with pollen, bedclothes, dusty parts of the house or animals, respectively, in combination with IgE antibodies against defined allergens.

Allergy associated symptoms of rhino-conjunctivitis (ARC): A history of itchy or watering eyes or runny nose either seasonal or on contact with animals, bedclothes or dusty parts of the house, combined with IgE antibodies against timothy or birch (pollen ARC), D. pteronyssinus (mite ARC), cat (cat ARC) or Cladosporium.

Asthma: An affirmative answer to the question: ”Have you ever had asthma?”

Asthmatic symptoms: Wheeze with breathlessness occurring in the absence of colds in the last 12 months.

Atopy: Presence of serum IgE antibodies > 0.35 kU/l against at least one allergen.

Current asthma : Asthma diagnosed by a doctor and attack of asthma during the last 12 months or currently taking asthma medication.

Bronchial hyperresponsiveness (BHR): A 20% decline in FEV_1 compared with the post-diluent value were the cut of point was $\leq$1.5 mg with the Mefar method. When other limits were used, this is indicated.

Bronchial responsiveness to methacholine (Slope): 100/(regression coefficient + 10), where the regression coefficient was calculated by regressing percentage fall in FEV_1 on $\log_{10}$ (methacholine dose)[45].

Skin allergy : Positive answer to the question: ”Have you ever had eczema or any kind of skin allergy?”

Subjects exposed to environmental tobacco smoke (ETS): Non-smokers who reported exposure to ETS on most days or nights during the last 12 months.

Visible mould: Affirmative answer to the question “Has there been mould or mildew on any surface, other than food, inside the home in the last 12 months?”

Wheeze: Affirmative answer to the question: “Have you had wheezing or whistling in your chest at any time in the last 12 months?”.

European Community Respiratory Health Survey (ECRHS)

ECRHS is a multicentre survey of the prevalence, determinants and management of asthma. A screening questionnaire, including seven questions relating to the 12 month prevalence of symptoms of asthma, was distributed to representative samples of 20-44 year old men and women in 48 centres, predominantly in Western Europe, followed by the tests for allergy, lung function measurements, tests of airway responsiveness, and blood and urine collection in the clinical phase of the study in 38 centres from 18 countries. The total number of subjects that responded to stage 1 was 137,619. The random sample of stage 2 comprised 18,811 subjects. A symptomatic stage 2 sample was included in 25 centres and comprised 2,998 subjects.

The three main objectives of the survey were:

1. To estimate the variation in the prevalence of asthma, asthma-like symptoms, atopic sensitisation and bronchial lability in Europe.
2. To estimate variation in exposure to known or suspected risk factors for asthma; to measure their association with asthma and to assess further the extent to which they explain variations in the prevalence across Europe.
3. To estimate the variation in treatment practice for asthma in the European Community.

Study areas (I-IV)

Following the protocol of the ECRHS [42], two areas were compared, i.e. Tartu in Estonia and Uppsala in Sweden. Both are university towns (population 110,000 and 167,000, respectively) with little industry. They are located at a similar latitude and they have a similar climate. The track of development of the countries parted 50 years ago and the study group in Tartu has been living in a socialist economy from birth. In 1993, the mean monthly salary was 80$ in Tartu and 2,612$ in Uppsala and the purchasing power of the salary for the inner market five times higher in Sweden than in Estonia (personal communication, Ministry of Economics in Estonia). The living area per inhabitant was 12 m^2 in Tartu, as compared to 40 m^2 in Uppsala. In Tartu 63% of people used gas stoves for cooking, as compared to less than 1% in Uppsala. There were also major differences in the use of asthma medication between the countries [204] (Figure 1).

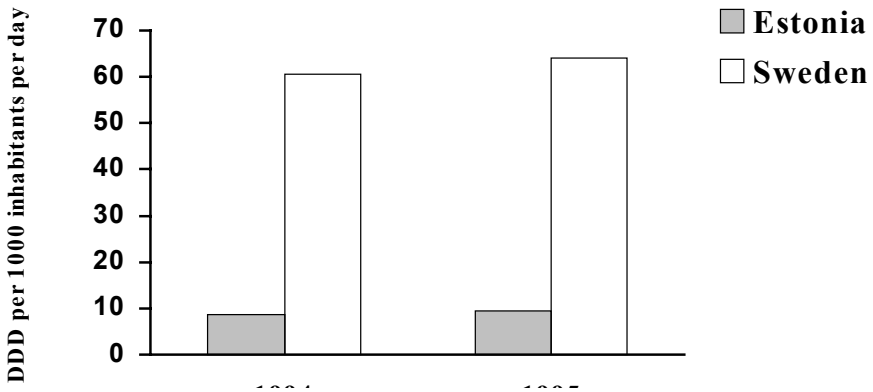

Figure 1. The use of anti-asthma drugs in Estonia and Sweden in DDD *per* 1,000 inhabitants [204].

Phase 1

Subjects (I)

Random samples, of 3000 individuals in Tartu and 3600 in Uppsala between ages 20 and 44, with equal number from either sex, were taken from the Civil Registers. To avoid linguistic problems, only people of Estonian nationality were selected in Tartu.

Questionnaire (I)

The ECRHS modified version of the International Union Against Tuberculosis and Lung Disease (IUATLD) questionnaire [75,205] was used. It included seven questions commonly related to the diagnosis of asthma. Questions on smoking history were added both in Uppsala and Tartu. The questionnaire was translated from English into Estonian and Swedish and re-translated into English. The Estonian questionnaire was also translated into Swedish for comparison. The questionnaires were mailed in December 1990 in Uppsala and in December 1993 in Tartu, together with a letter of explanation and a stamped reply envelope. Two additional questionnaires were sent to non-responders.

Phase 2

Subjects (II-IV)

A random sample of 800 persons in both centres was selected for further investigations, which included a structured interview and the determination of circulating IgE antibodies to allergens. Only individuals who had answered to the mailed questionnaire were invited to participate (n= 723 in Tartu, n=706 in Uppsala) (II-IV).

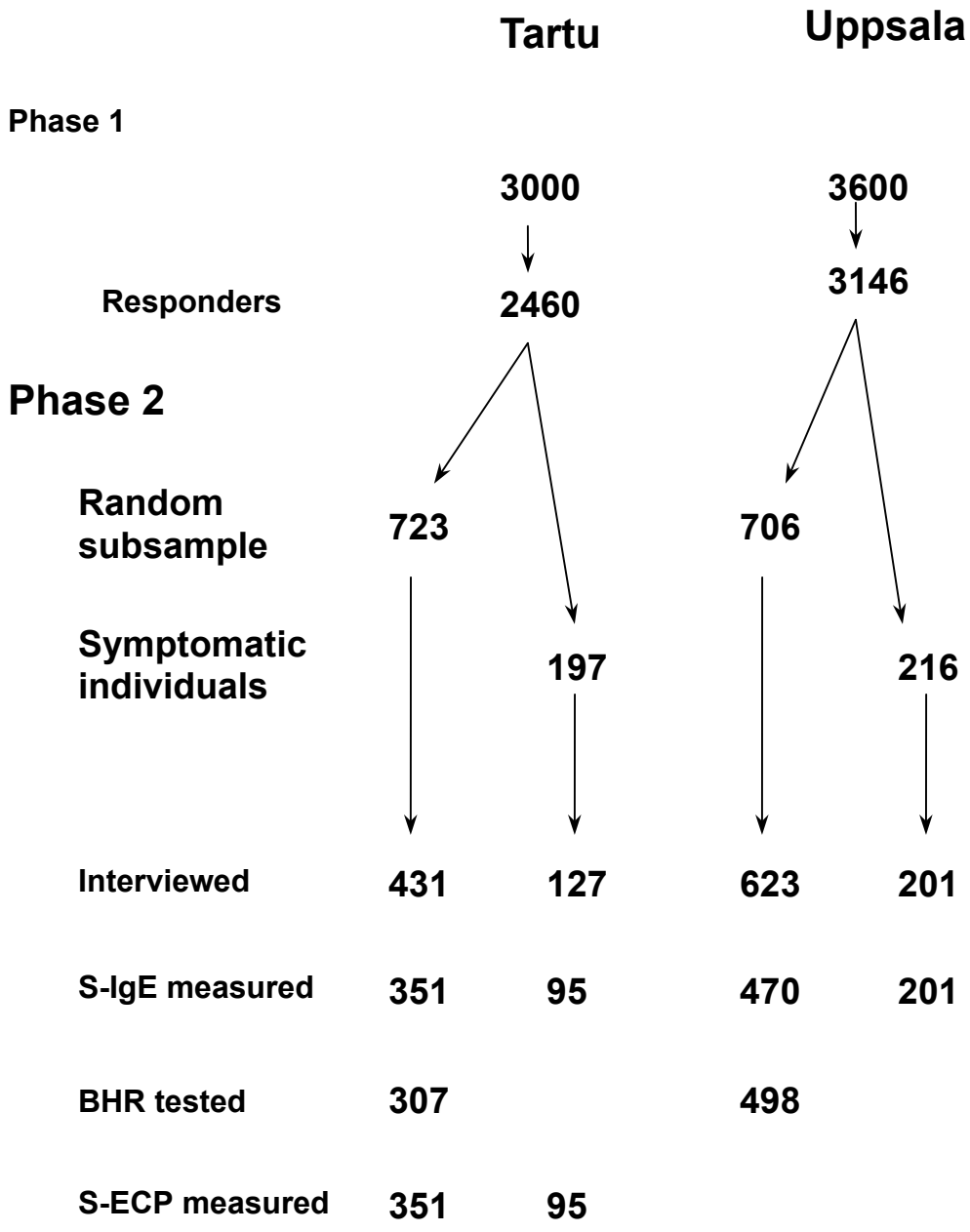

Figure 2. Selection of the subjects and response rates in different parts of the study.

In addition, a symptomatic sample comprising all persons from the original study group who reported use of asthma medication, attacks of asthma, or awakening because of shortness of breath and who were not already included in the random sample were invited to participate (n= 197 in Tartu and n= 216 in Uppsala) (II, IV). The study was conducted from April 1991 to February 1992 in Uppsala and from March 1994 to July 1995 in Tartu. The study flow is presented on figure 2.

Questionnaire (II-IV)

The ECRHS questionnaire for a structured interview was used [42,205,206]. The questionnaire was translated from English into Estonian and Swedish and re-translated into English. The interview included questions on symptoms, diagnoses, smoking history, parental smoking history, family structure, family history, early environment, education, employment, home environment, diet, use of medicines, attitudes to medicines and the use of health services. In addition individuals with pollen associated symptoms were asked about what time of the year the symptoms appeared. Allergy to birch and timothy were only considered when the symptoms appeared in the spring and summer, respectively.

Serum IgE levels and circulating IgE antibodies (II-IV)

Serum IgE levels and IgE antibodies against D. pteronyssinus, timothy, birch, cat and Cladosporium were determined by Pharmacia CAP™ system (Pharmacia, Uppsala, Sweden).

Serum ECP (IV)

The blood samples that were collected for ECP measurements were kept at 24^{o}C for 60 minutes before they were centrifuged. Sera were stored at –20^{o}C. The concentration of S-ECP was assayed with a double antibody radioimmuno assay (Pharmacia Diagnostics, Uppsala, Sweden).

Spirometry (II-V)

Forced expiratory volume in one second (FEV_1) was measured with Jäeger Flowscreen, Lilly type pneumotachograph (Erich JÄEGER GmbH & CoKG, Würzburg, Germany) in Tartu and with Spiro Medics computerised dry-rolling seal spirometer system 2130 (Sensor Medics, Anaheim, California, USA) in Uppsala. The predicted value was calculated for each subject [207].

Methacholine challenge test (II-V)

Two automatic inhalation synchronised dosimeter jet nebulisers were used. In the clinical phase of ECRHS (II-IV), Spira Elektro2 (Respiratory Care Centre, Hämeenlinna, Finland) [208] was

used in Tartu and Mefar MB3 dosimeter (Mefar, Brescia, Italy) [209] in Uppsala. The methods were compared in a separate analyses (V).

The Spira dosimeter was adjusted to nebulise for 0.5 seconds from the beginning of each inhalation. Patients controlled their tidal breathing with the flow indicator and volume digital readout to achieve a stable flow of 0.5 l/s with tidal volume 0.4-0.6 l. Employing the Mefar protocol, slow inspirations from functional residual capacity to total lung capacity with breath holding for at least three seconds were done. Aerosol delivery time was set to one second and pause time to six seconds.

FEV_1 was measured, before the challenge and either two (Mefar) or three minutes (Spira) after inhalation of normal saline and each dose of methacholine. The best of two attempts was registered. The decline of FEV_1 was calculated from the control value, i.e. the value of FEV_1 after four inhalations of saline (36 μg) using Mefar dosimeter and five inhalations (32 μg) using Spira-2. Methacholine challenges were terminated after a fall of FEV1 ≥ 20% from the control value or after the last dose inhaled. The test was not performed if the post diluent FEV_1 was reduced by more than 10% compared with the pre-diluent FEV_1. Appropriate transition equations were used to transform the Tartu data, making them comparable to those obtained in Uppsala. After transforming the data maximum cumulative dose administered using the Spira (2.3 mg) corresponded to 1.5 mg using that of the Mefar method. This cut-off point was used to define BHR.

Comparison of two dosimeter methods

Subjects (V)

Twenty-eight patients (16 males) with mild to moderate asthma were recruited through the Asthma Register of Tartu University Lung Clinic. The mean age was 30.5 years (range 17-43 years). All patients had a documented variation in forced expired volume in one second (FEV_1) of more than 15% after β_2-agonist and fulfilled the criteria of asthma (International Consensus Report on Diagnosis and Management of Asthma, 1992). They had to be symptomatic or on inhaled short acting β_2-agonists during two weeks preceding the investigations. None of them used any other asthma medication than either inhaled glucocorticoids, or short acting inhaled β_2-agonists, or both, and the dose regimen did not change during the study. Inhaled β_2-agonists

were withheld for at least five hours before each challenge. The prechallenge FEV_1 was 74% of predicted value in two and over 80% in all other cases.

Ethical considerations

All participants gave their informed consent. The respective local Ethics Committees approved the studies.

STATISTICAL ANALYSIS

The statistical analysis was performed with the Statistica 4.0 software package (Stat Soft Inc., Tulsa, USA) (I), the SAS System 6.11 software package (SAS Institute Inc., Cary, NC, USA) (II, III, V) and the StatView 5 software package (SAS Institute Inc, Cary, NC, USA) (IV).

Chi-square-test was used to analyse differences between groups. Linear regression was used to correlate two continuous variables and logistic and multiple linear regression for multiple variables. Logistic regression analysis was performed in order to estimate adjusted odds ratios when taking into account several variables. Odds ratios with 95% confidence intervals (CI) were determined. Log-transformation was performed to achieve a normal distribution of S-ECP and S-IgE. S-ECP values are presented as geometric mean ($\pm$SD). A p-value <0.05 was regarded as statistically significant. In paper V, after logarithmic transformation of PD_{20} values, paired t-test was used to compare measurements obtained with the two challenge methods. Linear regression was used to calculate transition equations. A measure of slope was calculated by regressing percentage fall in FEV_1 on $\log_{10}$ (dose). In order to satisfy the assumptions of standard statistical analyses, i.e. normality and homogeneity of variance, the regression coefficient was transformed. The term "slope" is used for the following transformed log slope: 100/(regression coefficient + 10). A p-value <0.05 was regarded as statistically significant.

RESULTS

Paper I

Asthmatic symptoms were less prevalent in Tartu (4.6% vs. 6.8%), as were attacks of asthma (1.9% vs. 3.3%), current asthma medication (0.7% vs. 4.9%), and allergic rhinitis (17.8% vs. 22.2%). The prevalence of current wheeze was higher and wheeze with breathlessness lower in Tartu than in Uppsala, while the prevalence of wheeze without a cold was similar. In Tartu, but not in Uppsala, wheezing and wheeze without a cold were more common among men than among women. Nocturnal respiratory symptoms (wakening with chest tightness or being woken by an attack of breathlessness or an attack of cough) were more prevalent in Tartu ($p<0.001$), especially among women. The prevalence of nocturnal symptoms also increased with age ($p<0.05$) in Tartu, but not in Uppsala. Age was inversely related to allergic rhinitis in Uppsala but not in Tartu. Current smoking was an invariable risk factor for all respiratory symptoms, except for attacks of asthma, both in Tartu and Uppsala. The prevalence of current smoking was higher in Tartu compared to Uppsala (45.9% vs. 33.8%). Allowing for age, sex, current smoking and self reported allergic rhinitis did not change the differences in symptom prevalence between the centres.

Paper II

The prevalence of atopy was 19% in Tartu and 32% in Uppsala. The major allergen in Tartu was house dust mite (10%) , while in Uppsala it was pollen (grass 18% and birch 15%). The prevalence of sensitisation to pollen was twice as low (11.5 vs. 23.2%) and the prevalence of pollen associated asthma symptoms was four times lower (1.7 vs. 6.8) in Tartu than in Uppsala, while sensitisation to pollen was an equally large risk factor for asthma in both centres.

Risk factors were estimated for nine outcome variables: three different conditions, i.e. atopic sensitisation, allergy associated symptoms of asthma and allergy associated symptoms of rhinoconjunctivitis, in association with three different allergens: mite, pets and pollen. Risk factors were different for different sensitises and some differences in them could be revealed between the two centres. For atopic sensitisation the risk factors were mainly demographic or genetic by nature, or associated with infant environment. Male gender and allergic heredity were most often associated with outcome variables, both in Tartu and Uppsala. Age was inversely related to cat and pollen associated symptoms of rhinoconjunctivitis in Uppsala. Pet in infancy was inversely associated with pollen atopy in Uppsala, while no association with atopic sensitisation and pet ownership in childhood could be revealed in Tartu. Current exposures had some oppo-

site effects on sensitisation, thus cat ownership was a risk factor for sensitisation to cat in Tartu, whereas pet ownership was inversely related to atopic sensitisation to pollen in Uppsala. Working as a manager, legislative official or an administrator was inversely related to sensitisation to pollen in Tartu, and was associated with an increased prevalence of IgE antibodies against cat in Uppsala. Additional current exposures as risk factors could be revealed for allergic respiratory symptoms associated with indoor allergens but not for pollen. In Tartu, use of open fire in the household tended to be a risk factor both for symptoms of asthma and allergic rhinitis associated with sensitisation to cat or mite. The age of the dwelling had an opposite effect on allergic respiratory symptoms in the two centres. In Tartu, living in house built after 1971, and in Uppsala, before 1971, increased the risk for symptoms.

The examined risk factors, however, did not explain the difference in prevalence between the centres, thus after controlling for several risk factors, living in Uppsala was still associated with OR of 2.1 for pollen atopy, 2.4 for pollen asthma and 5.4 for pollen ARC.

Paper III

The prevalence of BHR was higher in Tartu regardless how BHR was defined. Using the cut-off point 1.5 mg the prevalence of BHR was 19% in Tartu and 11% in Uppsala ($p<0.001$). Bronchial responsiveness estimated by mean slope was 7.2 and 7.8, respectively ($p<0.001$). Female gender, sensitisation to cat, current smoking, and visible mould in the home during the last 12 months were independent risk factors for BHR in Tartu. In Uppsala, BHR was significantly associated with total IgE levels, current smoking, and exposure to environmental tobacco smoke. In Tartu, the prevalence of BHR among current smokers was significantly higher only compared to lifelong non-smokers who were not exposed to ETS, while in Uppsala the prevalence of BHR was higher among passive smokers than among individuals not exposed to ETS. The prevalence of current asthma in subjects with BHR was 7% in Tartu and 23% in Uppsala. None of several other environmental factors, e.g., use of gas and open fire, age of the dwelling, water damage, childhood environmental factors like parental smoking, number of siblings and severe respiratory infection before five years of age or social class were significantly associated with BHR in the univariate analyses. When the two populations were combined in one multiple regression model, living in Tartu was an independent risk factor for BHR (OR 1.8; 95% CI 1.15-3.0).

Paper IV

The median S-ECP value was 8.0 µg/l in the random sample which was considerably lower than in an investigation with identical design in Uppsala [32]. The geometric mean of S-ECP was

higher in subjects with, than without atopy (10.2. vs. 8.9 µg/l, $p<0.01$) and in subjects with bronchial hyperresponsiveness (BHR) than in subjects without BHR (9.9 vs. 8.0 µg/l, $p<0.01$). The levels correlated weakly to forced expiratory volume in one second (FEV_1) ($r=0.13$, $p<0.01$) and were not independently correlated with respiratory symptoms, asthma or FEV_1 after adjusting for BHR, IgE, sensitisation and smoking. The specificity and sensitivity of a S-ECP value above the interquartile range (12.0 µg/l) for atopy were 32% and 80% respectively, for BHR 30% and 80%, for self reported asthma 63% and 97% and for wheeze 36% and 76%.

Paper V

The provocative dose of methacholine producing a 20% fall in forced expiratory volume in one second (PD20) was lower when determined by Spira than with the Mefar dosimeter. Transition equations calculated by linear regression analysis were: PD20mefar = exp10[0.897 + 0.678(logPD20spira)] and PD20spira = exp10[0.759 + 0.559 (log PD20mefar)].The slopes were calculated by regressing the percentage fall in FEV_1 on log10 (dose) and transformed as slope = 100/(regression coefficient + 10). The mean slope (95% CI) for Spira was 3.1 (2.6-3.7) and for Mefar 4.4 (3.6-5.1) ($P < 0.005$). Regression equations calculated by linear regression analysis were: slope(mefar) = 2.126 + 0.712 slope(spira) and slope(spira) = 1.551 + 0.365 slope(mefar).

DISCUSSION

The postal questionnaire study (paper I) showed high prevalence of respiratory symptoms including wheeze, but low prevalence of diagnosed asthma and asthma treatment in Tartu compared to Uppsala. As the prevalence of asthma like symptoms, i.e. having wheeze with breathlessness while not having a cold, was also low in Tartu, it seemed likely that the relatively low prevalence of asthma in Tartu is real. Results of the clinical phase of the study confirmed that the prevalence of atopic sensitisation and respiratory allergies (paper II) as well as the levels of serum ECP (paper IV) were low in Tartu, concordant to the notion of low prevalence of asthma. The high prevalence of BHR in Tartu concorded with the high prevalence of wheeze and nocturnal symptoms (paper III). The prevalence of current asthma in subjects with BHR was only 7% in Tartu but 23% in Uppsala. Furthermore, the association between BHR and atopic sensitisation was statistically significant only in Uppsala, indicating that atopy is less associated to BHR in Tartu than in Uppsala.

S-ECP was elevated in subjects with atopy and BHR, but the levels were not related to asthma symptoms or lung function when other asthma related variables were controlled for. It is, however, not surprising that after controlling for the effect of atopy and BHR, S-ECP no longer remains associated with asthma symptoms and self reported asthma, as atopy, elevated S-ECP, and BHR may share the same pathway to asthma: atopic sensitisation being the prerequisite to allergic inflammation, the latter marked by elevated S-ECP. Activation of eosinophils in its turn can result in increased BHR.

The difference in the prevalence of asthma like symptoms between Tartu and Uppsala was smaller than the difference in the prevalence of diagnosed asthma and even more so, asthma treatment. Hence, we have suggested that the low prevalence of self reported asthma in Tartu may partly be the reflection of low awareness of the disease in the society. The awareness of allergic diseases in Tartu seemed to be lower than in Uppsala, based on the results of the clinical phase of the study as well, as the difference in the prevalence of pollen associated respiratory symptoms, especially hay fever, was larger than the difference in the prevalence of sensitisation to pollen. Alternatively, there may be some unknown factors in Tartu that decrease the risk of expression of symptoms in allergen sensitised subjects.

Our results are in line with previous comparative studies between countries with affluent market economy and previous socialist countries, revealing low prevalence of allergies and asthma

in the latter both among children [52,57,59-61] and adults [62,63]. However, in contrast to previous comparisons of adult populations in East and West Germany, respiratory symptoms as well as BHR were high among Estonian adults. While generally the prevalence of BHR parallels that of self reported asthma and wheeze [45,51] the prevalence of BHR was also high among Estonian children [66]. It has recently been suggested that bronchial challenge might provide more valid information than questionnaires in epidemiological studies of asthma, when comparing populations which do not share the same language, culture, health care system, or perception and labelling of asthma symptoms [21]. Our results indicate that the validity of BHR as a marker of asthma may be low when target populations differ in regard to other possible causes of BHR, e.g. differences in environmental pollution and ETS. Thus, a method that is valid within a particular population may not be the most appropriate one for regional or international comparisons of asthma.

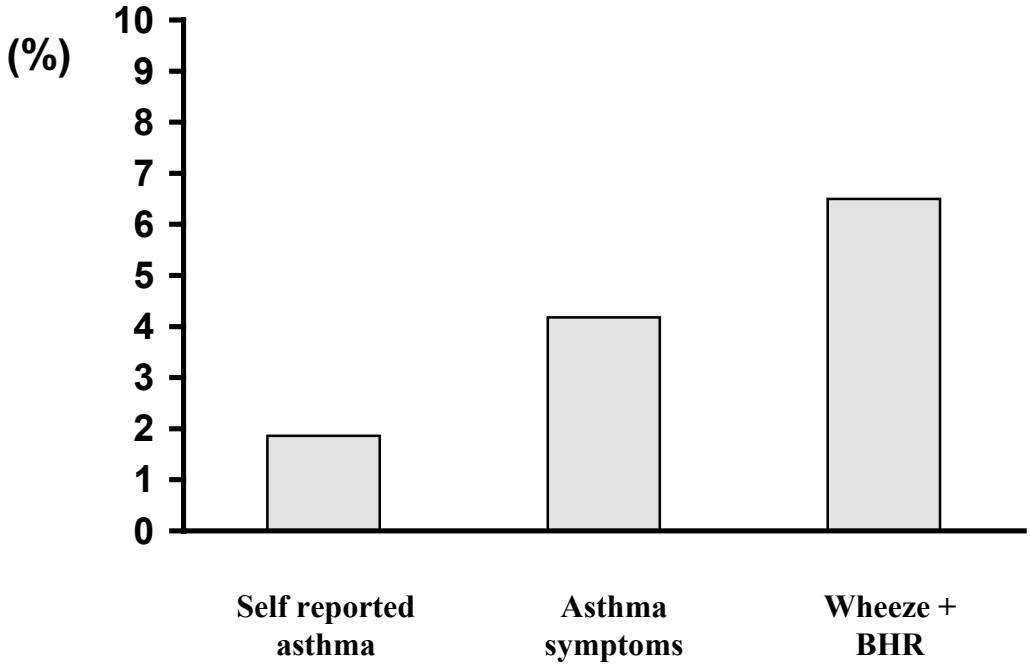

Figure 3. Prevalence of self reported asthma, wheeze with breathlesness without cold and current wheeze with BHR among young Estonian adults.

As shown in the figure 3, the prevalence estimates of asthma depend on what operational definition is used and what measurement techniques are employed. Disease prevalence *per se* is, however, important mainly from an economical point of view, to enable the planning of the health care.

Age was inversely related to self reported allergic rhinitis in Uppsala but there was no similar trend in Tartu. Age was inversely related to cat and pollen associated symptoms of rhinoconjunctivitis and there was also a tendency towards an inverse relationship between age and atopy in Uppsala but not in Tartu. Analysis of the data from three Swedish centres participating in ECRHS revealed this trend to be statistically significant [125]. This result supports the suggestion that the increasing prevalence of allergic diseases among children and young adults in Western Europe is caused by a cohort effect, the causes of which possibly date back to changes beginning in the mid 1950s [210]. However, the prevalence of atopic sensitisation among adults in Tartu was much higher than in a prevalence study in children [58], while in Sweden the prevalence was similar in children and in adults [52], indicating that expression of atopic sensitisation can occur later in life. Resampling of the 9-11-year schoolchildren in Leipzig four years after the German unification showed significant increase in the prevalence of atopy compared to the study conducted four years earlier [69]. These children were born about three years before unification and were therefore exposed to western living conditions only after the third birthday. Thus, factors operating very early in life may be particularly important for maturation of the immune system, whereas the development of atopic sensitisation may also be affected by environmental factors occurring beyond infancy.

A definition of high specificity, like doctor diagnosed asthma, should preferably be used, in estimating risk factors [5]. We could not use self reported asthma as an outcome, as there were only eight subjects with diagnosed asthma in Estonia in a random sample and additional 13 came from the enriched sample. The dependent variables we have used in risk factor analyses comprise respiratory symptoms, in the postal questionnaire data and BHR, atopic sensitisation and allergic respiratory symptoms from the clinical part of the study. For atopy and respiratory allergies nine outcome variables were used: atopic sensitisation to three different allergens: mite, pets and pollen, and allergy associated symptoms of asthma, and allergy associated symptoms of rhinoconjunctivitis associated with the same different allergens.

Women had more nocturnal symptoms than men in Tartu, while the prevalence of wheeze was higher among men. However, after controlling for current smoking, no gender difference in wheeze remained. The gender difference in reported night symptoms can be explained by higher BHR among women in Tartu. In analyses of the clinical phase of the study, female gender was a risk factor for BHR in Tartu after controlling for baseline lung function and other variables. This is in accordance with previous reports [83,84]. In an analysis of Leynaert *et al*. of

the French ECRHS data set greater bronchial responsiveness was observed in women than in men after adjusting for several variables for airway calibre and lung size as well as for body mass index [90]. In that study BHR in women was more related to smoking than in men. Thus, the excess of BHR in women may be related to a greater susceptibility to tobacco smoke. Women have been found to be more susceptible than men also to other environmental factors, as the use of gas for cooking is associated with respiratory symptoms suggestive of airway obstruction in women but not in men [91-93]. Men were more often sensitised to common allergens and, correspondingly, tended also to have more respiratory allergies.

Current smoking was a significant risk factor for BHR and for all respiratory symptoms, except for attacks of asthma, both in Tartu and Uppsala but not for atopic sensitisation and allergic respiratory symptoms. This is in concordance with many previous studies [75,77,84,84,100,114-116].

ETS was associated with a four-fold increased BHR in Uppsala, but was not significantly associated with BHR in Tartu. This was in contrast to the results of a cross sectional study involving 2600 schoolchildren (10-12 years) from Estonia, Poland and Sweden where maternal smoking was related to respiratory symptoms only in Estonia and Poland [59]. In the current study, subjects were classified as exposed to ETS if they reported exposure to ETS on most days or nights during the last 12 months. As smoking is not restricted in public places in Estonia unlike in Sweden, our questionnaire may underestimate the true exposure to ETS in Tartu. An association between exposure to tobacco smoke and sensitisation to mite but not to other allergens has been observed previously [124]. We found an association between maternal smoking in childhood with sensitisation to mite, and mite associated symptoms of asthma in Tartu.

An association between atopic sensitisation and BHR has been found in many studies [77,106,116,211]. Cat was the allergen most strongly related to BHR in Tartu. Pet ownership, both in infancy and at the time of the study, was inversely associated with atopic sensitisation, especially to pollen, and correspondingly also to pollen associated respiratory symptoms in Uppsala, while in Tartu current cat ownership was a risk factor for cat atopy. The inverse association of respiratory symptoms with current pet ownership in Uppsala can be explained by selection bias, i.e. sensitised individuals were less likely to keep pets, while in Tartu the awareness of pets as a risk factor for allergic diseases is probably lower. Pet ownership in childhood has, however, been found to be inversely related with atopic sensitisation, allergic rhinitis and asthma in later life also in other studies [109,113]. These findings have been explained by avoidance of pet keeping

by parents of atopic children, induced tolerance caused by the bigger allergic load in those having pets, but also as a marker of difference in lifestyle. We did not find a similar association in Tartu, furthermore, pet in infancy was a risk factor for mite associated symptoms of asthma. All these findings together are in favour of the explanation that pet ownership can be confounded by some other lifestyle aspects in some societies, while in other societies, like in Tartu, it does not serve as a marker of way of life.

Half of the population both in Tartu and Uppsala live in buildings built after the 1960s. The age of the home did not affect the concentration of house dust mites in one study where the association between the dwelling conditions and prevalence of house dust mites were analysed [212], while higher humidity and poor ventilation have been associated with high concentrations of house dust mites [213,214]. Energy saving measures during the last 20 years have changed the indoor climate in Sweden. The higher prevalence of mite associated symptoms of respiratory allergy in Uppsala among people living in older buildings may indicate that additional insulation of older houses, rather than using modern tight constructions and new building materials, could be responsible for mite associated respiratory symptoms. The energy crisis in the early 1970s did not have a major impact on the building standards in the Soviet Union as energy continued to be very cheap. Most of the houses built in Tartu after 1970 are standard 5-9-floor blocks of flats that are centrally heated and do not have mechanical ventilation systems. In Tartu living in houses built more recently tended to increase the risk of respiratory symptoms associated with indoor allergens. Allergen levels are similar, however, in older and more recently built houses, except for house dust mite, that was found in slightly higher concentrations in houses older than 25 years in Tartu [215].

Domestic gas use is the only indoor risk factor related to air quality that with some consistency has been found to be associated with BHR among adults [91-93]. Home dampness is, however, a recognised risk factor for respiratory symptoms in children [216-218]. Visible mould can be considered an indirect marker of humidity and bad ventilation. The association of BHR and visible mould indicates that indoor air quality has an impact on BHR in Tartu.

The low prevalence of atopy among the office workers in Tartu may appear surprising. The social structure in Estonia during the Soviet time did not resemble that of countries with market economy, however, since manual workers were often more well off than people with a higher education, i.e. university graduates. The observation does, therefore, not contradict suggestions

of an association between a high standard of living and an increased risk for atopic disease. This was also the case in Uppsala where allergy to cat was particularly common among those working as managers, legislative officials or administrators.

Allowing for measured exposures in multiple logistic regression analysis did not explain most of the differences in the prevalence. After controlling for several risk factors, living in Tartu was still associated with lower risk for atopy, symptoms of asthma and rhinoconjunctivitis, but with higher risk of nocturnal respiratory symptoms, wheezing and BHR. This indicates that other, not measured risk factors play the major role. The climate, vegetation and pollen seasons are similar in the two towns. As the levels of major indoor allergens are also similar in the two regions [212,215], the differences in prevalence cannot be explained by differences in exposure to allergens. No data on outdoor air pollution in Tartu are available from the beginning of the 1990s. As there is no major industry neither in Tartu nor in Uppsala, the main air pollution comes from vehicles. The difference in gasoline used in Tartu and Uppsala at the beginning of the 1990s may have resulted in differences in outdoor pollution between the two towns. The differences in air pollution can hardly be responsible for the differences we found between these two populations, however. Differences in lifestyle, that likely form the base of the differences in atopic sensitisation and allergies, are difficult to distract into measurable components. It is likely that asthma prevalence has increased because of something that is lacking in the modern environment, rather than through the positive reactions to some toxic factor.

Man is exposed to the environment mainly via lungs and gut, the organs with the biggest total surface area. The exposure is therefore mainly dependant on what we breathe and what we eat and probably also, on whom we live with. In fact, there are more bacteria in man's gut than there are cells in the human organism. This assembly is in constant change and one of the important communication lines between micro organisms and mammals is the immune system of the latter. Recent reports from studies in children indicate that roots in the increased prevalence of atopy and asthma may lie in the changed microflora of the gut [200,201,219].

Methodological implications

The methodological implications of the study are, that dose-response curves should be validated and transition equations calculated when different methods for measuring bronchial reactivity to inhaled agents are compared. This is true even while using apparently similar well-standardised dosimeter methods. If objective markers are used in asthma epidemiological studies, it is beneficial to encompass markers of cellular activity, like serum ECP, in addition to

physiological parameters, like BHR, to better describe the study subjects, especially when the populations compared differ in risk factors for those markers. As far as asthma remains poorly defined in epidemiological studies, postal questionnaires seem to be inexpensive and reliable tools in estimating the prevalence of the disease. However, in addition to self reported asthma, asthma related symptom complex rather than wheeze alone should be reported when studying populations with differences in the prevalence of other respiratory diseases and awareness of asthma.

Internal validity

The response rate in the questionnaire phase of the study was reasonably high in both centres, but slightly lower in Tartu than in Uppsala. Women responded more often than men both in Tartu and Uppsala. Current smoking was more common among the late responders than among early responders. Twenty-five percent of the non-responders in Uppsala were randomly chosen for a telephone interview [75]. The prevalence of symptoms was similar among those who could be reached and those who had answered the questionnaire. A similar analysis was not feasible in Tartu, because of the low number of private telephones.

The proportion of symptomatic persons selected to the second phase of the study was higher in Tartu than in Uppsala despite the lower prevalence of symptoms suggestive of asthma in the mailed questionnaire. This depended on the selection criteria, which in addition to attacks of asthma and current asthma medication comprised the symptom "woken by attack of breathlessness". The prevalence of this symptom was high in Tartu. The participation rate in the second phase of the ECRHS was lower in Tartu than in Uppsala. In Tartu, persons with self reported nasal allergies and symptoms suggestive of asthma appeared to be more willing to participate in the second phase of the study. This selection bias would however tend to overestimate the prevalence of allergy in Tartu, lowering the differences we have revealed between the two centres. Participants in the clinical phase of the study who had respiratory symptoms tended not to participate in BHR testing in Tartu, while the opposite was true in Uppsala. This selection bias would not significantly alter our findings on the prevalence of BHR in the two centres, as the prevalence of BHR was higher in Tartu. Despite our attempt to overcome the differences in the methods used in BHR testing by using the transition equations, obtained in a separate study (paper V), the possibility for a differential misclassification of BHR still remains. The probable result of this misclassification would be that milder BHR has been labelled in Tartu. This could dilute the effect but not affect our results in differences in risk factors.

External validity

As the study was conducted only in one centre in Estonia, a question can be raised on the external validity of the results. Large differences between centres in one country were revealed in the central analyses of the ECRHS data for respiratory symptoms as well as for BHR and atopic sensitisation, indicating that estimations of prevalence rates of a whole country, based on measurements of prevalence in one specific area should be interpreted with caution [220]. Our results have, however, in this respect been validated by a similar study in the age group 15-64 years, conducted in three areas in Estonia: the capital of the country, Tallinn, an agricultural island in the Baltic See, Saaremaa, and a heavily industrialised town located at the Russian border, Narva. In concordance to ECRHS in Tartu, the prevalence of wheeze in the last 12 months was high: among men 22,7% in Tallinn, 23,4% in Saaremaa and 26,4% in Narva and 19,7%, 19,3% and 21,5% respectively among women. The prevalence of physician diagnosed asthma was 2.0% with minor differences between centres [221].

Future aspects

As asthma prevalence in a population reflects both asthma incidence and the average duration of the condition, it is important to know the persistence of symptoms, the incidence of new cases and remissions and to study the factors that associate with incidence and remissions. Two currently ongoing projects, ECRHS II and Respiratory Health in Northern Europe (RHINE), that follow up different sub samples of ECRHS, have been designed to find answers to these questions.

CONCLUSIONS

1. Symptoms of asthma and hay fever were less common in Estonia than in Sweden, while respiratory symptoms in general were more common in Estonia. The differences in the prevalence of symptoms that were likely to be caused by allergy, diminished with age, indicating a probable cohort effect, the causes of which possibly date back to changes beginning in the mid 1950s. The prevalence of nocturnal respiratory symptoms increased with age in Tartu but not in Uppsala. Current smoking was a dominant risk factor for all respiratory symptoms, except attacks of asthma, both in Tartu and Uppsala (I).

2. The prevalence of BHR was higher and the prevalence of atopic sensitisation lower among young adults in Estonia, compared to Sweden. The biggest difference was in the prevalence of cat and pollen atopy. There was some difference between risk factors for BHR and atopy between Tartu and Uppsala, mostly of social and environmental origin. Theses differences, however, could not explain the differences in prevalence for most outcome variables. The low prevalence of hay fever and asthma in Tartu seemed to be partly explained by a lack of awareness of atopy and allergic diseases in the Estonian society (II-III).

3. In this Estonian population sample S-ECP was elevated in subjects with either BHR or atopy, but the levels were not related with asthma symptoms or lung function when other asthma related variables were controlled for. Our results indicate that the level of eosinophil activation is low in a population with a low prevalence of atopy, even when bronchial hyperresponsiveness is common (IV).

4. The estimated cumulative dose causing a 20% fall in FEV_1 was smaller and the decline of FEV_1 /log(dose) curve steeper, using the Spira, compared to Mefar protocol. The dose response of lung function to inhaled agents, even while using apparently similar well standardised dosimeter methods, should be validated and transition equations calculated before comparing the results (V).

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