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XXI CICLO

DETERMINANTS OF LUNG FUNCTION DECLINE  
IN ADULT ASTHMA

*Results from the European Community Respiratory Health Survey*

S.S.D. MED/01

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*Doubt is uncomfortable, certainty is ridiculous.*  
— **Voltaire (1694-1778)**



# Summary

## Introduction

Forced expiratory volume in 1 second (FEV<sub>1</sub>) is a predictor of mortality in the general, as well as in the asthmatic population. Subjects with asthma have a steeper decline in FEV<sub>1</sub> over time than subjects without, and some patients with severe asthma develop progressive airflow obstruction that is not fully reversible with currently available therapy. Moreover, a FEV<sub>1</sub> lower than predicted is a marker of poor asthma control.

## Aims

This thesis was aimed at shedding some light on the factors associated with the decline in FEV<sub>1</sub> in subjects with asthma, with particular attention paid to investigating the potential preventative effect of the use of inhaled corticosteroids (ICSs), as well as to the association of FEV<sub>1</sub> decline with body mass index (BMI) and change in body weight over time.

## Methods

We analysed data from an international, population-based cohort of subjects with asthma, identified in the European Community Respiratory Health Survey (ECRHS, 1991-1993) and followed up from 1999 to 2002. Spirometry was performed on both occasions, and FEV<sub>1</sub> decline was related to potential determinants evaluated at baseline and during the follow-up by random intercept linear regression models.

## Results

- In *asthmatic subjects with high (>100 kU/L) immunoglobulin E (IgE)*, the use of ICSs for 4 years or more during the follow-up was associated with a lower FEV<sub>1</sub> decline (23 mL/y; 95% CI: 8-38 compared with non-users). This association was not seen in *asthmatic subjects with lower IgE*.
- In *asthmatic subjects who did not have airflow obstruction (FEV<sub>1</sub>/FVC<0.70) at*

*baseline*, obese subjects had the lowest level of FEV<sub>1</sub> at baseline; a faster FEV<sub>1</sub> decline was observed for subjects with intermediate BMI than for lean and obese subjects; FEV<sub>1</sub> decline was associated with weight gain independently of baseline BMI, and this association was stronger in men (20, 95% CI: 10; 30, mL/year per kg gained) than in women (7, 95% CI: 2; 11, mL/year); these figures were about 60% greater than the decline found in men and women from the general population who took part in the ECRHS.

- In *asthmatic subjects with airflow obstruction at baseline*, the absence of allergen sensitization and a low BMI at baseline were associated with a faster FEV<sub>1</sub> decline, while weight gain was not associated with decline.

## Conclusions and clinical implications

- Our findings confirm the beneficial association between long-term treatment with ICSs and lung function decline in asthma. However, they suggest that asthmatic subjects with high IgE could maximally benefit from prolonged treatment with ICSs. As a consequence, it could be worth calibrating the corticosteroid dose according to a patient's level of total IgE, although further studies are needed to clarify this.
- The detrimental effect of weight gain on FEV<sub>1</sub> decline is particularly important in asthmatic subjects who still do not have an established airflow obstruction. This effect could be greater in subjects with asthma than in people from the general population. Accordingly, weight management in asthma and weight loss in overweight or obese asthmatic individuals are strongly recommended.
- Among asthmatic subjects with airflow obstruction at baseline, lean subjects without sensitization to allergens deserve particular attention, because they had the greatest decline in FEV<sub>1</sub>. Weight gain was not associated with decline, suggesting that mechanisms that are typical of milder asthma could be less important in severe asthma, while a serious long-lasting inflammation may have a crucial role.

# List of papers

## Paper I

de Marco R, **Marcon A**, Jarvis D, Accordini S, Bugiani M, Cazzoletti L, Cerveri I, Corsico A, Gislason D, Gulsvik A, Jøgi R, Martínez-Moratalla J, Pin I, Janson C; ECRHS Therapy Group. INHALED STEROIDS ARE ASSOCIATED WITH REDUCED LUNG FUNCTION DECLINE IN SUBJECTS WITH ASTHMA WITH ELEVATED TOTAL IgE. *J Allergy Clin Immunol*. 2007 Mar;119(3):611-7.

## Paper II

**Marcon A**, Corsico A, Cazzoletti L, Bugiani M, Accordini S, Almar E, Cerveri I, Gislason D, Gulsvik A, Janson C, Jarvis D, Martínez-Moratalla J, Pin I, and de Marco R; ECRHS Therapy Group. BODY MASS INDEX, WEIGHT GAIN AND OTHER DETERMINANTS OF LUNG FUNCTION DECLINE IN ADULT ASTHMA. *J Allergy Clin Immunol*, *in press*.



# Abbreviations

BHR, bronchial hyperresponsiveness

BMI, body mass index

COPD, chronic obstructive pulmonary disease

ECRHS, European Community Respiratory Health Survey

FEV<sub>1</sub>, forced expiratory volume in 1 second

FRC, functional residual capacity

FVC, forced vital capacity

ICS, inhaled corticosteroid

IQR, interquartile range

IgE, immunoglobulin E

SNP, single nucleotide polymorphism

Th2, T helper class 2 (cells)

TV, tidal volume

VC, vital capacity



# Chapter 1

## Introduction

### 1.1 Asthma: a complex disease

Asthma is a problem worldwide, with an estimated 300 million affected individuals.<sup>1,2</sup> The global prevalence of asthma ranges from 1% to 18% of the population in different countries.<sup>1</sup> Worldwide, approximately 180,000 deaths annually are attributable to asthma, although overall mortality rates have fallen since the 1980s.<sup>2</sup>

The Global Initiative for Asthma guidelines<sup>1</sup> give a brief and comprehensive definition of asthma: “Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment”.

Although a simple definition of asthma is appealing, asthma is unlikely to be a single disease, but rather a series of complex, overlapping individual diseases or phenotypes, each defined by its unique interaction between genetic and environmental factors.<sup>3</sup> These conditions include syndromes characterized by allergen-exacerbated, nonallergic, and aspirin-exacerbated factors along with syndromes best distinguished by their pathologic findings (eosinophilic, neutrophilic, pauci-granulocytic), response to therapy (corticosteroid resistant), and natural history (remodeling prone).<sup>3</sup> Once considered to be a purely allergic disorder dominated by T helper class 2 (Th2)-type lymphocytes, immunoglobulin E (IgE), mast cells, eosinophils, macrophages, and cytokines, the disease also involves local epithelial, mesenchymal, vascular and neurologic events that are involved in directing the Th2 phenotype to the lung and through aberrant injury-repair mechanisms to

remodeling of the airway wall.<sup>4</sup>

The presence of airway inflammation is a consistent feature of all the different asthma phenotypes. Acute inflammation is the response of vascularized tissue to injury: the inflammatory reaction is designed to protect the host and to restore tissue and its function to normal.<sup>5</sup> The airway inflammation in asthma is persistent even if symptoms are episodic, and the relationship between the severity of asthma and the intensity of inflammation is not clearly established.<sup>6,7</sup> The physiological effects of inflammation are most pronounced in medium-sized bronchi. The pattern of inflammation in the airways appears to be similar in all clinical forms of asthma, whether allergic, non-allergic or aspirin-induced, and at all ages.<sup>1</sup> The eosinophil is the most prominent inflammatory cell in the airways of asthma, although mast cells, CD4+ T lymphocytes, macrophages, and neutrophils are also higher in number.<sup>8</sup> Prominent neutrophilic inflammation also occurs in severe asthma. The number of neutrophils has been shown to be enhanced in the airways of patients with severe steroid-dependent asthma or in patients who died of asthma.<sup>9,10</sup>

Airway hyperresponsiveness is the excessive response of the airways to a variety of stimuli. It is thought that its presence, at some time in the natural history of this disorder, is essential for the diagnosis.<sup>11</sup> However, in epidemiological studies, airway hyperresponsiveness has been demonstrated to occur in asymptomatic people,<sup>12</sup> particularly atopic individuals, where its significance is uncertain. The airway narrowing leads to variable airflow limitation and intermittent symptoms. Airway hyperresponsiveness is linked to both inflammation and repair of the airways and is partially reversible with therapy. Its mechanisms are not fully understood.<sup>1,11</sup> Abnormalities of the airway smooth muscle, airway inflammation, airway remodelling, neural control, elaboration of mediators and alteration in the elastic loads against which the airway smooth muscle contracts have all been implicated, but none have given an entirely satisfactory explanation of this phenomenon.<sup>11</sup> Perhaps this is because there is no single underlying mechanism or there are different mechanisms in different asthma phenotypes.

## **1.2 The lungs**

The main function of the lungs is to provide continuous gas exchange between inspired air and the blood in the pulmonary circulation, supplying oxygen and removing carbon dioxide, which is then cleared from the lungs by subsequent expiration. Survival is dependent upon this process being reliable, sustained and

efficient, even when challenged by disease or an unfavourable environment.<sup>13</sup>

The respiratory tract extends from the mouth and nose to the alveoli. It may be seen as a branching tree-like structure, with about 17 levels of branching between the trachea and the respiratory bronchioles. As, on average, the number of branches doubles at each level, there are about 130,000 respiratory bronchioles. The part of the lung supplied by one of these first order respiratory bronchioles is called a "primary lobule". Each primary lobule contains about two thousand alveoli, and is about 3.5 mm in diameter. Branches within the primary lobule give rise to alveolar ducts, which in turn give off alveoli.<sup>14</sup> The alveoli provide an enormous surface area for gas exchange with pulmonary blood (between 50-100 m<sup>2</sup>) with a thin membrane across which gases must diffuse. The solubility of oxygen is such that its diffusion across the normal alveolar-capillary membrane is an efficient and rapid process.

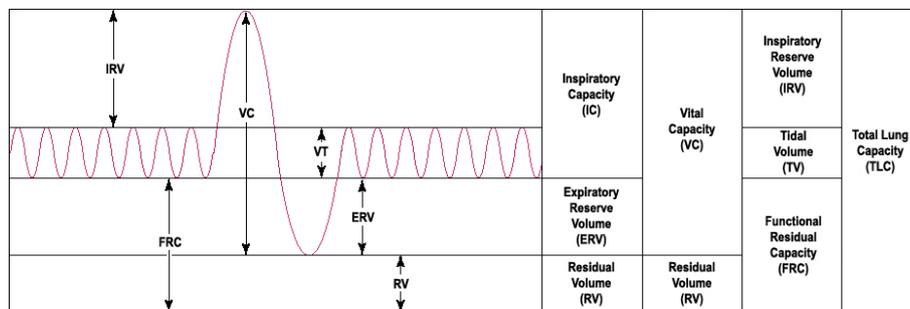
By convention, the upper airways are the extrathoracic ones (nasopharynx, mouth, larynx and extrathoracic trachea). The function of the upper airways is to filter airborne particles, humidify and warm the inspired gases. The lower airways (the intrathoracic trachea followed by bifurcations into the bronchi and bronchioli) are commonly divided into large and small airways. The small airways have a diameter of <2 mm.

The right lung is divided into 3 lobes (upper, middle and lower) whereas the left one has only 2 lobes (upper and lower), with further division into the bronchopulmonary segments (10 right, 9 left). In total there are up to 23 airway divisions between trachea and alveoli. The pleura is a double layer surrounding the lungs, the visceral pleura enveloping the lung itself and the parietal pleura lining the thoracic cavity. Under normal circumstances the interpleural space between these layers contains only a tiny amount of lubricating fluid. The lungs have a double blood supply, the pulmonary circulation for gas exchange with the alveoli and the bronchial circulation to supply the parenchyma (tissue) of the lung itself. Most of the blood from the bronchial circulation drains into the left side of the heart via the pulmonary veins and this deoxygenated blood makes up part of the normal physiological shunt present in the body.<sup>13</sup>

The lungs move in response to external forces. During normal inspiration these forces are the movement of the diaphragm, and movement of the chest wall by the intercostal muscles.<sup>14</sup> The various terms used to describe lung excursion (movement) during quiet and maximal respiration are shown in figure 1.1. Tidal volume (TV) is the lung volume representing the normal volume of air displaced between normal inhalation and exhalation when extra effort is not applied. Func-

tional residual capacity (FRC) is the volume of air in the lungs at the end of a normal expiration. The point at which this occurs (and hence the FRC value) is determined by a balance between the inward elastic forces of the lung and the outward forces of the respiratory cage (mostly due to muscle tone). FRC falls with obesity, pregnancy and anaesthesia, and when lying supine, though not with age. In the absence of respiratory effort, the lung volume will be positioned at the FRC. To move from this position and generate respiratory movement, two aspects need to be considered which oppose lung expansion and airflow and therefore need to be overcome by respiratory muscle activity. These are the airway resistance and the compliance of the lung and chest wall.

Figure 1.1: Lung volumes (source: Wikipedia).



Resistance of the airways describes the obstruction to airflow provided by the conducting airways (resulting largely from the larger airways down to division 6-7), plus a contribution from tissue resistance (produced by the friction generated when the tissues of the lung slide over each other during respiration). An increase in resistance, resulting from airway narrowing such as bronchospasm, leads to *obstructive airways disease* like asthma, bronchitis and chronic obstructive pulmonary disease (COPD).

Compliance denotes distensibility (stretchiness). In a clinical setting it refers to the lung and chest wall combined, and is defined as the volume change per unit pressure change. When compliance is low, the lungs are stiffer and therefore more effort is required to inflate the alveoli. Conditions that worsen compliance, such as pulmonary fibrosis, produce *restrictive lung disease*. Compliance also varies within the lung according to the degree of inflation. Poor compliance is seen at low volumes (because of difficulty with initial lung inflation) and at high volumes (because of the limit of chest wall expansion), with the best compliance in the mid-expansion range. Of the two barriers that oppose respiration, airway resistance and lung compliance, it is only the first of these that requires actual

work to be done to overcome it. Airway resistance to flow is present during both inspiration and expiration and the energy required to overcome it, which represents the actual work of breathing, is dissipated as heat. Although energy is required to overcome compliance in expanding the lung, it does not contribute to the actual work of breathing as it is not dissipated but converted to potential energy in the distended elastic tissues. Some of this stored energy is used for the function of breathing during expiration.

### **1.3 Spirometry**

Spirometry is a physiological test that measures how an individual inhales or exhales volumes of air as a function of time. The primary signal measured in spirometry may be volume or flow. Spirometry is invaluable as a screening test of general respiratory health, in the same way that blood pressure provides important information about general cardiovascular health.<sup>15</sup> The most important indexes measured by spirometry are the forced vital capacity (FVC), which is the volume delivered during an expiration made as forcefully and completely as possible starting from full inspiration, and the forced expiratory volume in one second (FEV<sub>1</sub>), which is the volume delivered in the first second of an FVC manoeuvre.<sup>15</sup> Vital capacity (VC) (see figure 1.1) is the maximum volume of air which can be exhaled or inspired during a slow manoeuvre. The %FEV<sub>1</sub>/FVC ratio is the FEV<sub>1</sub> expressed as a percentage of the FVC and gives a clinically useful index of airflow obstruction.<sup>16</sup> Spirometry can be undertaken with many different types of equipment, and requires the cooperation between the subject and the examiner, and the results obtained will depend on technical as well as personal factors.<sup>15</sup> This is the reason why there is a strong need to perform spirometry following standardized protocols, especially when it is used in epidemiological studies. The lung function protocol followed in the ECRHS is shown in Appendix A.

### **1.4 Lung function in asthma and airway remodeling**

Patients in particular may describe their current asthma status in terms of the presence or absence of symptoms, quality of life or minimum use, if any, of antiasthma medication. But, for the clinician, disease control is also characterized by the best possible level of lung function and the absence of signs of poorly controlled airway inflammation.<sup>17</sup> Many studies have consistently shown that lung function in patients with clinical asthma is less than predicted.<sup>17-19</sup> This may

reflect suboptimal treatment at the time of measurement, as well as (depending on the age of the patient) any combination of at least four factors: slower growth of lung-function; lower maximally attained level of lung function; earlier onset of decline of lung function; and accelerated decline of lung function. Even if the magnitude of the excess loss of lung function differs between studies, most studies have reported an excess decline in FEV<sub>1</sub> of 5-25 mL/yr in subjects with asthma, when compared to subjects without asthma.<sup>17</sup> Impaired pulmonary function is a predictor of mortality in the general,<sup>20</sup> as well as in the asthmatic population.<sup>21</sup> Lung function decline may result in persistent airflow limitation for some of the asthmatic individuals.

One of the factors contributing to the progressive loss of lung function in asthma is airway remodeling.<sup>22</sup> Airway remodeling includes an increase in overall wall thickness, an increase in airway fibrosis and smooth muscle mass, abnormalities in composition of the extracellular matrix, and an increase in vascularity.<sup>23,24</sup> Airway remodeling in asthma includes not only structural changes but also fundamental changes in the relationships between and among various airway constituents. Cell-cell interactions, the regulation of cells by extracellular matrix, and the modulation of the composition of matrix by cells have all been demonstrated to be altered.<sup>22</sup>

The process of remodeling per se is not necessarily abnormal. It is an alteration in size, mass, or number of tissue structural components that occurs during growth or in response to injury and/or inflammation. It may be "appropriate", as in the case of normal lung development or what occurs during the acute reaction to injury (e.g. wound healing); or "inappropriate", when it is chronic and associated with abnormally altered tissue structure and function, like, for example, in asthma, COPD, or fibrosing alveolitis.<sup>5</sup> In asthma, both inflammation and remodeling usually persist and the consequences are inappropriate for the maintenance of normal airway function. The reasons for the persistence are unknown, but they may be the result of repeated inhalation of allergens or exposure to high concentrations of allergens, or infections, or a genetically influenced abnormal host inflammatory response or defect in the repair process. The host responses in asthma may of course differ due to differences in genetic makeup or consequent to developmental abnormality or to the effects of perinatal environmental exposure.

It is generally believed that the accelerated decline in forced expiratory flow over time in obstructive lung diseases (like COPD and asthma), is the direct result of a switch from acute and episodic to chronic inflammation and to consequent parenchymal and airway wall remodeling, respectively.<sup>7</sup> However, as yet, there

is no convincing evidence that the remodeling process is dependent on the prior development of chronic inflammation.<sup>5</sup> It is equally plausible that the processes responsible for the development of chronic inflammation are distinct from those responsible for remodeling. Alternatively, an intrinsic abnormality of the airway structure or function may drive inflammation.<sup>22</sup>

## 1.5 Asthma genetics

Burke et al.<sup>25</sup> examined 33 studies from all geographic regions in the world. Most of these studies used large population-based samples to assess if family case history were a risk factor for asthma. Although there were many methodologic differences, the picture that emerged was consistent. A family history of asthma is a strong predictor of asthma risk. In fact, in most of the studies reviewed, the odds ratios for asthma fell between 2 and 4 when a first-degree relative had asthma.

Studies in identical twins have convincingly demonstrated that at least 50% of the susceptibility to asthma is determined by inherited predisposition.<sup>26</sup> It has also been clearly established that no single gene or even a small number of genes exert a decisive influence in determining this susceptibility. Asthma is essentially a polygenic disease in which many genetic variants determine small changes in immune responses or in the manner in which the airways respond to the environment.<sup>27,28</sup> Linkage (and positional cloning) and association studies have identified numerous candidate genes and chromosomal regions that may contribute to asthma risk.<sup>28,29</sup> Most of the genes currently known to modify asthma and allergy susceptibility have been identified through hypothesis-driven studies that sought to identify an association between variants such as single nucleotide polymorphisms (SNPs) in the main pathways that influence allergic inflammation and asthma or asthma-related (intermediate) phenotypes.<sup>30</sup>

On **chromosome 1**, A1 adenosine receptor (A1AR) and calcium-dependant chloride channel 1 (CLCA1) genes are likely to be important in the pathogenesis of asthma and other respiratory conditions. A1AR signaling may serve to regulate the severity of pulmonary inflammation and remodeling in chronic lung disease by controlling the levels of important mediators of inflammation and damage.<sup>29</sup> Adenosine contributes to mucus hypersecretion by airway epithelial cells and upregulates mucin expression. The pathway is initiated at the A1AR that transduces signals through a Ca<sup>2+</sup> activated Cl<sup>-</sup> channel and the epidermal growth factor receptor (EGFR). CLCA1 gene polymorphisms have been associated with childhood and adult asthma in the Japanese population.<sup>31</sup> The CLCA1 channel

may be responsible, in part, for the overproduction of mucus in asthmatic patients<sup>32</sup>, which is one of the reasons for fixed airflow obstruction in a subgroup of subjects with asthma. White et al.<sup>33</sup> reported their findings from a genome scan for asthma using the GAIN (Genetics of Asthma International Network) families. The study pinpointed a genetic locus on Chromosome 1q43 with subsequent fine mapping identifying OPN3. The function of OPN3 is unknown. White et al.<sup>33</sup>, however, showed that both the RNA and the protein of the gene are expressed in lung bronchial epithelia and immune cells, whereas siRNA knockdown suggested OPN3 might modulate T-cell responses.

On **chromosome 2**, studies have identified the IL-1RN gene as possibly being responsible for some alterations in the pathogenesis of asthma.<sup>34,35</sup> IL-1RN is a gene encoding the IL-1 receptor antagonist protein, an anti-inflammatory cytokine that plays an important role in maintaining the balance between inflammatory and anti-inflammatory cytokines. Another interesting gene in the region 2q14–32 is the dipeptidyl-peptidase 10 (DPP10) gene, discovered by positional cloning.<sup>35</sup> The protein encoded by this gene is involved in cleavage of terminal dipeptides from cytokines and chemokines.

The cytokine gene cluster on **chromosome 5** (5q31–33) is another gene rich region of the human genome.<sup>36</sup> Two genes in this region, ADRB2 and IL4R, have had many positive associations to asthma and asthma-related traits reported.<sup>30</sup> ADRB2 encodes the  $\beta_2$ -adrenergic receptor and contains several common genetic variations that affect gene expression and receptor function in vitro.<sup>37</sup>  $\beta_2$ -agonists are the most frequently prescribed form of medication in the treatment of the bronchoconstriction that is seen in asthma.<sup>37</sup> To date 55 single nucleotide polymorphisms (SNPs) for ADRB2 have been listed in public databases.<sup>38</sup> A number of these SNPs are nonsynonymous resulting in amino acid changes and functional investigations have suggested that certain SNPs are of importance with regard to the cellular response to  $\beta$ -agonists. IL4R is a key gene of the Th2 pathways as are its ligands, interleukin (IL) 4 and IL13. These two cytokines are the only known cytokines that are capable of inducing human IgE synthesis whereas IL13 is recognized to be the chief effector of the allergic immune response. The IL4R gene contains many SNPs with the majority being contained in exon 12 of the gene and coding for amino acid changes that have the potential to impact on receptor signalling.<sup>36</sup>

The human major histocompatibility (MHC) genes and many other genes that play an important role in the regulation of the immune system are found on **chromosome 6** (6p21).<sup>39</sup> The 6p21 region has shown strong linkage to atopic

phenotype and asthma in many studies<sup>40,41</sup>, and it is considered to be a major locus influencing allergic diseases. One of the genes that is likely to be involved in asthma and allergy is the TNF- $\alpha$  gene.<sup>42</sup> Tumour necrosis factor (TNF) is a proinflammatory cytokine that increases human airway tissue responsiveness.

**Chromosome 7** seems to be involved in asthma through the alteration of the IgE levels. The region involved is likely to be a 20cM region on chromosome 7p14-p15, which showed linkage for asthma and IgE.<sup>29</sup> Particularly, the orphan G protein-coupled receptor gene (GPRA, G-protein-coupled receptor for asthma susceptibility) has been associated with elevated IgE.<sup>43,44</sup>

On **chromosome 11**, the glutathione-S-transferase (GST) genes and other genes involved in the oxidation stress have been described to be involved in asthma and atopy.<sup>45,46</sup> Gene polymorphisms and differential expression levels of the GST genes have been associated with asthma, atopy and lung function.<sup>47</sup>

Several studies have shown linkage of **chromosome 12** (12q13-26) to asthma or related phenotypes in different populations.<sup>48,49</sup> The linkage shown spans a wide region, suggesting that more than one asthma susceptibility gene may be located on chromosome 12.<sup>50</sup> Oxidative stress, with the formation of reactive oxygen species, is a key component of inflammation. The nitric oxide synthase (NOS) enzymes are involved in the synthesis of nitric oxide (NO) from arginine. NO has been proposed to be a marker for airway inflammation in asthma. One of the enzymes producing NO is the neuronal nNOS, which is located on chromosome 12q32. Another region of the chromosome 12 that seems to have a role in asthma is the vitamin D receptor (VDR) gene.<sup>51</sup> A recent study of experimental models showed the importance of the vitamin D endocrine system in the generation of the Th2-driven inflammation in the lung.<sup>52</sup> IRAK-M was reported to be involved in the pathogenesis of early onset persistent asthma. The gene (on Chromosome 12q14) encodes a protein that is both a master regulator of NF- $\kappa$ B and inflammation, as well as being a negative regulator of the Toll-like receptor/IL1-R pathways, and it is expressed in the bronchial epithelium.<sup>53</sup>

On **chromosome 13**, there seem to be genes influencing IgE levels. Several studies confirmed linkage of atopy and the related phenotype to chromosome 13q14.<sup>54</sup> Plant homeodomain finger protein 11 (PHF11) is one of these genes, and it was identified through linkage analysis followed by positional cloning.<sup>30</sup> PHF11 is expressed in both T and B cells<sup>55</sup>, thus it is likely that this protein may regulate the transcription of lymphocyte genes involved in allergic inflammation.

Linkage of **chromosome 14** markers with asthma or related phenotypes has been illustrated in several reports and some gene associations have been de-

scribed.<sup>56,57</sup> Mansur et al.<sup>58</sup> reported the linkage and association of the D14S63 marker with total serum IgE levels in asthmatic families. Marker D14S63 is physically located 16 Mb from the prostaglandin D2 receptor (DP) gene (PTGDR). PTGDR is present on mast cells and eosinophils, which generate the effector molecules of the asthmatic diathesis.<sup>59</sup>

A disintegrin and metalloprotease (ADAM)33 gene on **chromosome 20** (20p13) has been recognized as a susceptibility gene for asthma through linkage studies followed by positional cloning.<sup>30,60,61</sup> ADAM33 consists of 22 exons that encode a signal sequence, prodomain, catalytic domain, disintegrin domain, cysteine-rich domain, EGF domain, transmembrane domain, and cytoplasmic domain with a long 3'-untranslated region.<sup>60</sup> From a functional standpoint, these different domains translate into different functions of ADAM33, which include activation, proteolysis, adhesion, fusion, and intracellular signaling.<sup>62</sup> ADAM33 belongs to a family of 40 ADAM proteins and, being a metalloprotease, the catalytic domain has a zinc binding site. ADAM33 mRNA is preferentially expressed in smooth muscle, fibroblasts, and myofibroblasts, but not in the bronchial epithelium or in inflammatory or immune cells.<sup>60</sup> Jongepier and colleagues investigated genetic and environmental factors that may contribute to accelerated decline in lung function in 200 patients with chronic asthma studied annually for 25 yr.<sup>63</sup> They showed that the rare allele of the S<sub>2</sub> polymorphism was significantly associated with excess decline in FEV<sub>1</sub> over time and concluded that this variant of ADAM33 was not only important in the development of asthma but also in disease progression, possibly related to enhanced airway remodeling. A further study by the same group<sup>64</sup> investigated whether polymorphic variation of ADAM33 could also predict an accelerated decline in baseline lung function at a population level. A total of 1,390 subjects from a Dutch cohort were genotyped for eight asthma-associated SNPs and these were analyzed in relation to baseline FEV<sub>1</sub> measured every 3 yr for 25 yr. Individuals homozygous for the minor alleles of SNPs S<sub>2</sub> and Q-1 and heterozygous for the SNP S<sub>1</sub> had a significantly accelerated decline in FEV<sub>1</sub> of 4.9, 9.6, and 3.6 ml/yr, respectively, when compared with the wild-type allele. A further analysis demonstrated a higher prevalence of the SNPs F+1, S<sub>1</sub>, S<sub>2</sub>, and T<sub>2</sub> in subjects with COPD at GOLD (Global Initiative for Chronic Obstructive Lung Disease) stage 2 or higher. Thus, in addition to asthma, it seems that polymorphic variation in ADAM33 also influences the rate of decline of lung function at a population level and more specifically in COPD. The selective expression of ADAM33 in mesenchymal cells, and the stronger linkage and association observed when conditioning asthma with bronchial hyperresponsiveness (BHR),<sup>60</sup> strongly sug-

gests that alterations in its activity may underlie abnormalities in the function of airway smooth muscle cells and fibroblasts linked to airway remodelling and BHR.<sup>65</sup>

Since completion of the human genome sequence, the toolkit available for complex disease gene identification has grown substantially.<sup>36</sup> Sequencing of the human genome in itself was a remarkable achievement. It also opened up the opportunity for comprehensive SNP identification and characterization of linkage disequilibrium patterns across the genome for populations of differing ethnic backgrounds. In hand with this has been the vast technological development in platforms for SNP genotyping and advances in the study of genome-wide gene expression. Consequently, it is now possible to genotype a single individual for up to a million SNPs simultaneously. This has opened up the possibility of conducting genome-wide association (GWA) studies. To date there have only been two GWA studies for asthma.<sup>66,67</sup>

The first, published in 2007 for childhood asthma, involved 994 patients and 1,243 nonasthmatics that were genotyped for more than 317,000 SNPs.<sup>66</sup> Seven out of the 12 SNPs showing association above the 1% false discovery rate threshold mapped to an interval on **chromosome 17** (17q21). Further SNP typing was conducted resulting in three SNPs jointly showing strong association to childhood asthma ( $P < 10^{-12}$ ). By coupling their genetic findings with genome-wide gene expression data from Epstein-Barr virus-transformed lymphoblastoid cells from children in the genotyped family samples<sup>68</sup>, the authors showed that the SNPs with the strongest association to asthma were consistently associated with levels of ORMDL3 transcripts.<sup>66</sup> Little is known about the protein that ORMDL3 encodes. ORMDL3 is a member of a conserved family of endoplasmic reticulum membrane proteins and the gene is expressed in a large range of tissues. The protein contains four transmembrane regions and, therefore, has the potential to act as a transporter. Several further publications<sup>69-72</sup> have confirmed the associations between asthma and the Chromosome 17q21 SNPs with one study highlighting that the locus is a risk factor for asthma in ethnically diverse populations.<sup>70</sup>

In 2008, Ober et al.<sup>67</sup> reported findings of a genetic influence on asthma and a chitinase-like protein known as YKL-40, which is encoded by the chitinase 3-like 1 (CHI3L1) gene (**chromosome 1**, region 1q32.1). It had previously been established that YKL-40 levels were increased in the lung and circulation of patients with severe asthma.<sup>73</sup> Using a carefully characterized founder population of European descent, the Hutterites, 500,000 genomewide SNPs were genotyped. Following further fine mapping, a promoter SNP in the CHI3L1 gene was found to be asso-

ciated not only with elevated serum YKL-40 levels but also asthma and measures of lung function. YKL-40 is produced at sites of inflammation in many cells and is secreted from vascular smooth-muscle cells and macrophages.<sup>74</sup> It is strongly upregulated in the airway epithelium and alveolar macrophages of patients with asthma.<sup>73</sup> Although the real function of YKL-40 remains to be established, the study of Ober et al.<sup>67</sup> has identified not only a further susceptibility gene for asthma that is of importance, but also an important biomarker that can be used as a measure of asthma susceptibility.

## 1.6 Inhaled corticosteroids and lung function decline

Inhaled corticosteroids (ICSs) are the mainstay of asthma treatment,<sup>1</sup> and are generally effective for the control of symptoms. Several studies suggest that they may also alter the natural history of the disease and have a role in the prevention of the structural airway changes associated with asthma and the progressive lung function decline that is characteristic of the disease.<sup>75</sup> The first study to suggest that ICSs may have an effect on lung function decline was one of the earlier studies of budesonide use on growth and pulmonary function.<sup>76</sup> In this controlled prospective study, Agertoft et al. measured growth and pulmonary function in children with asthma during long-term treatment with inhaled budesonide and compared these findings with those obtained from children not treated with corticosteroids. In the latter, an annual decrease in FEV<sub>1</sub> % predicted of 1-3% was seen. In contrast, FEV<sub>1</sub> improved significantly with time during budesonide treatment, both compared with the run-in period and with the control group (p<0.01).

The Childhood Asthma Management Program (CAMP) study was a randomized controlled trial of older children (age five to 12 years) with established mild to moderate asthma.<sup>77</sup> It was designed to evaluate whether continuous long-term treatment of budesonide 200 µg twice daily for a period of four to six years would safely produce an improvement in lung growth as compared with treatment for symptoms alone (placebo group: β<sub>2</sub>-agonist and prednisolone when needed). The trial did not show a significant difference between either treatment and placebo in the degree of change in the post-bronchodilator FEV<sub>1</sub>. However, compared with the placebo control group, the children assigned to receive budesonide had a significantly smaller decline in the ratio of pre-bronchodilator FEV<sub>1</sub>/FVC (0.2% vs. 1.8%, p=0.001).

Virtually all short term studies (weeks to a few years) on adults have shown

that ICSs benefit patients with chronic persistent asthma by decreasing airway inflammation, improving lung function, and reducing symptoms and airway hyperresponsiveness.<sup>78-81</sup> The regular use of ICSs was found to be associated with a decreased risk of fatal and near-fatal asthma,<sup>82,83</sup> and with a decrease in the risk of asthma hospitalisation.<sup>84</sup> However, only recently observational studies were performed to evaluate whether long-term treatment with ICSs also have a beneficial effect on lung function decline.<sup>85,86</sup> Lange et al.<sup>85</sup> studied subjects participating in the third and fourth examinations of the Copenhagen City Heart Study. Compared to subjects who did not use ICSs, subjects who used ICSs had a 18 mL/year significantly smaller decline in FEV<sub>1</sub> (p=0.013). Dijkstra et al.<sup>86</sup> reported that the use of ICSs was associated with a reduction in the annual decline in lung function in men, but not in women. They also showed that the beneficial effect of ICSs on lung function was dose-dependent, and that it was not present in men with >5 pack-years smoking. Given the acknowledged benefits of ICSs in asthma, randomized controlled trials that evaluate the effects of ICS use on lung function decline will not be feasible, and long-term observational studies will probably be the best available evidence.

## 1.7 Other determinants of lung function decline

The cross-sectional relationship existing between weight (or body mass index, BMI) and lung function in healthy individuals was first revealed very long time ago (1846),<sup>87</sup> and later replicated.<sup>88</sup> These papers showed that lung function, as measured by the VC, increased with weight until a certain value (e.g. 107% of average weight<sup>87</sup>), and decreased slightly with further increases in weight. The explanation of this relationship between weight and lung function was that the increase of lung function with weight may reflect increasing muscle force (muscularity effect), while the decrease with further weight increase probably reflects “the mere circumstance of fat preventing the mobility of the thoracic boundaries”<sup>87</sup> (obesity effect).<sup>88</sup>

Recently, the detrimental effect of weight gain on lung function decline has been shown in the general population.<sup>89</sup> Several mechanisms have been advocated to explain the negative effect of obesity and weight gain on lung function.<sup>90,91</sup> The first of these possibilities rests on simple mechanical considerations, where both static and dynamic factors come into play. Concerning static factors, increased abdominal and chest wall mass in the obese causes lower than normal FRC. Because lung volume is a major determinant of airway diameter, it is therefore likely that

obesity-related changes in FRC unload the airway smooth muscle and thereby allow it to shorten excessively when activated.<sup>92</sup> Concerning dynamic factors, the tidal action of spontaneous breathing imposes tidal strains on airway smooth muscle, and these tidal strains happen to be the most potent of all known bronchodilating agencies.<sup>93</sup> The obese individual breathes at higher frequencies but substantially smaller tidal volume compared with the lean individual,<sup>94</sup> and as a result, this potent bronchodilating mechanism is compromised and predisposes toward increased airway responsiveness compared with the lean individual. A second possible explanation for the influence of obesity on airway smooth muscle function concerns differences in the anatomy of the lungs and airways. For example, in children the mechanical load of obesity might affect lung growth, leading to reduced pulmonary function, a known risk factor for asthma. Obesity might also lead to more accelerated airway remodeling with each asthma exacerbation. Moreover, in obese individuals, even in the absence of an overt inflammatory insult, there is chronic, low-grade systemic inflammation characterized by increased circulating leukocytes and increased serum concentrations of cytokines, cytokine receptors, chemokines, and acute-phase proteins.<sup>90,91,95</sup> Despite the above considerations, the relationship between weight (or weight gain) and lung function decline has not been investigated thoroughly enough in the asthmatic population.

Smoking has been consistently shown to accelerate lung function decline in the general population,<sup>19,89</sup> and it is the main risk factor for COPD.<sup>16</sup> Active smoking is, therefore, likely to have a more deleterious effect on lung growth and senescence in asthmatics than in nonasthmatics. However, in both children and adults, an effect of smoking additional to the effect of asthma on longitudinal changes in lung function has been difficult to demonstrate.<sup>17</sup> In fact, although many studies<sup>19,96,97</sup> have found that asthmatic smokers have a steeper decline in lung function than asthmatic non-smokers, and that smoking is a risk factor for persistent airflow obstruction for subjects with asthma<sup>98</sup>, some studies could not show this association.<sup>18,99,100</sup> It is possible that these studies have included insufficient numbers of smokers with asthma to draw conclusions about the impact of cigarette smoking on outcome, perhaps because individuals with more susceptible airways, i.e. asthmatics, are less likely to take up smoking or quit at an early stage (the healthy smoker effect). Although the evidence is scarce, it is difficult not to assume that active smoking has a negative effect, additional to the effect of asthma, on longitudinal changes in lung function in both children and adults suffering from asthma.<sup>17</sup>

Passive exposure to cigarette smoke in childhood is a risk factor for wheezy

bronchitis, airway hyperresponsiveness, and symptomatic asthma.<sup>101</sup> In young adults, passive smoking was found to be associated with respiratory symptoms, asthma, a small but significant impairment of lung function and increased bronchial responsiveness.<sup>102,103</sup>

The duration of the disease seems to be associated with lung function decline in a non-linear way. Both newly diagnosed asthma and long-standing asthma appear to be associated with unfavourable longitudinal changes in lung function, possibly reflecting increased airway vulnerability initially, possibly owing to unopposed inflammation, and progressive airway remodelling due to chronic inflammation.<sup>17</sup> In fact, several studies have found that adult asthmatics may have an excessive annual decline in lung function prior to the time of diagnosis, and also in the first years following the onset of asthma.<sup>104,105</sup> However, in adult asthmatics, the degree of lung function impairment seems to be related to the duration of previous asthma. In other words, longer duration of the disease may be associated with increasing decline in lung function.<sup>99,106</sup> Accordingly, incomplete reversibility of airflow obstruction may be found in some, most probably a minority, patients with long-standing disease.<sup>98,107</sup> These observations suggest that long-standing airway inflammation may lead to, perhaps nonreversible, structural changes in the airway wall.

Atopy, defined as a positive skin test reactivity to inhalant allergens, is found in the majority of patients, especially children, with asthma, but the association between atopy and the outcome of asthma is not straightforward.<sup>17</sup> Allergic (also called atopic, or extrinsic) asthma usually develops in the second decade of life and frequently persists into adult years, but young patients with allergic asthma often enjoy a transient or even a permanent remission. Asthma beginning after the fourth decade is usually non-allergic (also called non-atopic, or intrinsic) and may include the aspirin triad. Its severity tends to increase with time.<sup>108</sup> Only a few long-term longitudinal studies have focused on the possible impact of atopy on longitudinal changes in lung function in patients with asthma. Evidence from longitudinal population studies indicates that skin test-positivity is associated with an accelerated decline in FEV<sub>1</sub>.<sup>109</sup> However, results from studies comparing subjects with atopic asthma and subjects with non-atopic asthma are conflicting. Some studies indicate that atopy is not associated with lung function decline in subjects with asthma,<sup>18,110,111</sup> although in one of these studies the authors conclude that asthmatic patients *with atopy and marked BHR* should be looked upon as patients with a risk of developing progressive airflow obstruction.<sup>111</sup> Conversely, other papers report that non-atopic asthma is a more severe

form of asthma than atopic asthma, and that it is associated with a steeper decline in lung function.<sup>99,112,113</sup> To explain these conflicting results, it can be observed that the patients with nonatopic asthma recruited in these studies were generally older than the patients with atopic asthma, and as lung function decline accelerates with age, the observed difference between atopic and nonatopic asthmatics might be, at least partly, explained by the difference in age between the two groups. Atopy is a known risk factor for symptomatic asthma, and in the nonasthmatic population it may also be a risk factor for an accelerated decline in lung function. However, in both children and adults with established asthma, according to Ulrik<sup>17</sup> it appears reasonable to conclude that atopy is not an independent determinant of prognosis with regard to lung function, suggesting that inflammatory processes in the airways of patients with asthma run their own courses irrespective of the subjects' atopic status. However, another possible explanation of these conflicting results on the association between atopy and lung function decline in asthma, could be the complexity of asthma: there is likely to be heterogeneity of asthma phenotypes even in the rather homogeneous atopic and non-atopic phenotypes.<sup>3</sup>

# Chapter 2

## Aims

The aim of this thesis was to increase knowledge about factors associated with lung function decline in asthma. We asked the following questions in a large, international, population-based cohort of subjects with asthma, followed up for 9 years in the European Community Respiratory Health Survey (ECRHS):

- is prolonged use of inhaled steroids associated with a reduced long-term decline in lung function?
- what are the main determinants of lung function decline in asthma? Do these determinants vary among asthmatic subjects with and asthmatic subjects without airflow obstruction?



# Chapter 3

## Methods

### 3.1 Study design of the ECRHS

ECRHS stage I<sup>114</sup> was an international postal questionnaire survey (see Appendix B), mainly carried out in 1990 and 1992, with randomly selected participants from the general population within administrative boundaries of 56 centres in 25 countries ([www.ecrhs.org](http://www.ecrhs.org)). From those who responded, a 20% random sample was invited to undergo a more detailed clinical examination in 1992-94 (stage 2). In addition, a sample (called symptomatic sample) consisting of subjects not already included in the random sample who reported asthma-like symptoms in the last 12 months, or individuals who were using asthma medication in stage 1, was also investigated. The ECRHS I aimed at describing variations in exposure to risk factors and their association with asthma and allergy and in analysing the variation in treatment practice in the international community. In ECRHS I stage 1 each centre invited about 1,500 men and 1,500 women for assessment of symptoms (respiratory symptoms, nasal symptoms, asthma). In ECRHS I stage 2, at least 300 men and 300 women from each centre were invited to participate in a detailed clinical examination. The main questionnaire in stage 2 (see Appendix C) included factors known or hypothesised to be of importance for asthma and allergy (family size, family history of the diseases, occupation, childhood and current exposure to pets, exposure to tobacco smoke, dampness, ventilation, use of soft furnishings, use of gas appliances) and use of health services and treatment for respiratory diseases. The main questionnaire was based on the validated Bronchial Symptoms Questionnaire of the International Union Against Tuberculosis and Lung Disease.<sup>114</sup> In addition, in stage 2, blood was taken for measurement of specific IgE to Der p1 (house dust mite), cat, Timothy grass, Cladosporium (mould), a local allergen, and total IgE. Forced expiratory volume in one second (FEV<sub>1</sub>), forced

vital capacity (FVC) and bronchial reactivity to metacholine were measured.

From 1998 to 2002, responders from 29 centres in 14 countries were invited to a follow-up study, the ECRHS II.<sup>115</sup> The ECRHS II stage 1 was a postal screening questionnaire (see Appendix D), while the ECRHS II stage 2 included a detailed clinical investigation (see Appendix E), additional questionnaires and a clinical examination. Lung function measurements (see Appendix A), methacholine challenge tests and blood samples for measurement of serum specific IgE were also performed. The present thesis includes longitudinal analyses using data from both ECRHS I stage 2 and ECRHS II. A flow chart of the ECRHS is shown in figure 3.1.

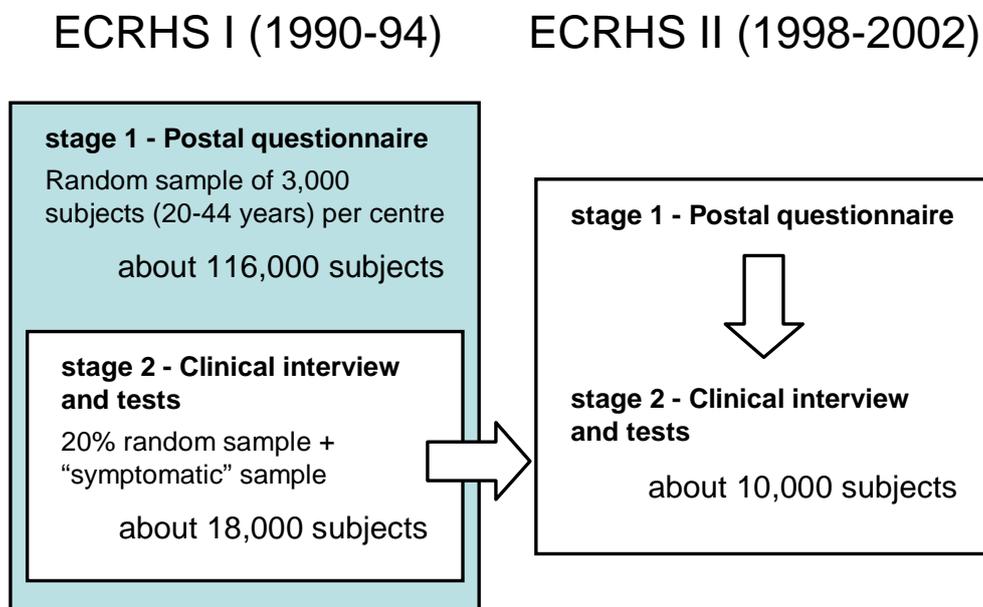


Figure 3.1: Flow-chart of the European Community Respiratory Health Survey.

## 3.2 Asthma definition and asthma phenotypes

Current asthma was defined in the ECRHS I as having reported asthma [EC1q13]\* confirmed by a doctor [EC1q13.1] and having had:

\*the reference questions (q#) and questionnaires (EC1=ECRHS I main questionnaire, EC2=ECRHS II main questionnaire) are indicated in squared brackets; e.g. "EC1q1" is the first question of the ECRHS I main questionnaire. See Appendix C for the ECRHS I main questionnaire and Appendix E for the ECRHS II main questionnaire.

- asthma-like symptoms (wheeze [EC1q1], nocturnal chest tightness [EC1q2], attacks of breathlessness following activity [EC1q4], at rest [EC1q3] or at night time [EC1q5], asthma attacks [EC1q13.5]) *and/or*
- having used inhaled [EC1q60]/oral [EC1q61] medicines because of breathing problems during the last 12 months.

In the paper on the determinants of lung function decline in asthma (paper II), two distinct phenotypes of asthma were separately investigated:

1. asthmatic subjects without airflow obstruction at baseline (milder asthma)
2. asthmatic subjects with airflow obstruction at baseline (more severe asthma)

Baseline airflow obstruction was defined as  $FEV_1/FVC < 0.70$ <sup>16</sup> at the first visit (ECRHS I). As a bronchodilator challenge test was not part of the ECRHS core protocol, the follow-up spirometry was used to confirm baseline airflow obstruction. Accordingly, 29 subjects with baseline airflow obstruction who had  $FEV_1/FVC \geq 0.70$  at follow-up were excluded from the analyses, in order to reduce the number of "false positives".<sup>116</sup>

### 3.3 Outcome

The maximum  $FEV_1$  and FVC of at least two and as many as five technically acceptable maneuvers were recorded, according to the American Thoracic Society criteria for reproducibility.<sup>117</sup>  $FEV_1$  % predicted and FVC % predicted were calculated on the basis of the equations by Quanjer et al.<sup>118</sup> The average decline in  $FEV_1$  (or FVC) during the follow-up was computed as the difference between  $FEV_1$  (or FVC) measured in the ECRHS I and II, divided by the duration of the follow-up (expressed in mL/year). % $FEV_1/FVC$  decline was also computed as the difference between % $FEV_1/FVC$  in the ECRHS I and II, divided by the duration of the follow-up (expressed in %/year).

### 3.4 Use of inhaled steroids

In both surveys, the participants were asked whether they had used ICSs in the last 12 months, and the type/brand of steroid was recorded [EC1q60.4, EC2q76.5 and nested questions]. In the ECRHS II, quantitative information was collected about ICS use during the follow-up (how many months per year, how many years

since the last survey a subject had been on ICSs [EC2q78 and nested questions]). The data on ICSs were combined to calculate the cumulative time of treatment during the follow-up. Subjects with asthma were stratified according to the time of steroid use into:

1. non-users,
2. people who had used ICSs for <8.7 months (1<sup>st</sup> tertile of time of ICS use distribution among users),
3.  $\geq 8.7$  months but <48 months (2<sup>nd</sup> tertile),
4.  $\geq 48$  months.

### 3.5 Other covariates considered in the analyses

The following covariates were considered in the analyses as potential determinants of FEV<sub>1</sub> decline or as potential confounding factors:

- baseline covariates:
  - sex;
  - age;
  - height (m);
  - Body Mass Index (BMI, kg/m<sup>2</sup>), the subject's weight was measured, and BMI was calculated as weight/height<sup>2</sup>;
  - educational level, low if a subject had completed full-time education before the age of 16 [EC1q32: *Are you a full time student?*, EC1q32.1: *At what age did you complete full time education?*];
  - occupation, coded as manual job, non-manual job, or other [EC1q32.2: *Are you currently employed or self-employed?* EC1q32.3: *What is your current or most recent job? Be as precise as possible*, EC1q32.4: *Are you or were you (TICK ONE BOX ONLY): a manager working for an employer?, a foreman or supervisor working for an employer?, working for an employer, but neither a manager, supervisor or foreman?, self-employed?*];
  - occupational risk [EC1q32.7: *Have you ever worked in a job which exposed you to vapours, gas, dust or fumes?*];

- duration of asthma, estimated as the difference between the age of the subject at the ECRHS I interview and the age when the first asthma attack occurred;
- total IgE (kU/L), measured according to a standardized protocol<sup>119</sup>;
- sensitization to allergens; specific IgE against house dust mite, cat dander, timothy grass, Cladosporium species, and a local allergen, were measured.<sup>119</sup> A subject was considered sensitized to any allergen if the assay result for at least one allergen was higher than 0.35 kU/L, the detection limit of the assay;
- hospitalizations and/or emergency department visits for breathing problems [EC1q69: *Have you ever visited a hospital casualty department or emergency room because of breathing problems?* and/or EC1q70: *Have you ever been seen by a doctor because of breathing problems or because of shortness of breath?*];
- pack-years, calculated for the period before the ECRHS I interview<sup>89</sup> [EC1q58: *Have you ever smoked for as long as a year? YES means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year*, EC1q58.1: *How old were you when you started smoking?*, EC1q58.2: *Do you smoke now, as of one month ago?* and all the nested questions asking detailed information about the intensity of smoking, EC1q58.3: *Have you stopped or cut down smoking?* and all the nested questions asking detailed information about the intensity of smoking before cutting down or having stopped smoking, and the age when the subject cut down or stopped smoking];
- environmental tobacco smoke (ETS) exposure, i.e. regular exposure to other people's smoke for 1 hour/day or more [EC1q59: *Have you been regularly exposed to tobacco smoke in the last 12 months? 'Regularly' means on most days or nights*, EC1q59.3: *How many hours per day are you exposed to other people's tobacco smoke?*];
- family history of atopic diseases (evaluated at baseline); based on the questions EC1q25: *Did your mother ever have asthma?*, EC1q26: *Did your mother ever have eczema, skin or nasal allergy or hay fever?*, EC1q27: *Did your father ever have asthma?*, EC1q28: *Did your father ever have eczema, skin or nasal allergy or hay fever?*, the following covariates were implemented:
  - family history of asthma (mother and/or father with asthma);

- family history of allergy (mother and/or father with eczema, skin or nasal allergy or hay fever);
- mother with asthma and/or eczema, skin or nasal allergy or hay fever;
- father with asthma and/or eczema, skin or nasal allergy or hay fever;
- follow-up covariates:
  - pack-years, calculated for the period between the ECRHS I and II<sup>89</sup> [EC2q74: *Have you ever smoked for as long as a year?*, EC2q74.1: *How old were you when you started smoking?*, EC2q74.2: *Do you smoke now, as of one month ago?* and all the nested questions, EC2q74.3: *Have you stopped or cut down smoking?* and all the nested questions];
  - BMI gain (kg/(m<sup>2</sup>year)), or weight gain (kg/year), during the follow up, computed as the difference between BMI/weight, measured in the ECRHS II and I, divided by the duration of the follow-up;
  - change in ETS exposure from the ECRH I, coded as unchanged, worsened, improved;
- lifetime covariates:
  - lifetime pack-years, calculated as the sum of the baseline and follow-up covariates.

### 3.6 Subjects included in the analyses

In the ECRHS I, 1348 subjects with current asthma had their lung function measured according to the ATS criteria (700 from the random and 648 from the symptomatic samples). While there were no differences in age, sex, smoking habits, FEV<sub>1</sub> and IgE levels at baseline (ECRHS I) in “random” and “symptomatic” subjects, the former had a slightly longer duration of the disease (17.8 vs 16.2 years), a lower percentage of manual workers (25% vs 35%) and of people reporting exposure to vapors, gas, dust or fumes in the workplace (44% vs 50%), a lower body mass index (BMI) (23 vs 24 kg/m<sup>2</sup>), and they included a smaller percentage of individuals with a low educational level (9% vs 15%) (p <0.01).

Of these 1348 individuals, 860 (64%) attended the second study (1999-2002), and were therefore eligible for the analyses. In the ECRHS II (1999-2002), some eligible subjects did not repeat spirometry (135 subjects), or had their lung function measured in a way that was not in conformity with the ATS criteria (32 subjects),

and were excluded from the analysis. Among those who had performed the lung function test, some had used inhaled long-acting  $\beta_2$ -agonists in the 12 hours before the test. As the bronchodilating effect of long-acting  $\beta_2$ -agonists can persist for 8-12 hours after use, they were also excluded (26 patients).

Finally, 667 subjects were included in the analyses (311 from the random and 356 from the symptomatic samples) in paper I (Appendix F).

In paper II (Appendix G), the above subjects were divided into two distinct asthma phenotypes:

- asthmatic subjects without airflow obstruction at baseline (milder asthma, n=544);
- asthmatic subjects with airflow obstruction at baseline (more severe asthma, n=94).

Accordingly, 29 subjects with baseline airflow obstruction who had  $FEV_1/FVC \geq 0.70$  at follow-up were excluded from the analyses.

On average, among the individuals included in the analyses, the follow-up time was 9 years (range: 6-11 years).



# Chapter 4

## Results

### 4.1 Inhaled steroid use and lung function decline

#### 4.1.1 Comparison between included and excluded subjects

Non-participants in the ECRHS II, or participants whose FEV<sub>1</sub> was unavailable, not in agreement with the ATS criteria or measured within 12 hours after the last administration of a long-acting  $\beta_2$ -agonist were excluded from the analyses (n=681). Subjects included in the analyses (n=667) were slightly older (mean, SD: 33.9, 7.2 vs 32.3, 7.2, p=0.0001) and comprised a lower percentage of current smokers (30% vs 39%, p=0.05) and of manual workers (29.1% vs 31.3%, p<0.0001) than subjects who were excluded.

#### 4.1.2 Use of inhaled steroids

The cumulative time of ICS use could be evaluated for 636 subjects. In detail, 297 (47%) had never been on ICSs, whereas 339 (53%) had used ICSs during the follow-up. Among ICS users, the median cumulative time of treatment was 1.4 (interquartile range, IQR: 6.3) years, with no significant difference between men and women (p=0.33). Among people who had used ICSs for  $\geq 4$  years, the median time of use was 8.2 (IQR: 2.0) years. The steroid that was most commonly used during the year before the follow-up visit (ECRHS II) was budesonide (51%), followed by beclomethasone (27%), and fluticasone (20%); and the majority of subjects used dry-powder inhalers (65%), one third used metered-dose inhalers, while only 1% used nebulizers.

### 4.1.3 Characteristics of steroid users and non-steroid users

At baseline, on average, asthmatic subjects treated with ICSs during the follow-up (table 4.1) had a worse lung function, higher levels of total IgE, a shorter duration of asthma, a greater prevalence of family asthma and of hospitalizations/emergency department visits than non-steroid users. Steroid users were more likely to be women and had a higher baseline BMI than non-steroid users, and they also comprised a lower percentage of current smokers at baseline (24% vs 36%).

Table 4.1: Baseline characteristics (and lifetime pack-years smoked) of the asthmatic subjects, stratified according to the use of ICSs during the follow-up. Data are provided as mean (standard deviation) or percentages (%), unless stated otherwise\*. Statistically significant ( $p < 0.05$ ) results are reported in bold.

	Inhaled corticosteroids		p-value
	Non-users	Users	
No. of subjects (%)	297 (46.7)	339 (53.3)	–
<b>Sex (% female)</b>	<b>45.4</b>	<b>63.7</b>	<b>&lt;0.001</b>
Age (years)	33.7 (7.1)	34.1 (7.3)	0.44
<b>BMI (kg/m<sup>2</sup>)</b>	<b>23.6 (3.6)</b>	<b>24.5 (4.9)</b>	<b>0.001</b>
<b>Smoking habits (lifetime pack-yrs)*</b>	<b>1.6 (15.6)</b>	<b>0 (10.0)</b>	<b>0.013</b>
Occupation			
(% non-manual)	47.5	49.0	
(% manual)	28.0	30.4	0.48
(% other)	24.5	20.6	
Occupational exposure (%)	47.6	48.4	0.85
<b>Duration of asthma* (yrs)</b>	<b>18.7 (19.1)</b>	<b>13.5 (17.9)</b>	<b>0.001</b>
<b>Family asthma (%)</b>	<b>19.3</b>	<b>31.1</b>	<b>0.001</b>
<b>Total IgE<sup>‡</sup> (log transformation)</b>	<b>1.84 (0.66)</b>	<b>1.96 (0.68)</b>	<b>0.033</b>
<b>Hospitalizations (%)</b>	<b>28.6</b>	<b>42.9</b>	<b>&lt;0.0001</b>
<b>FEV<sub>1</sub> (L)</b>	<b>3.7 (0.8)</b>	<b>3.2 (0.8)</b>	<b>&lt;0.0001</b>
<b>FEV<sub>1</sub> % predicted<sup>†</sup></b>	<b>101.6 (12.6)</b>	<b>95.0 (17.9)</b>	<b>&lt;0.0001</b>

\* median (IQR); p-values of non-parametric test reported;

‡ corresponding to a geometric mean of 69.6 kU/L in non-users and of 91.9 kU/L in users;

† predicted values of FEV<sub>1</sub> were calculated on the basis of sex, age and height.

#### 4.1.4 Decline in lung function

In subjects with asthma, the average decline in FEV<sub>1</sub> was 35 (95% CI: 29; 40) mL/year for men and 24 (95% CI: 20; 28) mL/year for women (figure 4.1). FEV<sub>1</sub> decline was positively associated with age (p trend=0.003), but not with baseline BMI and with lifetime exposure to active smoking (pack-years). FEV<sub>1</sub> decline was 32 (95% CI: 27; 36) mL/year and 25 (95% CI: 21; 30) mL/year for individuals exposed and not exposed to occupational risk, respectively. FEV<sub>1</sub> decline was unrelated to occupation, hospitalizations/emergency department visits, total IgE, family asthma, and asthma duration at baseline.

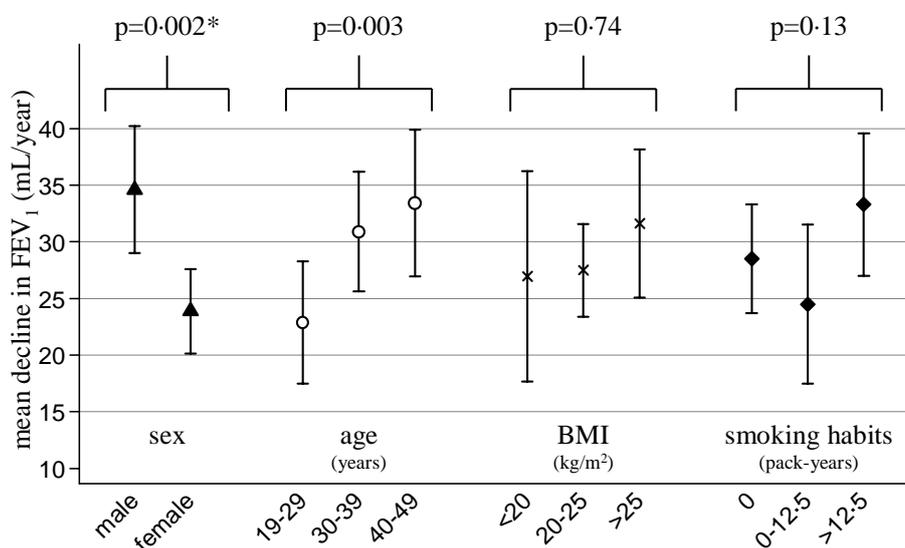


Figure 4.1: unadjusted mean annual decline in FEV<sub>1</sub> (with 95% CIs and p-value for trend) from 1991-1993 to 1999-2002, according to sex, age, and BMI at baseline, and lifetime pack-years smoked. \* Student *t* test.

FEV<sub>1</sub> decline was lower in subjects who had used ICSs for a longer time (p trend=0.025); it was 34 mL/year in non-users, 28 mL/year in subjects treated for <8.7 months, 24 mL/year in subjects treated for ≥8.7 months but <48 months, and 20 mL/year in subjects treated for ≥48 months.

When all the variables were considered simultaneously (table 4.2), the use of ICSs for ≥48 months was statistically significantly associated with a lower decline (11 mL/year, 95% CI: 0.1; 21, compared with non-users). A shorter therapy with steroids (<48 months) was also associated with a lower FEV<sub>1</sub> decline (about 4 mL/year), but it was not statistically significant. FEV<sub>1</sub> decline was greater for

older subjects (0.8 mL/year, 95% CI: 0.2; 1.4 for every additional year in subject age). None of the other covariates considered in the analysis was significantly associated with FEV<sub>1</sub> decline.

There was no statistically significant interaction between sex and ICS use, nor between lifetime pack-years smoked and ICS use, but a significant (p=0.02) interaction was found between having an elevated (>100 kU/L) level of total IgE and ICS use (figure 4.2). After adjusting for all the potential confounders, in subjects having total IgE >100 kU/L (47% of the subjects included in the analysis), the use of ICSs for ≥4 years was associated with a lower FEV<sub>1</sub> decline (23 mL/year, 95% CI: 8; 38, compared with non-users). In ICS users with elevated total IgE, the Pearson correlation coefficient between FEV<sub>1</sub> decline and total IgE was 0.07 (95% CI: -0.09; 0.22). When the analysis was repeated adjusting for bronchial hyperresponsiveness, coded as "present" (if a subject had PD<sub>20</sub> <1 mg), "absent", or "test not performed", the results were fully consistent with those presented (data not shown).

Table 4.2: multiple regression coefficients\* with 95% confidence intervals (CIs), and related p-values for the association between the mean decline in FEV<sub>1</sub> and sex, age and BMI at baseline and smoking habits and ICS use during the follow-up. Statistically significant (p<0.05) results are reported in bold.

covariate	coefficient (mL/year)	95% CI	p-value
Sex <sup>†</sup> (female)	-9.5	-20.4; 1.4	0.09
<b>Age<sup>‡</sup> (years)</b>	<b>0.8</b>	<b>0.2; 1.4</b>	<b>0.005</b>
BMI <sup>‡</sup> (kg/m <sup>2</sup> )	-0.4	-1.3; 0.5	0.40
Smoking habits <sup>‡</sup> (lifetime pack-years)	0.04	-0.2; 0.3	0.76
<b>Use of ICSs<sup>†</sup></b>			
<8.7 months	-3.9	-14.4; 6.5	0.46
8.7 months to 4 years	-4.8	-15.5; 6.0	0.38
<b>≥4 years</b>	<b>-10.7</b>	<b>-21.3; -0.1</b>	<b>0.048</b>

\* adjusted for height, occupation, exposure to occupational risk, duration of asthma, familiarity of asthma, total IgE, previous hospitalizations/emergency department visits; obtained through a two-level random intercept regression model fitted on subjects with complete information (n=511);  
<sup>†</sup> the coefficient represents the difference in the annual rate of FEV<sub>1</sub> decline (mL/year) between subjects with the characteristic and subjects belonging to the reference category (i.e.: "male" for variable sex and "no ICSs" for variable "use of ICSs");  
<sup>‡</sup> the coefficient represents the change in FEV<sub>1</sub> decline (mL/year) for a unit change in the covariate.

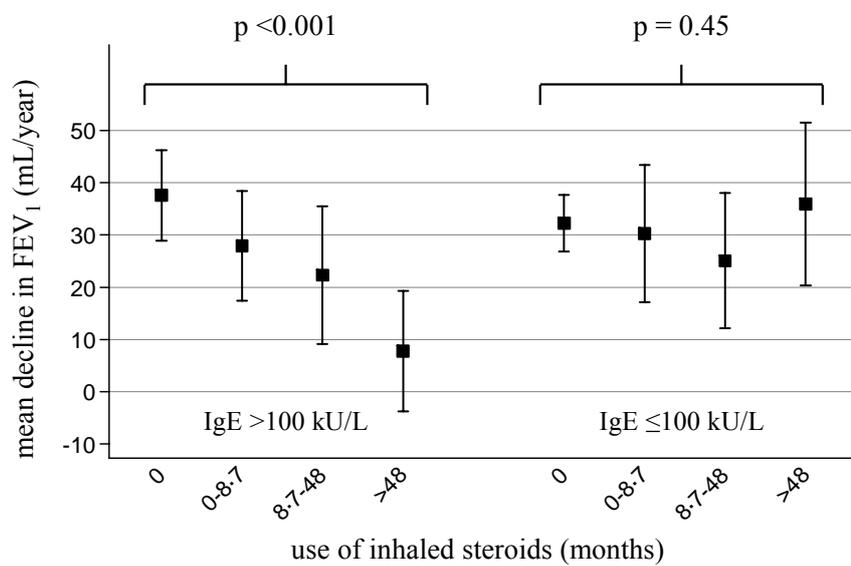


Figure 4.2: unadjusted mean annual decline in FEV<sub>1</sub> (with 95% CIs and p-value for trend), according to the level of total IgE\* and to the time of ICS use during the follow-up (non-users, 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> tertile).

\* Elevated (>100 kU/L) total IgE were present in 47% of the subjects included in the analysis.

## 4.2 Other determinants of lung function decline

### 4.2.1 Comparison between included and excluded subjects

Subjects excluded from the analyses (n=710) were younger (median age, IQR: 32, 12 vs. 34, 12 years,  $p<0.001$ ), and they were more likely to be current smokers (38% vs 30%,  $p=0.01$ ) than subjects who were included (n=638). However, the two groups were similar as regards all the other baseline covariates (including BMI: median, 23 kg/m<sup>2</sup> in both groups), as well as baseline FEV<sub>1</sub> and FVC.

### 4.2.2 Characteristics and lung function of the subjects included

Subjects with airflow obstruction at baseline (n=94, 15%) were more likely to be men (66% vs 41%,  $p<0.001$ ), to be older (median age, IQR: 36, 13 vs 33, 12 years,  $p=0.004$ ) and to have had asthma for a longer time (median duration of asthma, IQR: 22, 20 vs 15, 19,  $p<0.001$ ) than subjects without airflow obstruction (n=544, 85%). Moreover, they had a lower BMI gain during the follow-up (median, IQR: 0.15, 0.24 vs 0.18, 0.31 kg/m<sup>2</sup>year,  $p=0.01$ ). In subjects without baseline airflow obstruction, a high baseline BMI was associated with a lower baseline FEV<sub>1</sub>, both in men and in women (figure 4.3). Obese subjects (BMI range, 30 to 47 kg/m<sup>2</sup>) had a lower median FEV<sub>1</sub> than non-obese subjects (3.0 vs 3.5 L,  $p <0.001$ ), a lower median FVC (3.8 vs 4.2,  $p <0.001$ ) and a lower FEV<sub>1</sub>/FVC (0.80 vs 0.82,  $p=0.10$ ).

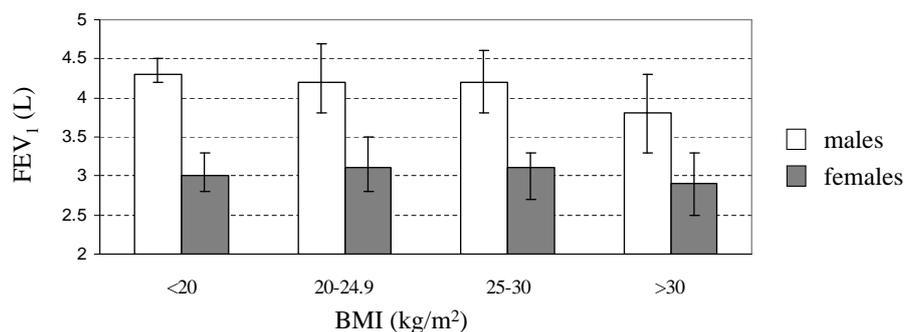


Figure 4.3: Baseline FEV<sub>1</sub> by categories of baseline BMI for men and women without airflow obstruction at baseline\*. Medians with IQRs are reported.

\* The number of subjects in the BMI categories <20, 20-24.9, 25-30, and >30 kg/m<sup>2</sup> are 12, 134, 71, 7 (men) and 51, 182, 52, 35 (women) respectively.

### 4.2.3 Determinants of lung function decline

FEV<sub>1</sub> decline and %FEV<sub>1</sub>/FVC decline were greater for subjects without airflow obstruction than for subjects with airflow obstruction (table 4.3). In asthmatic subjects without airflow obstruction at baseline, FEV<sub>1</sub> decline was associated with baseline BMI in a quadratic way (table 4.4, column 1). In other words, men and women with an intermediate BMI had the greatest FEV<sub>1</sub> decline (figure 4.4). Subjects with a high educational level had a faster FEV<sub>1</sub> decline than subjects with a low educational level (p=0.003) (table 4.4, column 1). Independently of baseline BMI, FEV<sub>1</sub> decline was 28 mL/year greater for every BMI unit (1 kg/m<sup>2</sup>) gained during 1 year of follow-up (p<0.001). Moreover, there was an interaction between sex and BMI gain (p=0.003): FEV<sub>1</sub> decline was 61.8 (95% CI: 32.0; 91.7, p<0.001) mL/year greater per BMI unit gained during 1 year of follow-up in men, and 20.2 (95% CI: 7.9; 32.6, p=0.001) mL/year greater per BMI unit gained in women (figure 4.5). This corresponds to a FEV<sub>1</sub> decline of 20.0 (95% CI: 10.4; 29.5, p<0.001) mL/year per kg gained during 1 year of follow-up in men, and to 6.9 (95% CI: 2.5; 11.4, p=0.002) mL/year per kg gained in women. In asthmatic subjects with airflow obstruction at baseline, the absence of allergen sensitization (p=0.035) and a lower BMI (p=0.008) at baseline were both associated with a faster FEV<sub>1</sub> decline (table 4.4, column 2). No significant association was found between exposure to active or passive smoking and FEV<sub>1</sub> decline, either in subjects with or in subjects without airflow obstruction.

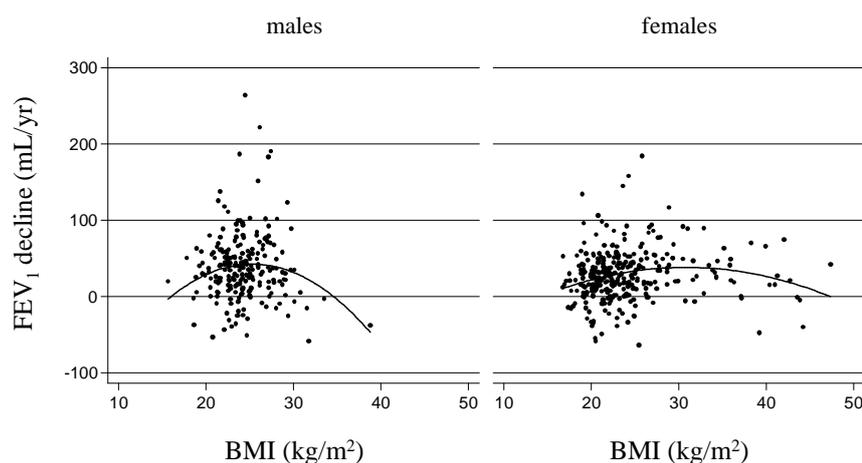


Figure 4.4: Plot of FEV<sub>1</sub> decline vs baseline BMI, and of the curve representing the FEV<sub>1</sub> decline predicted on the basis of a linear regression on BMI and BMI squared, in men and women without airflow obstruction at baseline.

Table 4.3: Baseline lung function and lung function decline of the asthmatic subjects, stratified by the presence of airflow obstruction at baseline. Data are provided as means (with 95% CIs). Statistically significant ( $p < 0.05$ ) results are reported in bold.

	Airflow obstruction at baseline		p-value
	No	Yes	
<b>FEV<sub>1</sub> % predicted (%)</b>	<b>102 (101; 103)</b>	<b>78 (75; 82)</b>	<b>&lt;0.001</b>
FVC % predicted (%)	107 (106; 108)	106 (102; 110)	0.97
<b>FEV<sub>1</sub>* (L)</b>	<b>3.6 (3.5; 3.6)</b>	<b>2.7 (2.6; 2.8)</b>	<b>&lt;0.001</b>
FVC* (L)	4.4 (4.4; 4.5)	4.4 (4.3; 4.5)	0.65
FEV <sub>1</sub> /FVC <sup>†</sup>	0.82	0.63	–
<b>FEV<sub>1</sub> decline* (mL/year)</b>	<b>33 (28; 37)</b>	<b>23 (14; 32)</b>	<b>0.04</b>
FVC decline* (mL/year)	22 (15; 29)	26 (15; 38)	0.43
<b>%FEV<sub>1</sub>/FVC decline<sup>†</sup> (%/year)</b>	<b>0.35 (0.29; 0.41)</b>	<b>0.16 (0.03; 0.29)</b>	<b>0.008</b>

\* adjusted for sex, age and height;

† adjusted for sex and age.

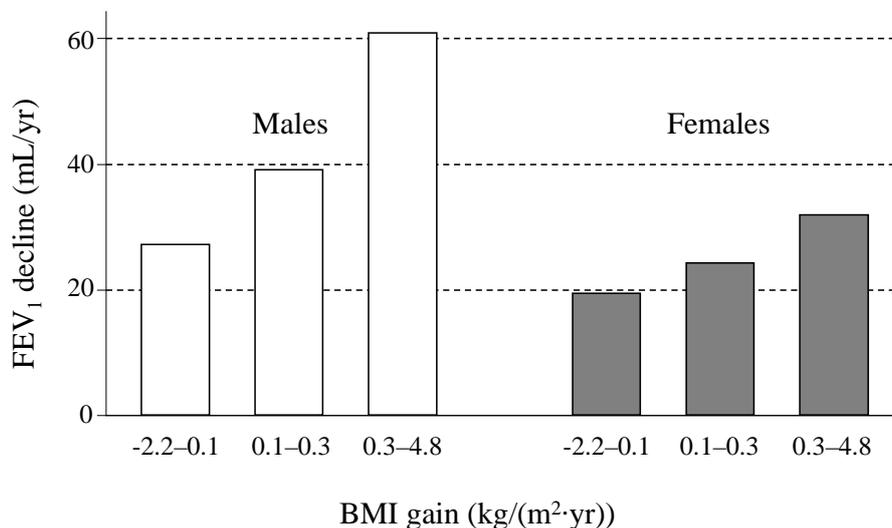


Figure 4.5: Mean FEV<sub>1</sub> decline\* by categories of BMI gain (according to tertiles) for men and women without airflow obstruction at baseline. P-value for trend:  $p < 0.001$  (men) and  $p = 0.001$  (women).

\* adjusted for all the covariates considered in the multivariate analysis.

Table 4.4: Multiple regression coefficients\* with 95% confidence intervals (CIs) for the association between FEV<sub>1</sub> decline and the covariates indicated, in asthmatic subjects stratified by the presence of airflow obstruction at baseline. Statistically significant results (p<0.05) are reported in bold.

	<b>Airflow obstruction at baseline</b>	
	<b>No (n=371)<sup>†</sup></b> estimate <sup>††</sup> (95% CI) (mL/year)	<b>Yes (n=76)<sup>†</sup></b> estimate <sup>††</sup> (95% CI) (mL/year)
<i>Baseline covariates</i>		
BMI (kg/m <sup>2</sup> )	<b>11.8 (5.4; 18.2)<sup>‡</sup></b>	<b>-4.6 (-8.1; -1.2)<sup>‡</sup></b>
BMI <sup>2</sup> ((kg/m <sup>2</sup> ) <sup>2</sup> )	<b>-0.2 (-0.3; -0.09)<sup>‡</sup></b>	-
Low educational level	<b>-18.7 (-30.8; -6.5)<sup>‡</sup></b>	20.2 (-15.2; 55.6)
Pack-years (1-unit increase)	-0.2 (-0.6; 0.3)	-0.7 (-2.2; 0.8)
Exposure to ETS	5.9 (-4.4; 16.2)	-29.3 (-62.2; 3.6)
Sensitization to any allergen	-4.0 (-13.1; 5.0)	<b>-29.2 (-56.3; -2.1)</b>
Duration of asthma (years)	-0.3 (-0.7; 0.01)	-0.4 (-1.5; 0.7)
Family asthma	-0.8 (-9.1; 7.6)	-21.4 (-47.8; 5.0)
<i>Follow-up covariates</i>		
BMI gain (kg/(m <sup>2</sup> ·year))	<b>28.1 (15.1; 41.1)<sup>‡</sup></b>	-7.4 (-51.4; 36.6)
Pack-years between surveys (1-unit increase)	-0.2 (-1.3; 1.0)	3.7 (-0.6; 8.0)
Change in ETS		
worsened vs. unchanged	-3.9 (-20.4; 12.6)	26.2 (-23.5; 75.8)
improved vs. unchanged	-6.8 (-17.9; 4.2)	13.7 (-22.6; 50.0)
<i>Intercept**</i>	38.9 (27.2; 50.6) <sup>‡</sup>	77.1 (44.9; 109.3) <sup>‡</sup>

\* adjusted also for sex, age, height, and for the interaction between total IgE and ICS use;

† number of subjects with complete information;

‡ p<0.01;

\*\* quantitative variables were centered around their mean to calculate the intercepts; the intercept represents the value of FEV<sub>1</sub> decline when all categorical variables are set to the reference category (e.g. sex = "male") and all the quantitative variables are set to their mean;

†† the estimate for different variables represents the difference in the annual decline in FEV<sub>1</sub> between those with and those without these characteristics (e.g. subjects without airflow obstruction at baseline who gained 1 BMI unit per year of follow-up had an additional decline in FEV<sub>1</sub> of 28.1 ml/year compared to those who did not gain weight).

### 4.3 Family history of atopic diseases and lung function decline

There were no statistically significant differences in the distribution of the family history of asthma and/or other allergic diseases in subjects without and with airflow obstruction at baseline (Table 4.5). Approximately 25% of the subjects included in the analysis had a family history of asthma, and 50% had a family history of eczema, skin or nasal allergy or hay fever.

No association was found between a family history of asthma and/or allergic diseases and the FEV<sub>1</sub> decline, either in subjects with or in subjects without airflow obstruction at baseline (Table 4.6).

Table 4.5: Family history of asthma and/or other allergic diseases, in asthmatic subjects stratified by the presence of airflow obstruction at baseline.

	Airflow obstruction at baseline		p-value
	No (n=544)	Yes (n=94)	
one/both parents with asthma	132 (26.1%)	23 (25.6%)	0.92
one/both parents with allergy	243 (49.2%)	43 (51.8%)	0.66
mother with asthma/allergy	174 (35.2%)	32 (37.6%)	0.66
father with asthma/allergy	150 (31.3%)	25 (29.8%)	0.78

Table 4.6: Regression coefficients\* with 95% confidence intervals (CIs) for the association between FEV<sub>1</sub> decline and family history of asthma and other allergic diseases, in asthmatic subjects stratified by the presence of airflow obstruction at baseline.

	Airflow obstruction at baseline	
	No (n=544) estimate (95% CI) (mL/year)	Yes (n=94) estimate (95% CI) (mL/year)
one/both parents with asthma	5.6 (-2.1; 13.3)	-13.3 (-38.3; 11.6)
one/both parents with allergy	-1.5 (-8.5; 5.5)	-4.4 (-25.4; 16.6)
mother with asthma/allergy	-2.1 (-9.3; 5.1)	-2.2 (-24.3; 19.9)
father with asthma/allergy	3.3 (-4.3; 10.9)	-12.5 (-36.0; 10.9)

\* adjusted for sex, age, and height.

# Chapter 5

## Discussion

### 5.1 Inhaled steroid use and lung function decline

In a large community-based sample of subjects with asthma from the general population, followed up for 9 years, we have shown that a lower decline in FEV<sub>1</sub> was associated with a prolonged use of inhaled steroids. This association was only observed in those with elevated total IgE levels. There was a dose-response relationship, with lower decline in those who had used inhaled steroids for the longest periods. The association was found in men and in women, as well as in smokers and in nonsmokers.

In the whole cohort, the average FEV<sub>1</sub> decline was 11 mL/y lower in subjects with asthma who reported that they had used ICSs for 4 years or more during the follow-up, compared with nonusers. The current finding is in line both with clinical trials<sup>80,81,120–122</sup>, and with 2 recent observational studies.<sup>85,86</sup> Our estimate of the prevented FEV<sub>1</sub> decline that was potentially attributable to ICSs (11 mL/y; 95% CI, 0.1; 21) is somewhat smaller than that reported in the study by Lange et al<sup>85</sup> (18 mL/y). This may be a result of the relatively young age of our cohort. In fact, the rate of lung function decline increases with age, as supported by our results (Table 4.2) and as previously reported<sup>106,123</sup>; accordingly, one could expect a greater benefit from ICSs in patients whose FEV<sub>1</sub> is declining more steeply.

#### 5.1.1 Interaction between total IgE and ICS use

An intriguing and original finding of our analysis was that a lower decline in lung function in long-term steroid users was observed only in subjects with asthma with elevated (>100 kU/L) total serum IgE levels, whereas subjects with low IgE seemed to be less responsive. There was no correlation between lung function

decline and total IgE in ICS users with elevated total IgE, supporting the idea that the association between lower FEV<sub>1</sub> decline and ICS use was an all-or-none association.

High serum total IgE levels are strongly associated with asthma, independently of specific allergic sensitization,<sup>124</sup> with disease severity,<sup>125</sup> and are a major contributing factor for the development of bronchial hyperresponsiveness in both allergic and nonallergic asthma.<sup>124</sup> Elevated total serum IgE levels reflect the nature of the underlying airway inflammation, and are associated with a higher eosinophil count and higher eosinophil cationic protein (a marker of eosinophil activation) in the blood.<sup>126</sup> Recently, anti-IgE treatment has been shown to be associated with significant and profound reductions in tissue eosinophils and in mast cells, as well as T cells and B cells, suggesting that blocking IgE may inhibit more chronic aspects of allergic inflammation.<sup>127,128</sup> Furthermore, total serum IgE is an independent predictor of immediate treatment response to ICSs,<sup>129</sup> and a decrease in total serum IgE is significantly correlated with an improvement in asthma symptom scores.<sup>130</sup>

We speculate that the lower decline of lung function in long-term ICS users with asthma is mainly a result of the effect of steroids on the eosinophils. These cells produce cytokines, chemokines, lipid mediators, and growth factors, and can also cause an increase in mucus production, resulting in subepithelial fibrosis and in an alteration of airway smooth muscle contractility.<sup>22</sup> Eosinophils are thought to be key effector cells contributing to both airway inflammation and remodeling in asthma.<sup>22</sup> Steroids are able to inhibit the mediator release from eosinophils, to decrease the eosinophil count by inducing apoptosis,<sup>131,132</sup> and to attenuate the levels of eosinophil progenitors in peripheral blood and, to some extent, in bone marrow.<sup>22,131,133–135</sup>

Furthermore, in subjects having low IgE levels, inflammation could be predominantly neutrophilic,<sup>134</sup> and it has been shown that neutrophils are not sensitive to the effect of steroids. Indeed, systemic steroids increase peripheral neutrophil counts, a fact that may reflect an increased survival time because of an inhibitory action on neutrophil apoptosis.<sup>136</sup>

### 5.1.2 Sex and smoking habits

In agreement with Lange et al,<sup>85</sup> but not with Dijkstra et al,<sup>86</sup> we found no evidence that the association between ICS use and lung function decline was different in men and in women.

The lack of association between smoking and lung function deterioration was

somewhat surprising. One possible explanation could be the young age of our cohort. Also, one could speculate that this finding could reflect the healthy smoker effect (only subjects with asthma with relatively good lung function at baseline smoke or start smoking, whereas subjects with more severe asthma refrain from smoking).

Previous studies have found that smoking interferes with the long-term beneficial effects of steroids.<sup>86,122,137,138</sup> The lack of interaction between smoking habits and ICS use found in our study probably reflects that the group of subjects selected for our analysis does not allow a full appreciation of the effect of smoking on lung function decline, and hence on its potential modification on the effect of ICSs.

### 5.1.3 Potential limitations

In evaluating the association between the long-term use of ICSs and the average decline in FEV<sub>1</sub>, we adjusted for different characteristics of the subjects (age, height, BMI, different hazardous exposures), as well as for variables related to asthma (duration and familiarity) and its severity (previous hospitalizations and/or emergency department visits, total IgE levels). Nevertheless, our results may be affected by some bias, given that the allocation of subjects to ICS treatment was not based on randomization but rather on indication. ICS users may differ from nonusers in some aspects that have not been measured. For example, subjects who get access to and regularly take prescribed medications may be more likely to adopt generally healthier lifestyles (such as healthy diet and exercise). High dietary intake of antioxidants has been associated with lung function,<sup>139</sup> but we have no information on this for our sample. On the other hand, one might expect subjects with more severe disease to be prescribed more medication. At baseline, the treated group had poorer lung function, higher rates of hospitalization for respiratory diseases, and higher prevalence of family asthma than the untreated group. Therefore, we might expect them to have a steeper decrease in lung function than subjects who did not receive treatment (the horse-racing effect).<sup>140,141</sup> If this is true, we have underestimated the association between ICS use and FEV<sub>1</sub> decline.

A potential limitation of the current study is that a self-reported doctor diagnosis of asthma was used to identify individuals with asthma. Although this definition may be open to some degree of misclassification, it has been proven to be highly specific,<sup>142</sup> so that only milder or undiagnosed asthmatics would not have been included in our study.

### 5.1.4 Points of strength

Compared with other recent longitudinal studies investigating the association between ICSs and lung function decline,<sup>85,86</sup> our study has some points of strength. First, the ECRHS sample was selected from the general population in an international setting. Second, in the current study, a cumulative time of ICS use was estimated for each individual in the follow-up period. Finally, the individuals who had used inhaled long-acting  $\beta_2$ -agonists in the 12 hours before lung function testing were excluded, thus reducing the risk of a bias because of the residual bronchodilating effect of these drugs.

### 5.1.5 Conclusion

In conclusion, the longitudinal analysis of the decline of lung function in a large, international, population based sample of subjects with asthma supports the hypothesis that the long-term use of ICSs might prevent the deterioration of lung function in individuals having elevated levels of total serum IgE, whereas lung function could be less influenced by steroids in subjects with lower levels of IgE.

This finding is not to be interpreted as an advise to prescribe ICSs only in subjects with asthma and elevated IgE levels. In fact, we investigated only the effect of a prolonged use of ICSs on lung function deterioration, whereas ICSs have been clearly shown to have favorable effects on several short-term outcomes. Moreover, a long-term use of ICSs has been demonstrated to reduce the risk of both asthma-related death<sup>82</sup> and hospitalization.<sup>143</sup> Our findings underline the importance of total IgE as a feature of asthma, not only because it helps to predict its severity<sup>125</sup> and prognosis, but also because it might influence decisions on longterm anti-inflammatory treatment. Further investigations are needed to clarify whether calibrating the corticosteroid dose according to the level of IgE is a feasible approach in asthma management.

## 5.2 Other determinants of lung function decline

In the group of individuals without airflow obstruction at baseline, a faster FEV<sub>1</sub> decline was observed in subjects whose BMI was intermediate than for lean and obese subjects. FEV<sub>1</sub> decline was associated with BMI gain independently of baseline BMI, and this association was stronger in men than in women. In the group of individuals with airflow obstruction at baseline, weight gain was not associated with decline.

The analyses were stratified by the presence of airflow obstruction at baseline to account for the heterogeneity of asthma phenotypes.<sup>144</sup> As a bronchodilator challenge test was not part of the ECRHS core protocol, we used the follow-up spirometry to confirm baseline airflow obstruction. The stratification by airflow obstruction at baseline seems to be supported a posteriori by our findings, as the role played by BMI and weight gain in FEV<sub>1</sub> decline was different in the 2 groups. Furthermore, a joint (subjects with + subjects without airflow obstruction at baseline) post-hoc analysis showed a significant interaction between BMI gain and the presence of airflow obstruction (p=0.001).

Compared to subjects with airflow obstruction, subjects without airflow obstruction had a faster FEV<sub>1</sub> decline and %FEV<sub>1</sub>/FVC decline during the follow-up. This may be due to the fact that a steep decline had already occurred before the first visit in subjects with airflow obstruction, as suggested by their lower baseline FEV<sub>1</sub>.

### **5.2.1 Baseline BMI and lung function decline in subjects without airflow obstruction at baseline**

A high baseline BMI was associated with a lower baseline FEV<sub>1</sub>, but not with a faster FEV<sub>1</sub> decline during the follow-up. Several studies have reported that obesity decreases lung volumes. In fact, the adipose tissue increases chest wall loading.<sup>90,91,145,146</sup> This would also explain the concomitant FVC decline observed in the obese (BMI>30 kg/m<sup>2</sup>). However, no evident restrictive pattern (defined by BMI≥30 kg/m<sup>2</sup> and FEV<sub>1</sub>/FVC>0.70 and FVC<80% predicted)<sup>98</sup> was found at baseline in any of the subjects.

In the absence of a weight reduction, there is no evident reason why the lung function decline in obese subjects should be blunted with respect to non obese subjects. However, asthma in the obese may be different from asthma in normal weight subjects. Obesity causes lower than normal functional residual capacity, with the consequence of unloading the airway smooth muscle and allowing it to shorten excessively when activated,<sup>91,92</sup> and a decrease in operational lung volume.<sup>147</sup> Moreover, breathing at lower tidal volumes inhibits the bronchodilating effect of tidal strain on the airway smooth muscle.<sup>91,93</sup> These mechanical and functional changes within the respiratory system may explain the observed link between obesity and bronchial asthma.<sup>148</sup> Our data support the hypothesis that asthma in the obese is less determined by inflammation than in the non-obese, in agreement with the reversible nature of lung function decline observed with

weight loss.<sup>149</sup> This hypothesis, if true, could lead to the implication that, in obese subjects with uncontrolled asthma, weight loss should be primarily targeted, and that it may be worth assessing the level of bronchial inflammation before increasing inhaled steroid doses. However, the faster FEV<sub>1</sub> decline seen in the non-obese compared to the obese could be due to the fact that obese subjects had a lower baseline FEV<sub>1</sub>, and that the process of decline may no longer be progressive after the FEV<sub>1</sub> had previously dropped to a considerable extent.

As subjects included and excluded from our analyses had a similar BMI, a selection bias in the obese subgroup, i.e. a lower participation rate among obese people with poor lung function, is not likely. Obese subjects tend to report doctor-diagnosed asthma, as well as asthma-like symptoms and use of bronchodilators, more frequently than non-obese subjects, even if they do not have an objective impairment of lung function.<sup>150</sup> In view of our epidemiological definition of asthma, one could hypothesize that asthma has been over-diagnosed in the obese subgroup. However, for subjects with BMI <20, 20-24.9, 25-30 and >30 kg/m<sup>2</sup>, the prevalences of family asthma were 15%, 26%, 29%, and 35% (p=0.11), respectively. Thus, the above explanation seems unconvincing.

## **5.2.2 BMI gain and lung function decline in subjects without airflow obstruction at baseline**

In asthmatic subjects without airflow obstruction, we found that BMI gain during the follow-up was associated with FEV<sub>1</sub> decline, and this association was stronger in men than in women. In men FEV<sub>1</sub> dropped by 20 (95% CI: 10; 30) mL/year for every kg gained during the follow-up, while in women FEV<sub>1</sub> decreased by 7 (95% CI: 2; 11) mL/year for every kg gained. This might be explained by the fact that men who gain weight tend to accumulate abdominal and visceral fat, while women who gain weight tend to accumulate peripheral fat.<sup>151</sup> Chinn et al<sup>89</sup> found that the FEV<sub>1</sub> decline in the general population of the ECRHS was about 40% lower than the decline we found in the asthmatic subjects. Therefore, one could speculate that the negative effect of weight gain on lung function is greater in subjects with asthma than in people from the general population.

The observed association between BMI gain and FEV<sub>1</sub> decline was independent of baseline BMI. This is also confirmed when we consider the group of subjects who were obese at baseline (n=42). Among them, 31 individuals gained weight during the follow-up (median BMI gain, [range]: 0.5, [0.04; 3.0] kg/m<sup>2</sup>) and 11 individuals decreased their weight (-0.2, [-2.2; -0.05] kg/m<sup>2</sup>). FEV<sub>1</sub> decline was

faster in the obese subjects who gained weight (median, IQR: 26, 48 mL/year) than in the obese subjects who decreased their weight (median, IQR: 5, 72 mL/year) ( $p=0.13$ ).

### **5.2.3 Educational level and lung function decline in subjects without airflow obstruction at baseline**

In the non-asthmatic population, a low socio-economic level is almost universally associated with worse health outcomes, including lung function.<sup>152</sup> In our data, asthmatic subjects without airflow obstruction who had a high educational level had a faster FEV<sub>1</sub> decline than those with a lower educational level. However, the latter had a low FEV<sub>1</sub> already at baseline (median FEV<sub>1</sub>: 3.1 vs 3.5 L). It is possible that a low educational level was associated with an early drop of FEV<sub>1</sub> and that, subsequently, the process of FEV<sub>1</sub> decline was no longer progressive.

### **5.2.4 Active smoking, passive smoking and lung function decline in subjects with/without airflow obstruction at baseline**

Many,<sup>19,96,97</sup> but not all,<sup>99</sup> studies have found that asthmatic smokers have a steeper decline in lung function than asthmatic non-smokers. Several factors could explain the lack of an association that was observed in our study. Firstly, our subjects were relatively young, and the detrimental effect of smoking could be greater at older ages.<sup>96</sup> Secondly, the exposure to active smoking of our cohort was relatively low. In the Copenhagen City Heart Study, about 70% of the asthmatic subjects were smokers,<sup>19</sup> and they had been exposed to smoking for more than 30 years on average.<sup>153</sup> In our study, only 30% were current smokers, and the exposure to active smoking tended to diminish significantly during the follow-up.<sup>154</sup> Finally, our finding could reflect the healthy smoker effect.

Passive smoking was found to be associated with lung function in several studies.<sup>102,103</sup> However, passive smoking was not related to FEV<sub>1</sub> decline in our cohort.

### **5.2.5 Determinants of lung function decline in subjects with airflow obstruction at baseline**

Among asthmatic subjects with airflow obstruction at baseline, those who were not sensitized to allergens had on average 29 mL/year more decline than those who were sensitized to allergens. This is in line with the finding that the rate of lung function decline is greater in subjects with intrinsic asthma than in those with extrinsic asthma,<sup>99</sup> and with the observation that inhaled steroids may be more effective in contrasting FEV<sub>1</sub> decline in asthmatic subjects with high levels of total IgE than in those with lower total IgE (see Section 4.1).

A 1-unit decrease in baseline BMI was associated with a 5 mL/year decrease in FEV<sub>1</sub> during the follow-up. In this group of subjects with airflow obstruction at baseline, leanness itself may be a marker of an early form of COPD.<sup>155</sup> No association was found between FEV<sub>1</sub> decline and BMI gain, probably because mechanisms that are typical of milder asthma (including the effect of weight gain) could be less important in severe asthma, while a serious long-lasting inflammation may play a crucial role. Moreover, subjects with airflow obstruction gained on average less weight than those without.

### **5.2.6 Conclusion**

In European asthmatic subjects without airflow obstruction at baseline, individuals with a high BMI had the lowest FEV<sub>1</sub> at baseline. Weight gain was positively associated with FEV<sub>1</sub> decline, independently of baseline BMI, and this association was stronger in men than in women. The detrimental effect of BMI gain on lung function might be greater for subjects with asthma than for subjects without asthma, but studies are needed to clarify this. Among asthmatic subjects with airflow obstruction at baseline, lean subjects without sensitization to allergens had the greatest FEV<sub>1</sub> decline, while weight gain was not associated with FEV<sub>1</sub> decline.

## **5.3 Family history of allergic diseases and lung function decline**

A family history of asthma is known to be a strong risk factor for asthma.<sup>25</sup> Some studies also found that a family history of atopic diseases other than asthma is a predictor of asthma risk, but results are inconsistent.<sup>25</sup>

The increased risk of developing asthma found in children whose parents have asthma may be due to the fact that they share some susceptibility genes for the disease. However, family history is also a proxy of a shared environment. In fact, one would expect that many of the environmental exposures experienced during childhood, and of potential importance for the occurrence of asthma, are shared by siblings who grow up in the same family environment. Such exposures include, for example, air pollution, number of siblings, parental smoking, mites, domestic pets, indoor air exposures, and factors associated with parental socio-economic status. However, the genetic resemblance of children to their parents seems to be the more likely explanation for the increased risk of asthma. In fact, studies on twins have shown that asthma risk to co-twins of affected twins is significantly greater for monozygotic than for dizygotic pairs.<sup>156</sup>

In our study, there was no association between a family history of asthma or other allergic diseases and lung function decline, neither in subjects with nor in subjects without baseline airflow obstruction. To our knowledge, no studies have investigated the association between a family history of asthma or allergy and lung function decline. The most obvious reason for the lack of an association is that parental asthma or allergy are not involved in the course of the disease. The genes implied in asthma susceptibility may be different from those implied in lung function decline. As an example, it is known that SNPs on the ADAM33 gene are also involved in accelerated lung function decline in the non-asthmatic population.<sup>64</sup> It may be argued that family asthma is too rough an indicator of asthma susceptibility in our population. However, this explanation seems to be unlikely, as the same indicator was confirmed to be a risk factor for asthma incidence in the general population of the ECRHS.<sup>157</sup>



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# **Appendix A**

## **ECRHS II Lung function protocol and questionnaire**



# THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY II



## ECRHS II

### LUNG FUNCTION PROTOCOL, DATA SHEETS AND LUNG FUNCTION QUESTIONNAIRE

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# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## LUNG FUNCTION TESTS

### CRITERIA FOR TESTING

#### **Criteria for baseline spirometry**

The purpose of baseline spirometry is to record an accurate Forced Expiratory Volume in one second (FEV<sub>1</sub>) and Forced Vital Capacity (FVC) from every subject who attends the testing centre.

#### ACCEPTANCE CRITERIA:

Any subject who is able to attend the testing centre.

#### EXCLUSION CRITERIA:

*If the subject smokes:* Lung function testing should be carried out at least one hour after the last cigarette has been smoked.

*If the subject has used an inhaler:* Lung function testing should be carried out at least one hour after the use of any inhaler.

*If the subject has used an inhaler that is not a beta-2-agonist or an anticholinergic inhaler in the last one to four hours:* Lung function testing is carried out and the data recorded.

*If the subject has used an inhaler that is a long acting Beta-2-agonist in the last 8 hours:* If the subject is willing to come back at another time when they have not taken their long acting Beta-2-agonist, another appointment should be made. HOWEVER – this may be difficult for them to do, in which case, testing should proceed and medication used should be recorded.

*If the subject has used an inhaler that is a beta-2-agonist or an anticholinergic inhaler in the last one to four hours:* If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used should be recorded.

*If the subject has taken an oral beta-2-agonist or an oral theophylline or an oral antimuscarinic within the last eight hours:* If the subject is willing to come back another time for lung function testing, another appointment should be made. If the subject is unable or reluctant to return another time, testing should proceed and the medication used recorded.

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*If the subject has had a respiratory tract infection in the last three weeks:* Another appointment should be made unless the subject is unwilling to come back, in which case testing should continue. The number of days elapsed since the end of the respiratory infection should be recorded.

If, after a total of nine attempts, a subject is unable to produce a technically satisfactory manoeuvre, no FEV<sub>1</sub> or FVC will be recorded.

### **Predicted FEV<sub>1</sub> values**

Normal FEV<sub>1</sub> values will be calculated using the following equations:

Males:  $4.30 H - 0.029 A - 2.49$

Females:  $3.95 H - 0.025 A - 2.60$

where

H = height in metres

A = age in years (range 25-44).

These equations are only valid for subjects over the age of 25. Subjects aged 20-24 should have their expected FEV<sub>1</sub> calculated as if their age is 25.

### **Criteria for methacholine challenge**

The aim of methacholine challenge is for subjects to inhale increasing concentrations of methacholine solutions and to monitor any change in FEV<sub>1</sub> by repeated spirometric testing.

ACCEPTANCE CRITERIA: Any subject who fulfils all three of the following criteria is accepted:

- 1) has been able to perform at least 2 technically satisfactory manoeuvres during baseline spirometry
- 2) has signed a consent form for methacholine challenge,
- 3) is not in the categories for exclusion (see below).

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**EXCLUSION CRITERIA:** Any subject who fulfils any one of the following criteria is excluded from methacholine challenge:

- 1) has had a heart attack in the last three months,
- 2) has any heart disease for which he/she is taking medication,
- 3) has epilepsy for which he/she is taking medication,
- 4) is pregnant,
- 5) is breast feeding,
- 6) is taking a beta-blocker for any reason (including eye drops).

These criteria will be assessed by the Lung Function Questionnaire.

In addition, any subject who fulfils either of the following is excluded:

- 7) has an FEV<sub>1</sub> less than 70% of the predicted value,
- 8) has an FEV<sub>1</sub> less than 1.5 litres.

FEV<sub>1</sub> is the maximum assessed during the baseline spirometry.

### **Criteria for bronchodilator challenge**

The FEV<sub>1</sub> and FVC will be measured following the administration of 400ug salbutamol by metered dose inhaler (MDI) via a Volumatic spacer.

**ACCEPTANCE CRITERIA:** Any subject who fulfils all of the following criteria is accepted:

- 1) has produced technically satisfactory FEV<sub>1</sub> and FVC manoeuvres,
- 2) refuses to undergo or is excluded from methacholine challenge,
- 3) has signed a consent form for bronchodilator challenge,
- 4) is not excluded by the following exclusion criteria.

**EXCLUSION CRITERIA:** Any subject who fulfils any one of the following criteria is excluded:

- 1) has had a heart attack in the last three months,
- 2) has any heart disease for which he/she is taking medication,
- 3) has epilepsy for which he/she is taking medication,
- 4) is pregnant,
- 5) is breast feeding,
- 6) is taking a beta-blocker for any reason (including eye drops).

These conditions will be assessed by the Lung Function Questionnaire.

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## **Making the appointment for testing**

*Ideally, lung function testing should be performed:*

- 1) more than four hours after the use of a beta-2-agonist or anticholinergic inhaler,
- 2) more than eight hours after inhaled long acting beta-2-agonist, oral beta-2-agonist or theophylline or oral antimuscarinic.

*When the appointment for lung function testing is made the fieldworker should determine if the subject is taking any of the following medications:*

- 1) beta-2-agonist inhaler (short or long acting),
- 2) anticholinergic inhaler,
- 3) oral beta-2-agonist,
- 4) oral theophylline,
- 5) oral antimuscarinic.

If the subject is taking any of these medications (or any other inhaler) an appointment time should be agreed that will cause the least disruption to the subject's normal dosing schedule.

*One simple way of ensuring compliance with these instructions is to:*

- 1) avoid early morning appointments for those using inhalers,
- 2) fix a time for an appointment and then ask the subject to take their inhalers four hours before and oral medication eight hours before testing. Ask them to avoid taking their long acting beta-2-agonist if possible.

The fieldworker should ensure that the subject has not had a respiratory tract infection in the three weeks prior to testing and should advise the subject not to smoke for one hour prior to coming to the testing centre. A letter should be sent to the subject explaining this.

## **Subjects who have not followed guidelines**

Those who have had a cigarette in the last hour should have the lung function test delayed until one hour has elapsed. (Most subjects will be in the centre for at least one hour.)

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*Those who have had an inhaler in the last four hours or oral medication (or long acting beta-2-agonist) in the last eight hours may fall into one or more of the following categories:*

- 1) misunderstood the instructions,
- 2) forgot the instructions,
- 3) ignored the instructions,
- 4) may have symptoms too severe to follow the instructions.

Lung function testing may still be carried out unless the subject is excluded for other reasons, and recent medication should be noted in the Lung Function Questionnaire.

### THE FORCED EXPIRATORY MANOEUVRE

#### **General guidelines**

*All forced expiratory manoeuvres will be performed:*

- 1) sitting, legs uncrossed
- 2) with noseclip on,
- 3) using a plastic or cardboard mouthpiece without teethgrips,
- 4) tight clothing should be loosened.

*Two types of forced expiratory manoeuvre will be used in this protocol:*

- 1) During baseline spirometry and bronchodilator challenge FVC will be measured and all subjects must exhale fully.
- 2) During methacholine challenge only the FEV<sub>1</sub> needs to be recorded and the technician may interrupt the exhalation when this has been achieved.

*A technically unsatisfactory manoeuvre (FEV<sub>1</sub> or FVC) is defined as:*

- 1) an unsatisfactory start of expiration characterised by excessive hesitation or false start
- 2) coughing during the first second of the manoeuvre, thereby affecting the measured FEV<sub>1</sub> value, or any cough that interferes with the accurate measurement of FVC
- 3) Valsalva Manoeuvre (glottis closure)
- 4) A leak in the system or around the mouthpiece
- 5) An obstructed mouthpiece, e.g. the tongue in front of the mouthpiece.

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Manoeuvres which have these faults are technically unsatisfactory and are rejected as failed attempts.

*Evidence of poor compliance is shown by:*

- 1) greater than 200ml (NB in ERCHS I this was 5%, this has been changed in line with current ATS criteria) variation in FEV<sub>1</sub> between blows
- 2) greater than 150 mL or 5% FVC back-extrapolated volume
- 3) peak expiratory flow that is less than 85% of the best record
- 4) expiratory time that is less than six seconds

If these features are noted technicians should encourage the subject to produce a better reading but the blows should not be excluded as failed attempts on these criteria alone.

A manoeuvre may only be rejected as a failed attempt if it is 'technically unsatisfactory'. Manoeuvres with evidence of 'poor compliance' only should not be rejected.

*The above protocol is consistent with current ATS guidelines (Am J Respir Crit Care Med 1995;152:1107-1136). These state that 'The only criterion for unacceptable performance is fewer than two acceptable curves. No spirogram should be rejected solely on the basis of its poor reproducibility.....elimination of data from subjects who fail to meet ATS reproducibility criteria may result in population bias by excluding subjects who have abnormal lung function'*

### **Instructions to subjects**

Some of the subjects will never have used any form of lung function testing equipment before and others will be very familiar with it.

Technicians should explain to the subject that the aim of the test is to find out how much air can be blown out of the lungs and how forcefully it can be blown out.

*This can be done by asking the subject to follow these steps:*

- 1) Take in as deep breath as possible when full-
- 2) Place the mouthpiece in his/her mouth.
- 3) Close his/her lips tightly around the mouthpiece.
- 4) Blast or blow through the mouthpiece into the spirometer, blowing air out as hard, fast, smoothly and completely as possible.

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The subject should continue to push out air actively for as long as possible (FVC manoeuvre) or until the technician tells him/her to stop (FEV<sub>1</sub> manoeuvre). During this time the technician must offer positive encouragement to push or squeeze out more air.

### **Baseline spirometry**

- 1) Ensure that it is appropriate to perform lung function testing.
- 2) Demonstrate the manoeuvre to all subjects at least once (more often if he/she appears uncertain).
- 3) Ask the subject to carry out five FVC manoeuvres.
- 4) Record the FEV<sub>1</sub> and FVC and Peak Expiratory Flow (in litres per second) from at least two and up to five technically satisfactory manoeuvres.
- 5) If the subject has failed to produce two technically satisfactory manoeuvres after five attempts, the technician should show them again how to conduct the manoeuvre and allow them four more attempts.
- 6) Any subject who is unable to produce two technically satisfactory manoeuvres after nine attempts should not be tested further and no FEV<sub>1</sub> / FVC data should be recorded.
- 7) The number of rejected attempts should be recorded as appropriate on the Lung Function Data Collection Sheet.

### **Methacholine challenge**

During methacholine challenge the subject may need to perform 30 or more expiratory manoeuvres and, to minimise exhaustion, the forced expiration will be abandoned each time after one second when the FEV<sub>1</sub> has been recorded.

- 1) Two minutes after inhalation from the dosimeter up to five attempts should be made to record an FEV<sub>1</sub>
- 2) As soon as two technically satisfactory manoeuvres have been achieved these readings are recorded. The next dose can be given as soon as possible after the completion of these measurements.
- 3) Further testing should be abandoned if the subject is unable to produce two technically satisfactory manoeuvres within five attempts.

If a reversal of bronchoconstriction needs to be carried out then the procedure is the same as the bronchodilator challenge.

### **Bronchodilator challenge**

## LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

A bronchodilator challenge will be given to those who do not undergo methacholine challenge. Any subject who has more than a 10% fall in FEV<sub>1</sub> from baseline during the methacholine challenge test should have their bronchoconstriction reversed at the end of the test and before leaving the test centre, by the same method.

The salbutamol inhaler should be shaken and inserted into the volumatic. One puff should be activated and the subject asked to place their lips around the volumatic and to inhale and exhale five times. The salbutamol inhaler should be activated again and five inhalations/exhalations performed. This should be repeated two more times so that a total of 400ug of salbutamol has been delivered. Subjects who are known asthmatics and familiar with Volumatic usage can self-administer this dose.

The FEV<sub>1</sub> and FVC are measured 10 minutes after the administration of bronchodilator. During the bronchodilator challenge FVC manoeuvres will be used. Up to nine attempts may be made to obtain two technically satisfactory recordings after the inhalation of bronchodilator.

### THE METHACHOLINE SOLUTIONS

#### **Source and supply**

Methacholine (Provocholine) will be obtained from Methapharm.

#### **The Diluent**

Saline buffered with phosphate to obtain physiological pH can be used as a diluent. Phenol must not be used as a preservative because of concerns regarding its safety. Citric acid/citrate buffer must not be used. Preservatives should be avoided.

#### **Session number and order in session**

Each time the nebulisers are filled with fresh methacholine solution a new session of testing is said to have started. Each session should be sequentially numbered. Each challenge within each testing session should also be sequentially numbered and recorded on the Lung Function Data Collection Sheet.

At the beginning of a session all nebulisers contain 3 mL methacholine. Six subjects are tested and their order in session is 1-6. After the 6th person has been tested the 12.5 mg/mL solution is discarded, the nebuliser is cleaned and dried, and 3 mL of fresh 12.5 mg/mL solution is added. Six more subjects are tested and they are numbered 7-12. After the 12th person has been tested all solutions are discarded and the nebulisers are cleaned. The next session begins when new solutions are added. A session may be extended over one night only by placing the nebulisers containing solutions upright in the fridge, covered with parafilm.

### THE *MEFAR MB3* DOSIMETER

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## **Quality control of Mefar dosimeter nebuliser output**

The methacholine challenge protocol has been written assuming that each single inhalation delivers approximately 0.01 mL solution to the mouth.

All Mefar nebulisers in the study will be calibrated in Melbourne prior to use in the study.

## **Pressure control Mefar**

The driving pressure of the Mefar dosimeter should be checked before the study starts and every four weeks thereafter. The method for checking and adjusting the driving pressure is available at:

‘[http://www.med.monash.edu.au/medicine/alfred/research/respiratory\\_medicine/newpage.htm](http://www.med.monash.edu.au/medicine/alfred/research/respiratory_medicine/newpage.htm).’

Pressure control forms should be returned to the co-ordinating centre at completion of the study.



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*Each nebuliser should be colour coded for the solution it will contain as follows:*

- 1) BLACK 12.5mg/mL
- 2) RED 6.25mg/mL
- 3) YELLOW 1.56mg/mL
- 4) BLUE 0.39mg/mL
- 5) WHITE Diluent

### **Setting up the Mefar dosimeter**

3 mL of methacholine solution should be placed in the appropriate nebuliser. A dry sterile mouthpiece should be connected for each new subject.

*The Mefar should be set at:*

- 1) inhalation time: 1 second
- 2) pause time: 6 seconds

### **The standard inhalation**

*The sequence of inhalation is:*

- 1) Slow expiration to functional residual capacity.
- 2) Place lips around mouthpiece to produce airtight seal.
- 3) Slow inspiration to total lung capacity.
- 4) Hold breath for at least three seconds.
- 5) Remove mouthpiece and exhale.

The procedure is repeated after six seconds until sufficient inhalations for the dose have been performed. Inhalations may be performed on consecutive breaths if desired. Spirometric testing is carried out two minutes after the dose. As soon as two FEV<sub>1</sub> manoeuvres have been recorded, the test is continued with the next dose.

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## **The end of the testing session**

Solutions remaining in the nebulisers must be discarded and under no circumstances should they be returned to the storage containers. All nebulisers must be cleaned and dried. All mouthpieces must be cleaned, sterilised and thoroughly rinsed to ensure that there is no sterilising solution left on the surface.

## THE METHACHOLINE PROTOCOL

### **Instructions for baseline spirometry**

Perform full FVC manoeuvres as described previously for 'Baseline spirometry' (The forced expiratory manoeuvre). Record INITIAL FEV<sub>1</sub> and FVC. Calculate the BEST INITIAL FEV<sub>1</sub> as a percentage of the total predicted.

### **Measurement of control (post-diluent) FEV<sub>1</sub>**

The control FEV<sub>1</sub> is the FEV<sub>1</sub> measured following the inhalation of diluent. Four inhalations of diluent (WHITE nebuliser) are given, as described in 'The standard inhalation'.

Perform FEV<sub>1</sub> manoeuvres as described in 'Methacholine challenge' (The forced expiratory manoeuvre). Record CONTROL (POST-DILUENT) FEV<sub>1</sub>. Calculate BEST CONTROL FEV<sub>1</sub> as a percentage of the BEST INITIAL FEV<sub>1</sub>.

*If the BEST CONTROL FEV<sub>1</sub> is less than 90% of the BEST INITIAL FEV<sub>1</sub> methacholine challenge is not carried out.* Bronchoconstriction should be reversed by administering 400 µg salbutamol by MDI via a Volumatic and full FVC manoeuvres should be repeated.

*If the BEST CONTROL FEV<sub>1</sub> is within 10% of the best initial FEV<sub>1</sub>.* Calculate 80% of the BEST CONTROL FEV<sub>1</sub>. Calculate 90% of the BEST CONTROL FEV<sub>1</sub>. Methacholine challenge may now be conducted following either the short or long protocol.

### **Dosing Schedule**

In ECRHS I centres were able to decide whether to use one of two dosing schedules for methacholine challenge. In ECRHS II only one method shall be used (Method 2 from ECRHS I protocol).

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## Choice of long or short protocol

Each subject can be challenged on the short or long protocol. The long protocol will increase by doubling doses and the short by quadrupling doses. Subjects most likely to react to methacholine should be tested on the long protocol. Subjects who are unexpectedly reactive and have been allocated to the short protocol may switch to the long protocol during the challenge to avoid severe bronchoconstriction. The choice of protocol for each subject will be assessed by the Main Questionnaire. The questions to be used to direct subjects to the long protocol may be decided locally, but the following are recommended:

*Subjects who answered 'YES' to any one of Questions 1, 2, 3, 5, 11 or 14 in the Main Questionnaire, that is any subject who has:*

- 1) had wheezing or whistling in their chest in the last 12 months (Q1)
- 2) woken with tightness of chest in the last 12 months (Q2)
- 3) had an attack of shortness of breath during the day while at rest in the last 12 months (Q3)
- 4) been woken by an attack of shortness of breath in the last 12 months (Q4)
- 5) trouble with their breathing (Q11)
- 6) ever had asthma (Q14)

## Methacholine challenge protocol

CONC (mg/mL)	No of inhalations:		CUMULATIVE DOSE (mg)	DOSE LEVEL (As per ECRHS I)
	LONG	SHORT		
0.39	2		0.0078	3
0.39	2	4	0.0156	4
1.56	1		0.0312	5
1.56	2	3	0.0625	6
6.25	1		0.125	7
6.25	2	3	0.25	8
12.5	2		0.5	9
12.5	4	6	1.0	10
12.5	8	8	2.0	11

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### Changing from Long to Short protocol

If, during the short protocol, the FEV<sub>1</sub> falls 10% or more from the best control FEV<sub>1</sub>, the subject should change protocol and receive the next dose level on the long protocol.

For example: A subject following the short protocol shows a fall of 10% after Dose 4 (four inhalations of 0.39 mg/mL). They should inhale Dose 5 (one inhalation of 1.56 mg/mL) next.

#### *Short protocol:*

Change to long protocol if FEV<sub>1</sub> falls below 90% of the BEST CONTROL FEV<sub>1</sub>. Go to next dose level on long protocol.

STOP challenge if FEV<sub>1</sub> falls below 80% of the BEST CONTROL FEV<sub>1</sub>

#### *Long protocol:*

STOP challenge if FEV<sub>1</sub> falls below 80% of the BEST CONTROL FEV<sub>1</sub>

### Completion of test

The methacholine challenge is complete when a cumulative dose 2 mg of methacholine has been reached.

#### *It is stopped sooner if:*

- 1) there is greater than 10% fall in FEV<sub>1</sub> from the **BEST BASELINE FEV<sub>1</sub>** following inhalation of diluent,
- 2) there is greater than 20% fall in FEV<sub>1</sub> from the **BEST CONTROL FEV<sub>1</sub>** following inhalation of any concentration of methacholine solution,
- 3) the subject is not able to perform two technically satisfactory manoeuvres in five attempts following any dose level,
- 4) the subject does not wish to carry on.

Subjects may complain of mild chest tightness, coughing or wheezing but if lung function does not demonstrate a 20% fall in FEV<sub>1</sub> this is not an indication to stop the test.

## LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

### **Reversal of bronchoconstriction**

Four hundred micrograms of salbutamol will be given via a volumatic (see above for bronchodilator challenge)  
Perform full FVC manoeuvres as described in 'Methacholine challenge' 10 minutes after administration.

Record the POST-BRONCHODILATOR FEV<sub>1</sub> and FVC.

Calculate the BEST POST-BRONCHODILATOR FEV<sub>1</sub> as a PERCENTAGE of the BEST INITIAL FEV<sub>1</sub>.

If the best post-bronchodilator FEV<sub>1</sub> is more than 90% of the best initial FEV<sub>1</sub> the test is over.

*EACH CENTRE SHOULD PREPARE PROTOCOLS TO BE FOLLOWED IN THE EVENT OF A SUBJECT NOT RETURNING TO WITHIN 10% OF THE BASELINE.*

### BRONCHODILATOR CHALLENGE PROTOCOL

Four hundred micrograms of salbutamol are administered by MDI as described in 'Bronchodilator challenge'.  
Perform full FVC manoeuvres as described in 'Baseline spirometry'. Record the POST-BRONCHODILATOR FEV<sub>1</sub>  
AND FVC.

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

Area Number			
Personal Number			
Sample			
Date			
	DAY	MONTH	YEAR

**BEFORE STARTING THIS QUESTIONNAIRE PLEASE ASK THE FOLLOWING QUESTIONS**

Have you had a cigarette in the last <b>hour</b> ?	YES <input type="checkbox"/>	NO <input type="checkbox"/>
Have you used an inhaler (puffer) in the last <b>hour</b> ?	YES <input type="checkbox"/>	NO <input type="checkbox"/>

**IF 'YES' DELAY LUNG FUNCTION TESTS UNTIL ONE HOUR AFTER THE LAST CIGARETTE OR INHALER USE (RESPONSES DO NOT HAVE TO BE INCLUDED IN DATA RECORDER)**

- |   |   |
|---|---|
| 1. How many times have you been woken at night with shortness of breath in the last <i>two weeks</i> ?          | NUMBER<br><input type="text"/>                              |
| 2. During the last <i>two weeks</i> , has your breathing been   | TICK ONE BOX ONLY   |
| (a) worse than usual?   | 1 <input type="checkbox"/>                                  |
| (b) same as usual?  | 2 <input type="checkbox"/>                                  |
| (c) better than usual?  | 3 <input type="checkbox"/>                                  |
| 3. Have you had wheezing or whistling in your chest <u>in the last 3 days</u> ?                                 | NO YES<br><input type="checkbox"/> <input type="checkbox"/> |
| 4. Have you woken up with a feeling of tightness in your chest <u>in the last 3 days</u> ?                      | <input type="checkbox"/> <input type="checkbox"/>           |
| 5. Have you been woken by an attack of shortness of breath <u>in the last 3 days</u> ?                          | <input type="checkbox"/> <input type="checkbox"/>           |
| 6. Have you been woken by an attack of coughing <u>in the last 3 days</u> ?                                     | <input type="checkbox"/> <input type="checkbox"/>           |
| 7. Have you had an attack of asthma <u>in the last 3 days</u> ?   | <input type="checkbox"/> <input type="checkbox"/>           |
| 8. Have you taken any medicine (including inhalers, aerosols or tablets) For asthma <u>in the last 3 days</u> ? | <input type="checkbox"/> <input type="checkbox"/>           |

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9. Have you had any symptoms of hay fever or nasal allergy in the **last 3 days?**

NO	YES
10. have you had a respiratory infection in the **last 3 weeks?**

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**IF 'NO' GO TO QUESTION 11**

**IF 'YES' AND THE SUBJECT IS WILLING TO COME BACK, STOP AND MAKE A NEW APPOINTMENT. IF NOT, PROCEED WITH QUESTION 10.1**

- 10.1 How many days ago did it end? 

DAYS	

11. Have you used an inhaler in the last **24 hours?**

NO	YES

**IF 'NO' GO TO QUESTION 12, IF 'YES' - :**

- 11.1 What inhaler(s) did you use and for how many hours did you use it?

	DRUG		HOURS	
_____				
_____				
_____				
_____				

**IF THE SUBJECT HAS USED A BETA-2-AGONIST INHALER OR AN ANTI-MUSCARINIC INHALER IN THE LAST FOUR HOURS, CONSIDER: -**

**a) WAITING UNTIL FOUR HOURS SINCE LAST USE HAS ELAPSED**

**b) RESCHEDULING FOR ANOTHER DAY IF THE SUBJECT IS WILLING, IF NEITHER OF THESE IS POSSIBLE, PROCEED.**

12. Have you used any other medicine (including pills, capsules or suppositories) to help your breathing, or any oral anti-muscarinic in the **last 24 hours?**

NO	YES

**IF 'NO' GO TO QUESTION 13, IF YES: -**

- 12.1 What medicine(s) did you take and how many hours ago did you take it?

	DRUG		HOURS	
_____				
_____				
_____				
_____				

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**IF THE SUBJECT HAS TAKEN AN ORAL BETA-2-AGONIST, AN ORAL THEOPHYLLINE OR AN ORAL ANTI-MUSCARINIC, CONSIDER RESCHEDULING FOR ANOTHER DAY IF THE SUBJECT IS WILLING, IF THIS IS NOT POSSIBLE, PROCEED.**

- |  | NO                       | YES                      |
|--|--------------------------|--------------------------|
| 13. Have you had a heart attack in the last <b>three months</b> ?                                | <input type="checkbox"/> | <input type="checkbox"/> |
| 14. Are you currently taking any medicine(s) for your heart?                                     | <input type="checkbox"/> | <input type="checkbox"/> |
| 15. Are you currently taking any medicines for epilepsy?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 16. Are you currently taking any medicine containing beta-blockers, <b>including eye-drops</b> ? | <input type="checkbox"/> | <input type="checkbox"/> |

**IF 'YES' TO ANY QUESTIONS 13-16 MEASURE BASELINE SPIROMETRY ONLY, DO NOT CHALLENGE.**

For women only:

- |                             | NO                       | YES                      |
|-----------------------------|--------------------------|--------------------------|
| 17. Are you pregnant?       | <input type="checkbox"/> | <input type="checkbox"/> |
| 18. Are you breast feeding? | <input type="checkbox"/> | <input type="checkbox"/> |

**IF 'YES' TO QUESTIONS 17 OR 18 MEASURE BASELINE SPIROMETRY ONLY, DO NOT CHALLENGE.**

For all subjects:

- |   | NO                       | YES                      |
|---|--------------------------|--------------------------|
| 19. Would you like us to notify your GP of the results of any test? | <input type="checkbox"/> | <input type="checkbox"/> |

**END**

FIELDWORKER NUMBER

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## Coding for lung function questionnaire

*The same general rules apply as for the main questionnaire.*

Questions with NO / YES

- 1 NO
- 2 YES

Questions with 'TICK ONE BOX ONLY' instruction:

The number of the box ticked is the code for that answer.

### **QUESTION 11.1 Inhalers in the last 24 hours**

Drug grouping should be consistent with those used for main questionnaire.

- 1 Beta-2-against (short acting)
- 2 Beta-2-against (long acting)
- 3 Non-specific adrenoreceptor agonists
- 4 Anticholinergic inhalers
- 5 Inhaled steroids
- 6 Sodium cromoglycate
- 7 Nedocromil
- 8 Compound bronchodilators
  
- 98 Not coded
- 99 Not known

### **QUESTION 12.1 Oral medications**

Drug grouping should be consistent with those used for main questionnaire.

- 1 Beta-2-against
- 2 Non-specific adrenoreceptor agonist
- 3 Oral anticholinergics/antimuscarinics
- 4 Oral methylxanthines
- 5 Oral steroids
- 6 Oral antihistamines
- 7 Oral compound bronchodilators
- 8 Oral-antileukotrienes
  
- 98 not coded
- 99 not known

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

Area Number

Personal Number

Sample

Date


DAY      MONTH      YEAR

DAY      MONTH      YEAR

METRES

1. Subject's Height

METRES

2. Subject's Weight

KILOGRAMS

3. Subject's Age

AGE    
Male Female

4. Subjects sex

5. Time of Day

'24 hrs' HOURS MINUTES  
  •

6. **PREDICTED FEV<sub>1</sub>**

•

Each subject now has five attempts to produce a technically satisfactory manoeuvre. If after five attempts there have not produced at least two manoeuvres – four more attempts are allowed  
*MAXIMUM NUMBER OF ATTEMPTS ALLOWED IS NINE ATTEMPTS*

7. **INITIAL FEV<sub>1</sub> and FVC**

	FEV <sub>1</sub>	FVC	PEFR (L/s)
1	<input type="text"/>	<input type="text"/>	<input type="text"/>
2	<input type="text"/>	<input type="text"/>	<input type="text"/>
3	<input type="text"/>	<input type="text"/>	<input type="text"/>
4	<input type="text"/>	<input type="text"/>	<input type="text"/>
5	<input type="text"/>	<input type="text"/>	<input type="text"/>

7.1 Number of rejected attempts

8. **Best INITIAL FEV<sub>1</sub> as % of predicted FEV<sub>1</sub>**

**IF BEST INITIAL FEV<sub>1</sub> IS**      **a) less than 70% PREDICTED or**  
**b) less than 1.5 LITRES**

**LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE**

**GO TO BRONCHODILATOR CHALLENGE – DO NOT DO METHACHOLINE  
CHALLENGE**

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## METHACHOLINE CHALLENGE

Give four inhalations of diluent. Two minutes later record FEV<sub>1</sub>

9. **CONTROL FEV<sub>1</sub>** following inhalation of diluent.

9.1 Record two technically satisfactory manoeuvres

1	<input type="text"/>	•	<input type="text"/>	<input type="text"/>
2	<input type="text"/>	•	<input type="text"/>	<input type="text"/>

9.2 Number of rejected attempts

10. **BEST CONTROL (post-diluent) FEV<sub>1</sub>** as % of INITIAL FEV<sub>1</sub>

%

**IF BEST CONTROL FEV<sub>1</sub> < 90% OF BEST INITIAL FEV<sub>1</sub>  
STOP METHACHOLINE CHALLENGE AND GO TO REVERSAL OF  
BRONCHOCONSTRICTION**

Choice of methacholine long or short protocol

NO YES

DID THE SUBJECT ANSWER 'YES' TO QUESTIONS 1,2,3,5,11 or 14  
OF THE MAIN QUESTIONNAIRE?

**IF 'NO' GO TO SHORT PROTOCOL  
IF 'YES' GO TO LONG PROTOCOL**

11. Will the subject follow the short or long protocol?

LONG SHORT

**CODING:** 1 LONG, 2 SHORT

### **SHORT PROTOCOL:**

**CHANGE TO LONG PROTOCOL if FEV<sub>1</sub> falls to < 90% of CONTROL FEV<sub>1</sub>**

**STOP METHACHOLINE CHALLENGE if FEV<sub>1</sub> fall to < 80% of CONTROL FEV<sub>1</sub>**

90% of CONTROL FEV<sub>1</sub>

•

### **LONG PROTOCOL:**

**STOP METHACHOLINE CHALLENGE if FEV<sub>1</sub> fall to < 80% of CONTROL FEV<sub>1</sub>**

80% of CONTROL FEV<sub>1</sub>

•

**THESE DATA NOT REQUIRED BY CO-ORDINATING CENTRES**

12. METHACHOLINE BATCH NUMBER

NUMBER OF SESSIONS

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

ORDER IN SESSIONS

--	--

DOSE LEVEL	CUMULATIVE CUM DOSE (mg)	NEBULISER CONCEN	NO. INHALATIONS		FEV <sub>1</sub>		FEV <sub>1</sub>		Rejected attempts
			SHORT	LONG					
3	0.0078	0.39	...	2	<input type="text"/>				
4	0.0156	0.39	4	2	<input type="text"/>				
5	0.0312	1.56	...	1	<input type="text"/>				
6	0.0625	1.56	3	2	<input type="text"/>				
7	0.125	6.25		1	<input type="text"/>				
8	0.25	6.25	3	2	<input type="text"/>				
9	0.5	12.5		2	<input type="text"/>				
10	1.0	12.5	6	4	<input type="text"/>				
11	2.0	12.5	8	8	<input type="text"/>				

TICK ONE

BOX ONLY

13. Why was methacholine challenge stopped

- a) end of test reached (2mg inhaled)
- b) >20% fall in FEV<sub>1</sub> occurred
- c) two satisfactory manoeuvres not achieved
- d) subject asked to stop
- e) other \_\_\_\_\_

1	
2	
3	
4	
5	

# LUNG FUNCTION PROTOCOL, DATA SHEETS AND QUESTIONNAIRE

## IF SUBJECTS FEV<sub>1</sub> HAS FALLEN BY MORE THAN 10%

### Reversal of bronchoconstriction

GIVE 400 µG SALBUTOMAL VIA VOLUMATIC AND 10 MINUTES LATER RECORD FEV<sub>1</sub>

#### 14. FEV<sub>1</sub> and FVC

14.1 Record first two technically satisfactory manoeuvres  
( up to five attempts)

	FEV <sub>1</sub>			FVC	
<input type="checkbox"/>					
<input type="checkbox"/>					

14.2 Number of rejected attempts

15 Best POST-BRONCHODILATOR FEV<sub>1</sub> as % of initial FEV<sub>1</sub>

16 Has the subject's FEV<sub>1</sub> returned to within 10% of initial FEV<sub>1</sub>

	NO	YES
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**IF 'YES' THE SUBJECT MAY LEAVE THE CENTRE  
IF 'NO' FURTHER ACTION MUST BE TAKEN TO RESTORE BASELINE LUNG  
FUNCTION**

### BRONCHODILATOR CHALLENGE ONLY

GIVE 400 µg SALBUTAMOL VIA VOLUMATIC AND 10 MINUTES LATER RECORD FEV<sub>1</sub>

#### 17. FEV<sub>1</sub> and FVC

17.1 Record first two technically satisfactory manoeuvres  
up to 9 attempts)

		FEV <sub>1</sub>			FVC		
<input type="checkbox"/>	•	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	•	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	•	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	•	<input type="checkbox"/>	<input type="checkbox"/>

17.2 Number of rejected attempts

**END**

FIELDWORKER NUMBER



## **Appendix B**

### **ECRHS I Screening questionnaire**



**ECRHS APPENDIX A 1 Screening Questionnaire**

Area number 

--	--	--	--

 1-3  
 Personal number 

--	--	--	--	--

 4-8  
 Sample 

--	--	--

 9

**TO ANSWER THE QUESTIONS PLEASE CHOOSE THE APPROPRIATE BOX  
 IF YOU ARE UNSURE OF THE ANSWER PLEASE CHOOSE 'NO'**

1. Have you had wheezing or whistling in your chest at any time in the last 12 months? NOYES   10

**IF 'NO' GO TO QUESTION 2, IF 'YES':**

1.1. Have you been at all breathless when the wheezing noise was present? NO YES   11

1.2. Have you had this wheezing or whistling when you did not have a cold? NOYES   12

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months? NOYES   13

3. Have you been woken by an attack of shortness of breath at any time in the last 12 months? NO YES   14

4. Have you been woken by an attack of coughing at any time in the last 12 months? NOYES   15

5. Have you had an attack of asthma in the last 12 months? NO YES   16

6. Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma? NOYES   17

7. Do you have any nasal allergies including hay fever? NO YES   18

8. What is your date of birth? DAY MONTH YEAR       19-24

9. What is today's date? DAY MONTH YEAR       25-30

10. Are you male or female? MALE FEMALE   31

**THANK YOU FOR YOUR HELP**  
**If you don't mind being telephoned at home or at work by one of the study team, please write your telephone number below:**  32  
 33  
 34

(DAY).....(EVE).....



# **Appendix C**

## **ECRHS I Main questionnaire**



**ECRHS APPENDIX B 1 Main Questionnaire**

Area number					1-3
Personal number					4-8
Sample					9
Date					10-15
	DAY	MONTH	YEAR		

**I AM GOING TO ASK YOU SOME QUESTIONS. AT FIRST THESE WILL BE MOSTLY ABOUT YOUR BREATHING. WHEREVER POSSIBLE, I WOULD LIKE YOU TO ANSWER 'YES' OR 'NO'.**

Wheeze and tightness in the chest

CARD 2

1. Have you had wheezing or whistling in your chest at any time in the last **12 months**? NO YES  
  16

**IF 'NO' GO TO QUESTION 2, IF 'YES':**

1.1 Have you been at all breathless when the wheezing noise was present? NO YES  
  17

1.2. Have you had this wheezing or whistling when you did **not** have a cold? NO YES  
  18

2. Have you woken up with a feeling of tightness in your chest at any time in the last **12 months**? NO YES  
  19

Shortness of breath

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last **12 months**? NO YES  
  20

4. Have you had an attack of shortness of breath that came on **following** strenuous activity at any time in the last **12 months**? NO YES  
  21

5. Have you been woken by an attack of shortness of breath at any time in the last **12 months**? NO YES  
  22

Cough and phlegm from the chest

6. Have you been woken by an attack of coughing at any time in the last **12 months**? NO YES  
  23

7. Do you **usually** cough first thing in the morning in the winter? NO YES  
  24  
**[IF DOUBTFUL, USE QUESTION 8.1 TO CONFIRM]**

8. Do you **usually** cough during the day, or at night, in the winter? NO YES  
  25

**IF 'NO' GO TO QUESTION 9, IF 'YES':**

8.1 Do you cough like this on most days for as much as three months each year? NO YES  
  26

**ECRHS APPENDIX B 1 Main Questionnaire**

9. Do you *usually* bring up any phlegm from your chest first thing in the morning in the winter? **[IF DOUBTFUL, USE QUESTION 10.1 TO CONFIRM]** NO YES  
  27

10. Do you *usually* bring up any phlegm from your chest during the day, or at night, in the winter? NO YES  
  28

**IF 'NO' GO TO QUESTION 11, IF 'YES':**

10.1 Do you bring up phlegm like this on most days for as much as three months each year? NO YES  
  29

Breathing

11. Do you ever have trouble with your breathing? NO YES  
  30

**IF 'NO' GO TO QUESTION 12, IF 'YES':**

11.1 Do you have this trouble TICK ONE BOX ONLY  
a) continuously so that your breathing is never quite right? 1   
b) repeatedly, but it always gets completely better? 2   
c) only rarely? 3  31

12. Are you disabled from walking by a condition *other than* heart or lung disease? NO YES  
  32

**IF 'YES' STATE CONDITION \_\_\_\_\_ AND GO TO QUESTION 13, IF 'NO':**

12.1 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? NO YES  
  33

**IF 'NO' GO TO QUESTION 13, IF 'YES':**

12.1.1 Do you get short of breath walking with other people of NOYES your own age on level ground?   34

**IF 'NO' GO TO QUESTION 13, IF 'YES':**

12.1.1.1 Do you have to stop for breath when walking at your own pace on level ground? NO YES  
  35

Asthma

13. Have you ever had asthma? NO YES  
  36

**IF 'NO' GO TO QUESTION 14, IF 'YES':**

13.1 Was this confirmed by a doctor? NO YES  
  37

**ECRHS APPENDIX B 1 Main Questionnaire**

13.2 How old were you when you had your first attack of asthma? YEARS  
  38-39

13.3 How old were you when you had your most recent attack of asthma? YEARS  
  40-41

13.4.1-6 Which months of the year do you usually have attacks of asthma?

13.4.1 January / February NO YES  
  42

13.4.2 March / April   43

13.4.3 May / June   44

13.4.4 July / August   45

13.4.5 September / October   46

13.4.6 November / December   47

13.5 Have you had an attack of asthma in the last **12 months**? NO YES  
  48

**IF 'NO' GO TO QUESTION 13.6, IF 'YES':**

13.5.1 How many attacks of asthma have you had in the last **12 months**? NUMBER  
  49-50

13.6 Are you currently taking any medicines, including inhalers, aerosols or tablets, for asthma? NO YES  
  51

Other conditions

14. Do you have any nasal allergies, including hay fever? NO YES  
  52

15. Have you ever had eczema or any kind of skin allergy? NO YES  
  53

16. Are you allergic to any insect stings or bites? NO YES  
  54

**IF 'NO' GO TO QUESTION 17, IF 'YES':**

16.1 Which insect? \_\_\_\_\_   55-56

16.2.1-3 What kind of reaction do you have?

16.2.1 breathing difficulty, feeling faint, nausea or fever NO YES  
  57

16.2.2 redness, itching or swelling at the site of the sting   58

16.2.3 other: \_\_\_\_\_   59

17. Have you ever had any difficulty with your breathing after taking medicines? NO YES  
  60

**IF 'NO' GO TO QUESTION 18, IF 'YES':**

**ECRHS APPENDIX B 1 Main Questionnaire**

17.1 Which medicines? \_\_\_\_\_ 1  61  
 2  62

Your parents' smoking

18. Did your father ever smoke regularly during your childhood? NO YES DONT  
   63  
 DONT  
 19. Did your mother ever smoke regularly during your childhood, or before you were born? NO YES KNOW  
   64

**IF 'NO' OR 'DONT KNOW' GO TO QUESTION 20, IF 'YES':**

19.1 When your mother was pregnant, in particular with you, did she  
 a) stop smoking before pregnancy? TICK ONE BOX ONLY  
 b) cut down or stop during pregnancy? 1   
 c) smoke as usual during pregnancy? 2   
 d) don't know 3  65  
 4

More about yourself

20. When were you born? DAY MONTH YEAR  
   66-71

21. What country were you born in? \_\_\_\_\_  72-74

22. Are you male or female? MALE FEMALE  
  75

23. How many brothers do or did you have? NUMBER  
 76-77

**INTERVIEW TYPE?**

At centre face to face TICK ONE BOX ONLY  
 At home face to face 1   
 By telephone 2  78  
 3

23. continued... CARD NUMBER  79-80

**IF 'NONE' GO TO QUESTION 24, IF 'YES':**

23.1 How many *older* brothers? CARD 3 DUPLICATE 1-9 NUMBER  10-11  
 23.2 How many *younger* brothers?  12-13  
 23.3 How many of your brothers ever had asthma?  14-15  
 23.4 How many of your *other* brothers ever had eczema, skin or nasal allergy or hay fever? NUMBER  16-17  
 NUMBER



**ECRHS APPENDIX B 1 Main Questionnaire**

- a) a manager working for an employer? 1
- b) a foreman or supervisor working for an employer? 2
- c) working for an employer, but neither a manager, supervisor or foreman? 3
- d) self-employed? 4  43

32.5 Does being at work ever make your chest tight or wheezy?  NO  YES 44

32.6 Have you ever had to change or leave your job because it affected your breathing?  NO  YES 45

**IF 'NO' GO TO QUESTION 32.7 IF 'YES':**

32.6.1 What was this job? [Be as precise as possible]

\_\_\_\_\_    46-48

32.7 Have you ever worked in a job which exposed you to vapours, gas, dust or fumes?  NO  YES 49

**IF 'NO' GO TO QUESTION 33, IF 'YES':**

32.7.1 What was or is this job? [Be as precise as possible]  
If current job write 'current job'

\_\_\_\_\_    50-52

Your home

33. How many years have you lived in your present home? YEARS   53-54

34. How many years have you lived in \_\_\_\_\_? [Insert area name] YEARS   55-56

35. When was your present home built? TICK ONE BOX ONLY

- a) before 1960? 1
- b) 1961-1970? 2
- c) 1971-1980? 3
- d) 1981 or later? 4
- e) don't know 5  57

36. Which best describes the building in which you live? TICK ONE BOX ONLY

- a) a mobile home or trailer? 1
- b) a one family house detached from any other house? 2
- c) a one family house attached to one or more houses? 3
- d) a building for two families? 4

**ECRHS APPENDIX B 1 Main Questionnaire**

- e) a building for three or four families? 5
- f) a building for five or more families? 6
- g) a boat, tent or van 7
- e) other: \_\_\_\_\_ 8  58

37.1-3 Does your home have any of the following?

- |                         |                             |                              |    |
|-------------------------|-----------------------------|------------------------------|----|
| 37.1 central heating    | NO <input type="checkbox"/> | YES <input type="checkbox"/> | 59 |
| 37.2 ducted air heating | <input type="checkbox"/>    | <input type="checkbox"/>     | 60 |
| 37.3 air conditioning   | <input type="checkbox"/>    | <input type="checkbox"/>     | 61 |

38.1-7 Which of the following fuels do you use for heating or for hot water?

- |                                   |                             |                              |    |
|-----------------------------------|-----------------------------|------------------------------|----|
| 38.1 open coal, coke or wood fire | NO <input type="checkbox"/> | YES <input type="checkbox"/> | 62 |
| 38.2 open gas fire                | <input type="checkbox"/>    | <input type="checkbox"/>     | 63 |
| 38.3 electric heater              | <input type="checkbox"/>    | <input type="checkbox"/>     | 64 |
| 38.4 paraffin heater              | <input type="checkbox"/>    | <input type="checkbox"/>     | 65 |
| 38.5 gas-fired boiler             | <input type="checkbox"/>    | <input type="checkbox"/>     | 66 |
| 38.6 oil-fired boiler             | <input type="checkbox"/>    | <input type="checkbox"/>     | 67 |
| 38.7 other: _____                 | <input type="checkbox"/>    | <input type="checkbox"/>     | 68 |

39. What kind of stove do you *mostly* use for cooking?

- a) coal, coke or wood (solid fuel)?
- b) gas?
- c) electric?
- d) paraffin?
- e) other: \_\_\_\_\_

TICK ONE BOX ONLY

- 1
- 2
- 3
- 4
- 5  69

DON'T KNOW

- NO  YES  DON'T KNOW  70

40. Do you have an extractor fan over the cooker?

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 41, IF 'YES':**

40.1 When cooking, do you use the fan

- a) all of the time?
- b) some of the time?
- c) none of the time?

TICK ONE BOX ONLY

- 1
- 2
- 3  71

DON'T KNOW

- NO  YES  DON'T KNOW  72

40.2 Does the fan take the fumes outside the house?

41.1-5 Does the room which you use most at home during the day

41.1 have fitted carpets covering the whole floor?

- NO  YES  73

**ECRHS APPENDIX B 1 Main Questionnaire**

41.2 contain rugs?	<input type="checkbox"/>	<input type="checkbox"/>	74
41.3 have double glazing?	<input type="checkbox"/>	<input type="checkbox"/>	75
41.4 have curtains?	<input type="checkbox"/>	<input type="checkbox"/>	76
41.5 have upholstered or soft furnishings?	<input type="checkbox"/>	<input type="checkbox"/>	77
	BLANK		78
	CARD NUMBER		<input type="checkbox"/> <input type="checkbox"/> 79-80
	CARD 4		
42.1-5 Does your bedroom	DUPLICATE 1-9		
	NO	YES	
42.1 have fitted carpets covering the whole floor?	<input type="checkbox"/>	<input type="checkbox"/>	10
42.2 contain rugs?	<input type="checkbox"/>	<input type="checkbox"/>	11
42.3 have double glazing?	<input type="checkbox"/>	<input type="checkbox"/>	12
42. continued...			
	NO	YES	
42.4 have curtains?	<input type="checkbox"/>	<input type="checkbox"/>	13
42.5 have upholstered or soft furnishings?	<input type="checkbox"/>	<input type="checkbox"/>	14
	NO	YES	
43. Do you sleep with the windows open at night during winter?	<input type="checkbox"/>	<input type="checkbox"/>	15
<b>IF 'NO' GO TO QUESTION 44, IF 'YES':</b>			
43.1 Do you sleep with the windows open			
		TICK ONE BOX ONLY	
a) all of the time?		1 <input type="checkbox"/>	
b) sometimes?		2 <input type="checkbox"/>	
c) only occasionally?		3 <input type="checkbox"/>	16
		DONT	
44. Has there ever been any water damage to the building or its contents, for example, from broken pipes, leaks or floods?	NO	YES	KNOW
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			17
<b>IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 45, IF 'YES':</b>			
44.1 Has there been any water damage in the last <i>12 months</i> ?	NO	YES	DONT KNOW
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			18
45. Do you have a basement or cellar?		NO	YES
		<input type="checkbox"/>	<input type="checkbox"/>
			19
<b>IF 'NO' GO TO QUESTION 46, IF 'YES':</b>			
45.1 Does water ever collect on the basement floor?	NO	YES	DONT KNOW
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			20
<b>IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 46, IF 'YES':</b>			
45.1.1 Has this happened in the last <i>12 months</i> ?	NO	YES	
	<input type="checkbox"/>	<input type="checkbox"/>	21
		DONT	
46. Has there ever been any mould or mildew on any surface, other than	NO	YES	KNOW

**ECRHS APPENDIX B 1 Main Questionnaire**

food, inside the home?    22

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 47, IF 'YES':**

46.1.1-6 Which rooms have been affected?

	NO	YES	
46.1.1 bathroom(s)	<input type="checkbox"/>	<input type="checkbox"/>	23
46.1.2 bedroom(s)	<input type="checkbox"/>	<input type="checkbox"/>	24
46.1.3 living area(s)	<input type="checkbox"/>	<input type="checkbox"/>	25
46.1.4 kitchen	<input type="checkbox"/>	<input type="checkbox"/>	26
46.1.5 basement or attic	<input type="checkbox"/>	<input type="checkbox"/>	27
46.1.6 other: _____	<input type="checkbox"/>	<input type="checkbox"/>	28

46.2 Has there been mould or mildew on any surfaces inside the home in the last **12 months**?  NO  YES 29

47. Do you use a humidifier, including any humidifier built into your heating system?  NO  YES  DON'T KNOW 30

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 48, IF 'YES':**

47.1 What kind of humidifier do you use?

	TICK ONE BOX ONLY	
a) humidifier built into heating system	1 <input type="checkbox"/>	31
b) portable cold mist (ultrasonic or spinning disc)	2 <input type="checkbox"/>	
c) portable hot mist vaporiser	3 <input type="checkbox"/>	
d) other: _____	4 <input type="checkbox"/>	

47.2 Under what circumstances do you use your humidifier?  
ONE

	TICK BOX ONLY	
a) only when someone is ill - in their room	1 <input type="checkbox"/>	32
b) to humidify the house	2 <input type="checkbox"/>	
d) other: _____	3 <input type="checkbox"/>	

Animals, dust and feathers

48. Do you keep a cat?  NO  YES 33

**IF 'NO' GO TO QUESTION 49, IF 'YES':**

48.1 Is your cat ever allowed into your bedroom?  NO  YES 34

48.2 Do all your cats stay outside the house?  NO  YES 35

49. Do you keep a dog?  NO  YES 36

**IF 'NO' GO TO QUESTION 50, IF 'YES':**

NO YES

**ECRHS APPENDIX B 1 Main Questionnaire**

49.1 Is your dog ever allowed into your bedroom?

<input type="checkbox"/>	<input type="checkbox"/>	37
--------------------------	--------------------------	----

49.2 Do all your dogs stay outside the house?

<input type="checkbox"/>	<input type="checkbox"/>	38
--------------------------	--------------------------	----

NO YES

50. Do you keep any birds?

<input type="checkbox"/>	<input type="checkbox"/>	39
--------------------------	--------------------------	----

**IF 'NO' GO TO QUESTION 51, IF 'YES':**

50.1 Are any of these birds kept inside the house?

<input type="checkbox"/>	<input type="checkbox"/>	40
--------------------------	--------------------------	----

51.1-12 When you were a child did anyone in your household keep any of the following pets?

51.1 cats

<input type="checkbox"/>	<input type="checkbox"/>	41
--------------------------	--------------------------	----

51.2 dogs

<input type="checkbox"/>	<input type="checkbox"/>	42
--------------------------	--------------------------	----

51.3 horses

<input type="checkbox"/>	<input type="checkbox"/>	43
--------------------------	--------------------------	----

51.4 birds

<input type="checkbox"/>	<input type="checkbox"/>	44
--------------------------	--------------------------	----

51.5 guinea pigs

<input type="checkbox"/>	<input type="checkbox"/>	45
--------------------------	--------------------------	----

51.6 hamsters

<input type="checkbox"/>	<input type="checkbox"/>	46
--------------------------	--------------------------	----

51. continued...

51.7 mice

<input type="checkbox"/>	<input type="checkbox"/>	47
--------------------------	--------------------------	----

51.8 rats

<input type="checkbox"/>	<input type="checkbox"/>	48
--------------------------	--------------------------	----

51.9 rabbits

<input type="checkbox"/>	<input type="checkbox"/>	49
--------------------------	--------------------------	----

51.10 gerbils

<input type="checkbox"/>	<input type="checkbox"/>	50
--------------------------	--------------------------	----

51.11 ferrets

<input type="checkbox"/>	<input type="checkbox"/>	51
--------------------------	--------------------------	----

51.12 other: \_\_\_\_\_

<input type="checkbox"/>	<input type="checkbox"/>	52
--------------------------	--------------------------	----

52.1-6 When you are near animals, such as cats, dogs or horses, near feathers, including pillows, quilts or duvets, or in a dusty part of the house, do you *ever*

52.1 start to cough?

<input type="checkbox"/>	<input type="checkbox"/>	53
--------------------------	--------------------------	----

52.2 start to wheeze?

<input type="checkbox"/>	<input type="checkbox"/>	54
--------------------------	--------------------------	----

52.3 get a feeling of tightness in your chest?

<input type="checkbox"/>	<input type="checkbox"/>	55
--------------------------	--------------------------	----

52.4 start to feel short of breath?

<input type="checkbox"/>	<input type="checkbox"/>	56
--------------------------	--------------------------	----

52.5 get a runny or stuffy nose or start to sneeze?

<input type="checkbox"/>	<input type="checkbox"/>	57
--------------------------	--------------------------	----

52.6 get itchy or watering eyes?

<input type="checkbox"/>	<input type="checkbox"/>	58
--------------------------	--------------------------	----

Trees, grass, plants, flowers and pollen

53.1-6 When you are near trees, grass or flowers, or when there is a lot of pollen about, do you *ever*

53.1 start to cough?

<input type="checkbox"/>	<input type="checkbox"/>	59
--------------------------	--------------------------	----

53.2 start to wheeze?

<input type="checkbox"/>	<input type="checkbox"/>	60
--------------------------	--------------------------	----

53.3 get a feeling of tightness in your chest?

<input type="checkbox"/>	<input type="checkbox"/>	61
--------------------------	--------------------------	----

**ECRHS APPENDIX B 1 Main Questionnaire**

- |   |                          |                          |    |
|---|--------------------------|--------------------------|----|
| 53.4 start to feel short of breath?                 | <input type="checkbox"/> | <input type="checkbox"/> | 62 |
| 53.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> | 63 |
| 53.6 get itchy or watering eyes?                    | <input type="checkbox"/> | <input type="checkbox"/> | 64 |

**IF 'YES' TO ANY OF THE ABOVE:**

- |   |                          |                          |    |
|---|--------------------------|--------------------------|----|
| 53.1.1-4 Which time of year does this happen? |                          |                          |    |
| 53.1.1 winter                                 | <input type="checkbox"/> | <input type="checkbox"/> | 65 |
| 53.1.2 spring                                 | <input type="checkbox"/> | <input type="checkbox"/> | 66 |
| 53.1.3 summer                                 | <input type="checkbox"/> | <input type="checkbox"/> | 67 |
| 53.1.4 autumn                                 | <input type="checkbox"/> | <input type="checkbox"/> | 68 |

Diet

54. How often do you eat pre-packaged food, such as tinned food or pre-prepared frozen meals?
- |                           |   |                          |    |
|---------------------------|---|--------------------------|----|
| a) every day or most days | 1 | <input type="checkbox"/> | 69 |
| b) at least once a week   | 2 | <input type="checkbox"/> |    |
| c) less than once a week  | 3 | <input type="checkbox"/> |    |

55. How often do you drink sweet fizzy drinks?
- |                           |   |                          |    |
|---------------------------|---|--------------------------|----|
| a) every day or most days | 1 | <input type="checkbox"/> | 70 |
| b) at least once a week   | 2 | <input type="checkbox"/> |    |
| c) less than once a week  | 3 | <input type="checkbox"/> |    |

56. Do you take snacks between meals?
- |                          |                          |    |
|--------------------------|--------------------------|----|
| <input type="checkbox"/> | <input type="checkbox"/> | 71 |
|--------------------------|--------------------------|----|

**IF 'NO' GO TO QUESTION 57, IF 'YES':**

- 56.1-3 Which of the following would you have as a snack at least *once a week*?
- |   |                          |                          |    |
|---|--------------------------|--------------------------|----|
| 56.1 savoury biscuits or crisps           | <input type="checkbox"/> | <input type="checkbox"/> | 72 |
| 56.2 sweets, chocolates or sweet biscuits | <input type="checkbox"/> | <input type="checkbox"/> | 73 |
| 56.3 fruit or vegetables                  | <input type="checkbox"/> | <input type="checkbox"/> | 74 |

57. Have you ever had an illness or trouble caused by eating a *particular* food or foods?
- |                          |                          |    |
|--------------------------|--------------------------|----|
| <input type="checkbox"/> | <input type="checkbox"/> | 75 |
|--------------------------|--------------------------|----|

**IF 'NO' GO TO QUESTION 58, IF 'YES':**

- 57.1 Have you nearly always had the same illness or trouble after eating this type of food?
- |                          |                          |    |
|--------------------------|--------------------------|----|
| <input type="checkbox"/> | <input type="checkbox"/> | 76 |
|--------------------------|--------------------------|----|

BLANK 77-78

**ECRHS APPENDIX B 1 Main Questionnaire**

CARD NUMBER

--	--

79-80

**IF 'NO' GO TO QUESTION 58, IF 'YES':**

CARD 5  
DUPLICATE 1-9

57.1.1 What type of food was this? [List up to 3]

---



---



---


10-11  
12-13  
14-15

57.1.2.1-6 Did this illness or trouble include

57.1.2.1 a rash or itchy skin?

57.1.2.2 diarrhoea or vomiting?

57.1.2.3 runny or stuffy nose?

57.1.2.4 severe headaches?

57.1.2.5 breathlessness?

57.1.2.6 other: \_\_\_\_\_

NO	YES

16  
17  
18  
19  
20  
21

Smoking

58. Have you ever smoked for as long as a year?

NO	YES

22

['YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year]

**ECRHS APPENDIX B 1 Main Questionnaire**

**IF 'NO' GO TO QUESTION 59, IF 'YES':**

58.1 How old were you when you started smoking? YEARS  
  23-24

58.2 Do you **now** smoke, as of *one month ago*? NO YES  
  25

**IF 'NO' GO TO QUESTION 58.3.1, IF 'YES':**

58.2.1-4 How much do you **now** smoke on average

58.2.1 number of cigarettes per day NUMBER  
  26-27

58.2.2 number of cigarillos per day   28-29

58.2.3 number of cigars a week   30-31

58.2.4 pipe tobacco in a) ounces / week   32-33

b) grams / week   34-36

58.3 Have you stopped or cut down smoking? NO YES  
  37

**IF 'NO' GO TO QUESTION 58.4, IF 'YES':**

58.3.1 How old were you when you stopped or cut down smoking? YEARS  
  38-39

58.3.2.1-4 **On average** of the entire time you smoked, before you stopped or cut down, how much did you smoke?

58.3.2.1 number of cigarettes per day NUMBER  
  40-41

58.3.2.2 number of cigarillos per day   42-43

58.3.2.3 number of cigars a week   44-45

58.3.2.4 pipe tobacco in a) ounces / week   46-47

b) grams / week   48-50

58.4 Do you or did you inhale the smoke? NO YES  
  51

59. Have you been **regularly** exposed to tobacco smoke in the last **12 months**? ['Regularly' means on most days or nights] NO YES  
  52

**IF 'NO' GO TO QUESTION 60, IF 'YES':**

59.1 Not counting yourself, how many people in your household smoke regularly? NUMBER  
  53-54

59.2 Do people smoke regularly in the room where you work? NO YES  
  55

59.3 How many hours per day are you exposed to **other people's** tobacco smoke? HOURS  
  56-57

Medicines and inhalers

**ECRHS APPENDIX B 1 Main Questionnaire**

60. Have you used any **inhaled** medicines to help your breathing at any time in the last **12 months**? NO YES  
  58

**IF 'NO' GO TO QUESTION 61, IF 'YES':**

60.1-6 Which of the following have you used in the last **12 months**?

60.1 **beta-2-agonist inhalers** NO YES  
  59

60.1.1 If used, which one? \_\_\_\_\_   60-61

60.2 **non-specific adrenoreceptor agonist inhalers** NO YES  
  62

60.2.1 If used, which one? \_\_\_\_\_  63

60.3 **anti-muscarinic inhalers** NO YES  
  64

60.3.1 If used, which one? \_\_\_\_\_  65

60.4 **inhaled steroids** NO YES  
  66

60.4.1 If used, which one? \_\_\_\_\_  67

60.5 **other inhalers (non-steroid, single drug)** NO YES  
  68

60.5.1 If used, which one? \_\_\_\_\_  69

60.6 **inhaled compound inhalers** NO YES  
  70

60.6.1 If used, which one? \_\_\_\_\_  71

61. Have you used any **pills, capsules, tablets** or **medicines**, other than inhaled medicines, to help your breathing at any time in the last **12 months**? NO YES  
  72

**IF 'NO' GO TO QUESTION 62, IF 'YES':**

61.1-6 Which of the following have you used in the last **12 months**?

61.1 **oral specific beta-2-agonists** NO YES  
  73

61.1.1 If used, which one? \_\_\_\_\_  74

61.2 **oral non-specific adrenoreceptor agonists** NO YES  
  75

61.2.1 If used, which one? \_\_\_\_\_  76

61.3 **oral anti-muscarinic drugs** NO YES  
  77

61.3.1 If used, which one? \_\_\_\_\_  78

**ECRHS APPENDIX B 1 Main Questionnaire**

CARD NUMBER   79-80  
 CARD 6  
 DUPLICATE 1-9

61.4 **oral methylxanthines**  NO  YES 10

60.4.1 If used, which one? \_\_\_\_\_  11

61.5 **oral steroids**  NO  YES 12

61.5.1 If used, which one? \_\_\_\_\_   13-14

61.6 **oral antihistamines**  NO  YES 15

61.6.1 If used, which one? \_\_\_\_\_  16

61.7 **oral compound bronchodilators (no sedatives)**  NO  YES 17

61.7.1 If used, which one? \_\_\_\_\_  18

61.8 **oral compound bronchodilators with sedatives**  NO  YES 19

61.8.1 If used, which one? \_\_\_\_\_  20

61.9 **other oral medications**  NO  YES 21

61.9.1 If used, which one? \_\_\_\_\_  22

62. Have you ever been vaccinated for allergy at any time in your life?  NO  YES  DON'T KNOW 23

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 63, IF 'YES':**

62.1 Have you been vaccinated for allergy in the last *12 months*?  NO  YES 24

63. Have you had any other **injections** to help your breathing at any time in the last *12 months*?  NO  YES 25

**IF 'NO' GO TO QUESTION 64, IF 'YES':**

63.1 What injections? \_\_\_\_\_  26  
 \_\_\_\_\_  27

64. Have you had any suppositories to help your breathing at any time in the last *12 months*?  NO  YES 28

**IF 'NO' GO TO QUESTION 65, IF 'YES':**

**ECRHS APPENDIX B 1 Main Questionnaire**

64.1 What suppositories? \_\_\_\_\_  29  
 \_\_\_\_\_  30

65. Have you used any other **remedies** to help your breathing at any time in the last **12 months**?  NO  YES 31

**IF 'NO' GO TO QUESTION 66, IF 'YES':**

65.1 What remedies? \_\_\_\_\_  32  
 \_\_\_\_\_  33  
 \_\_\_\_\_  34

66. Do you take drugs every day to help your breathing even if you don't feel short of breath?  NO  YES 35

**IF 'NO' GO TO QUESTION 67, IF 'YES':**

66.1 Which drugs? \_\_\_\_\_   36-37  
 \_\_\_\_\_   38-39  
 \_\_\_\_\_   40-41  
 \_\_\_\_\_   42-43  
 \_\_\_\_\_   44-45  
 \_\_\_\_\_   46-47  
 \_\_\_\_\_   48-49  
 \_\_\_\_\_   50-51

67. Do you take any drugs **only** for attacks of breathlessness?  NO  YES 52

**IF 'NO' GO TO QUESTION 68, IF 'YES':**

67.1 Which drugs? \_\_\_\_\_   53-54  
 \_\_\_\_\_   55-56  
 \_\_\_\_\_   57-58  
 \_\_\_\_\_   59-60  
 \_\_\_\_\_   61-62  
 \_\_\_\_\_   63-64

67.2 Do you take these drugs **TICK ONE BOX ONLY**  
 a) at the onset of the attack? 1  65  
 b) only when the attack becomes more severe? 2

68. Has your doctor ever prescribed medicines, including inhalers, for your breathing?  NO  YES 66

**IF 'NO' GO TO QUESTION 69, IF 'YES':**

**ECRHS APPENDIX B 1 Main Questionnaire**

68.1 If you are prescribed medicines for your breathing, do you *normally* take

- a) all of the medicine?
- b) most of the medicine?
- c) some of the medicine?
- d) none of the medicine?

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

67

68.2 *When your breathing gets worse*, and you are prescribed medicines for your breathing, do you normally take

- a) all of the medicine?
- b) most of the medicine?
- c) some of the medicine?
- d) none of the medicine?

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

68

68.3 Do you think it is bad for you to take medicines all the time to help your breathing?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

69

68.4 Do you think you should take as much medicine as you need to get rid of *all* your breathing problems?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

70

69. Have you ever visited a hospital casualty department or emergency room because of breathing problems?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

71

70. Have you ever spent a night in hospital because of breathing problems?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

72

**IF 'NO' GO TO QUESTION 71, IF 'YES':**

70.1 How many times in the last *12 months*?

NUMBER

<input type="text"/>	<input type="text"/>
----------------------	----------------------

73-74

71. Have you ever been seen by a doctor because of breathing problems or because of shortness of breath?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

75

**IF 'NO' GO TO END, IF 'YES':**

71.1 When was the last time you were seen by a doctor because of breathing problems or because of shortness of breath?

- a) within the last seven days
- b) more than seven days ago but within the last four weeks
- c) more than four weeks ago but within the last 12 months
- d) more than a year ago

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

76

71.2 *Where* were you seen?

TICK ONE BOX ONLY

**ECRHS APPENDIX B 1 Main Questionnaire**

- a) by a GP at home
- b) by a GP in his office or surgery
- c) by a specialist at home
- d) by a specialist in his office or hospital outpatients department
- e) in a casualty department or emergency room
- f) admitted to a hospital ward

1	
2	
3	
4	
5	
6	

77

**END**

FIELDWORKER NUMBER

--

78

CARD NUMBER

--	--

79-80

## **Appendix D**

### **ECRHS II Screening questionnaire**



# THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY II



## ECRHS II

### SCREENING QUESTIONNAIRE

**Project Leaders:**

Prof Peter Burney  
Dr Deborah Jarvis

For further information:

[www.ecrhs.org](http://www.ecrhs.org)



ECRHS II was funded by  
the European Commission  
as part of their quality of  
Life Programme

*Note:* Researchers using these materials are requested to cite the source appropriately

# ECRHS II Screening Questionnaire

Area number	<input type="text"/>	1-3		
Personal number	<input type="text"/>	4-8		
Sample			<input type="text"/>	9

**TO ANSWER THE QUESTIONS PLEASE CHOOSE THE APPROPRIATE BOX  
IF YOU ARE UNSURE OF THE ANSWER PLEASE CHOOSE 'NO'**

1. Have you had wheezing or whistling in your chest at any time in the last 12 months? NO YES  
  10

**IF 'NO' GO TO QUESTION 2, IF 'YES':**

1.1. Have you been at all breathless when the wheezing noise was present? NO YES  
  11

1.2. Have you had this wheezing or whistling when you did not have a cold? NO YES  
  12

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months? NO YES  
  13

3. Have you been woken by an attack of shortness of breath at any time in the last 12 months? NO YES  
  14

4. Have you been woken by an attack of coughing at any time in the last 12 months? NO YES  
  15

5. Have you had an attack of asthma in the last 12 months? NO YES  
  16

6. Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma? NO YES  
  17

7. Do you have any nasal allergies including hay fever? NO YES  
  18

8. What is your date of birth? DAY MONTH YEAR  
   19-24

9. What is today's date? DAY MONTH YEAR  
   25-30

10. Are you male or female? MALE FEMALE  
  31

**THANK YOU FOR YOUR HELP**

**If you don't mind being telephoned at home or at work by one of the study team, please write your telephone number below:**

<input type="text"/>	32
<input type="text"/>	33
<input type="text"/>	34

(DAY).....(EVE).....

# **Appendix E**

## **ECRHS II Main questionnaire**



# THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY II



## ECRHS II

### MAIN QUESTIONNAIRE

**Project Leaders:**

Prof Peter Burney  
Dr Deborah Jarvis

**For further information:**

[www.ecrhs.org](http://www.ecrhs.org)



ECRHS II was funded by  
the European Commission  
as part of their Quality of  
Life Programme

*Note:* Researchers using these materials are requested to cite the source  
appropriately

Centre number				
Personal number				
Sample				
Date				
	DAY	MONTH	YEAR	

**I AM GOING TO ASK YOU SOME QUESTIONS. AT FIRST THESE WILL BE MOSTLY ABOUT YOUR BREATHING. WHEREVER POSSIBLE, I WOULD LIKE YOU TO ANSWER 'YES' OR 'NO'.**

1. Have you had wheezing or whistling in your chest at any time in the last **12 months**? NO YES

**IF 'NO' GO TO QUESTION 2, IF 'YES':**

1.1 Have you been at all breathless when the wheezing noise was present? NO YES

1.2. Have you had this wheezing or whistling when you did **not** have a cold? NO YES

2. Have you woken up with a feeling of tightness in your chest at any time in the last **12 months**? NO YES

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last **12 months**? NO YES

4. Have you had an attack of shortness of breath that came on **following** strenuous activity at any time in the last **12 months**? NO YES

5. Have you been woken by an attack of shortness of breath at any time in the last **12 months**? NO YES

**IF NO GO TO Q6, IF YES**

5.1 Have you been woken by an attack of shortness of breath in the last **3 months**? NO YES

**IF NO GO TO Q6, IF YES**

5.1.1 **On average** have you been woken by an attack of shortness of breath **at least once a week in the last 3 months**? NO YES

**IF NO GO TO Q6, IF YES**

5.1.1.1 How many times a week **on average** have you been woken by shortness of breath in the **last 3 months**? TIMES

6. Have you been woken by an attack of coughing at any time **in the last 12 months**? NO YES

7. Do you **usually** cough first thing in the morning in the winter? NO YES  
   
**[IF DOUBTFUL, USE QUESTION 8.1 TO CONFIRM]**

8. Do you **usually** cough during the day, or at night, in the winter? NO YES

**IF 'NO' GO TO QUESTION 9, IF 'YES':**

8.1 Do you cough like this on most days for as much as three months each year? NO YES

9. Do you *usually* bring up any phlegm from your chest first thing in the morning in the winter? NO YES

**[IF DOUBTFUL, USE QUESTION 10.1 TO CONFIRM]**

10. Do you *usually* bring up any phlegm from your chest during the day, or at night, in the winter? NO YES

**IF 'NO' GO TO QUESTION 11, IF 'YES':**

10.1 Do you bring up phlegm like this on most days for as much as three months each year? NO YES

11. Do you ever have trouble with your breathing? NO YES

**IF 'NO' GO TO QUESTION 12, IF 'YES':**

11.1 Do you have this trouble TICK ONE BOX ONLY  
a) continuously so that your breathing is never quite right? 1   
b) repeatedly, but it always gets completely better? 2   
c) only rarely? 3

12. Are you disabled from walking by a condition *other than* heart or lung disease? NO YES

**IF 'YES' STATE CONDITION \_\_\_\_\_ AND GO TO QUESTION 13,**

**IF 'NO':**

12.1 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? NO YES

**IF 'NO' GO TO QUESTION 13, IF 'YES':**

12.1.1 Do you get short of breath walking with other people of your own age on level ground? NO YES

**IF 'NO' GO TO QUESTION 13, IF 'YES':**

12.1.1.1 Do you have to stop for breath when walking at your own pace on level ground? NO YES

**13. FOR WOMEN ONLY - MEN GO TO Q14**

Have you ever noticed that you had respiratory symptoms (such as wheeze, tightness in your chest or shortness of breath) at a particular time of your monthly cycle?

TICK ONE BOX ONLY  
yes, in the week before my period 1   
yes, during my period 2   
yes, in the week after my period 3   
yes, another time of the month 4   
does not apply to me (i.e., amenorrhoeal) 5   
No 6

NO YES

14. Have you ever had asthma?

**IF 'NO' GO TO QUESTION 15, IF 'YES':**

14.1 Was this confirmed by a doctor?    
NO YES  
YEARS

14.2 How old were you when you had your first attack of asthma?    
YEARS

14.3 How old were you when you had your most recent attack of asthma?

14.4.1-6 Which months of the year do you usually have attacks of asthma?

14.4.1 January / February    
14.4.2 March / April    
14.4.3 May / June    
14.4.4 July / August    
14.4.5 September / October    
14.4.6 November / December    
NO YES

14.5 Have you had an attack of asthma in the last **12 months**?    
**IF NO GO TO 14.8, IF YES**  
ATTACKS

14.6 How many attacks of asthma have you had in the last **12 months**?    
ATTACKS

14.7 How many attacks of asthma have you had in the last **3 months**?

14.8 How many times have you woken up because of your asthma in the last **3 months**? TICK ONE BOX ONLY

every night or almost every night 1   
more than once a week, but not most nights 2   
at least twice a month, but not more than once a week 3   
less than twice a month 4   
not at all 5

14.9. How often have you had trouble with your breathing because of your asthma in the last **3 months**? TICK ONE BOX ONLY

continuously 1   
about once a day 2   
at least once a week, but less than once a day 3   
less than once a week 4   
not at all 6

14.10 Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?    
NO YES

14.11 Do you have a peak flow meter of your own?    
NO YES

**IF 'NO' GO TO QUESTION 14.12 , IF 'YES':**

14.11.1 How often have you used it over the last 3 months? TICK ONE BOX ONLY

- never 1
- some of the days 2
- most of the days 3

14.12 Do you have written instructions from your doctor on how to manage your asthma if it gets worse or if you have an attack? NO YES

14.13. **FOR WOMEN ONLY - MEN GO TO Q15**

Have you ever noticed that your asthma got worse with your monthly cycle? TICK ONE BOX ONLY

- Yes, in the week before my period 1
- Yes, during my period 2
- Yes, in the week after my period 3
- Yes, another time of the month 4
- Does not apply to me (i.e., amenorrhoeal) 5
- No 6

14.14 Have you been pregnant (at least 25 weeks) since your asthma started? NO YES

***IF NO GO TO Q15, IF YES***

14.14.1. What happened to your asthma during your pregnancies?

TICK ONE BOX ONLY

- got better 1
- got worse 2
- stayed the same 3
- not the same for all pregnancies 4
- don't know 5

15. Do you have any nasal allergies, including hay fever? NO YES

***IF NO GO TO Q16, IF YES***

15.1 How old were you when you first had hay fever or nasal allergy? YEARS

16. Have you ever had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu? NO YES

***IF NO GO TO Q17, IF YES***

16.1. Have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu ***in the last 12 months?*** NO YES

***IF NO GO TO Q17, IF YES***

16.1.1. Has this nose problem been accompanied by itchy or watery eyes? NO YES

16.1.2. In which months of the year did this nose problem occur? NO YES

- January
- February
- March
- April
- May
- June
- July

August  
September  
October  
November  
December

<input type="checkbox"/>	<input type="checkbox"/>

17. *Since the last survey* have you used any medication to treat nasal disorders? NO YES

**IF NO GO TO Q18, IF YES**

17.1 Have you used any of the following nasal sprays for the treatment of your nasal disorder?

{SHOW LIST OF STEROID NASAL SPRAYS}

**IF NO GO TO Q17.2, IF YES**

17.1.1 How many years have you been taking this sort of nasal spray?

YEARS

17.1.2 Have you used any of these nasal sprays *in the last 12 months*?

NO YES

17.2 Have you used any of the following pills, capsules, or tablets for the treatment of your nasal disorder?

{SHOW LIST OF ANTIHISTAMINES}

**IF NO GO TO Q18, IF YES**

17.2.1 How many years have you been taking these sort of pills, capsules or tablets?

YEARS

17.2.2 Have you used any of these pills, capsules or tablets *in the last 12 months*?

NO YES

18. Have you *ever* had eczema or any kind of skin allergy?

NO YES

19. Have you *ever* had an itchy rash that was coming and going for at least 6 months?

NO YES

**IF 'NO' GO TO QUESTION 20, IF 'YES':**

19.1. Have you had this itchy rash *in the last 12 months*?

NO YES

**IF 'NO' GO TO QUESTION 20, IF 'YES':**

19.1.1. Has this itchy rash *at any time* affected any of the following places:  
the folds of the elbows, behind the knees, in front of the ankles  
under the buttocks or around the neck, ears or eyes

NO YES

20. Have you ever had any difficulty with your breathing after taking medicines?

NO YES

**IF 'NO' GO TO QUESTION 21, IF 'YES':**

20.1-2 Which medicines? \_\_\_\_\_

20.1.   
20.1.2

21. How old was your mother when you were born?

YEARS

22. How many times did you move house during the first five

years of your life?  
 None  
 Once  
 more than once

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>

23. Were you hospitalised before the age of two years for lung disease?

NO	<input type="checkbox"/>	YES	<input type="checkbox"/>
YEARS			

24. At what age did you first attend a school, play school, day care or nursery?

<input type="checkbox"/>
--------------------------

25. How many *other* children regularly slept in your bedroom before *you were five years old*?

CHILDREN
<input type="checkbox"/>

I would now like to ask you some questions on the type of jobs that you have done.

I am interested in each one of the jobs that you have done for more than 3 consecutive months since the time we last contacted you (in 1991/2). These jobs may be outside the house or at home, full time or part time, paid or not paid, including self employment, for example in a family business. Please include part time jobs only if you had been doing them for more than 8 hours per week.

Q26. Are you currently

TICK ONE BOX ONLY

Employed (including military service)	1	<input type="checkbox"/>
Self employed	2	<input type="checkbox"/>
Unemployed, looking for work	3	<input type="checkbox"/>
Not working because of poor health	4	<input type="checkbox"/>
Full-time house-person	5	<input type="checkbox"/>
Full time student	6	<input type="checkbox"/>
Retired	7	<input type="checkbox"/>
Other	8	<input type="checkbox"/>

***IF EMPLOYED OR SELF EMPLOYED OR A FULL TIME HOUSEPERSON GO TO Q28***

27. Have you been employed in any job for three continuous months or longer since the last survey?

NO	<input type="checkbox"/>	YES	<input type="checkbox"/>
----	--------------------------	-----	--------------------------

***IF YES NOW GO TO OCCUPATIONAL MATRIX***

**Appendix B 1 – ECRHS II Main Questionnaire**

Centre number 

--	--	--	--	--

  
 Personal number 

--	--	--	--	--	--	--	--

  
 Sample 

--	--	--	--

  
 Date 

--	--	--	--	--	--	--	--

  
DAY MONTH YEAR

Q 28. If you had more than one job in the same company, or if you were doing more than one job at the same time, we would like to talk about them separately. Please start with your current or last job.

JOB	Q28.1. What is (was) the title of your current (last) job?	Q28.2. What did the firm, company or organisation do or what services did it provide?	Q28.3. In what month and year did you start working in this job?				Q28.4. In what month and year did you stop working in this job?			
	<i>OCCUPATION</i>	<i>INDUSTRY</i>	MONTH	YEAR			MONTH	YEAR		
JOB 1										
JOB 2										
JOB 3										
JOB 4										
JOB 5										
JOB 6										
JOB 7										
JOB 8										
JOB 9										
JOB 10										

**Appendix B 1 – ECRHS II Main Questionnaire**

29. Have any of these jobs ever made your chest tight or wheezy? NO YES

***IF YES, (tick no or yes for each job)***

	NO		YES	
Job 1?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 2?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 3?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 4?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 5?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 6?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 7?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 8?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 9?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 10?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

30. Have you had to leave any of these jobs because they affected your breathing? NO YES

***IF YES, (tick no or yes for each job)***

	NO		YES	
Job 1?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 2?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 3?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 4?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 5?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 6?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 7?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 8?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 9?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Job 10?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

31. Since the last survey have you been involved in an accident at home, work or elsewhere that exposed you to high levels of vapours, gas, dust or fumes? NO YES

**IF YES,**

31.1 Did you experience respiratory symptoms immediately following this exposure? NO YES

**IF YES**

31.1.1 Could you describe to me what it was? \_\_\_\_\_

Centres performing the extra occupational modules should at this point introduce the modular introductory questionnaire and complete modules as appropriate.

32. At what age did you complete full time education? YEARS  
   
**If full time student enter 88**

33. How often do you usually exercise so much that you get out of breath or sweat? TICK ONE BOX ONLY

every day	1	<input type="checkbox"/>
4-6 times a week	2	<input type="checkbox"/>
2-3 times a week	3	<input type="checkbox"/>
once a week	4	<input type="checkbox"/>
once a month	5	<input type="checkbox"/>
less than once a month	6	<input type="checkbox"/>
never	7	<input type="checkbox"/>

34. How many hours a week do you usually exercise so much that you get out of breath or sweat? TICK ONE BOX ONLY

none	1	<input type="checkbox"/>
about ½ hr	2	<input type="checkbox"/>
about 1 hour	3	<input type="checkbox"/>
about 2-3 hours	4	<input type="checkbox"/>
about 4-6 hours	5	<input type="checkbox"/>
7 hours or more	6	<input type="checkbox"/>

35. Do you avoid taking vigorous exercise because of wheezing or asthma? NO YES

36. When was your present home built? YEAR

37. Do you live in the same home as when you were last surveyed? NO YES

**IF YES GO TO QUESTION 38, IF NO**

37.1. How many times have you moved since you were last surveyed? TIMES

37.2. How many years have you lived in your current home? YEARS

37.3 Where do you currently live? TICK ONE BOX ONLY

a different home, but still in the study sampling area	1	<input type="checkbox"/>
outside the sampling area but still in the same country	2	<input type="checkbox"/>
a different country	3	<input type="checkbox"/>

37.3.1. **IF A DIFFERENT COUNTRY** Which country?

**Appendix B 1 – ECRHS II Main Questionnaire**

37.4 Which best describes the building in which you live? **TICK ONE BOX ONLY**

- |   |   |                          |
|---|---|--------------------------|
| a) a mobile home or trailer?                          | 1 | <input type="checkbox"/> |
| b) a one family house detached from any other house?  | 2 | <input type="checkbox"/> |
| c) a one family house attached to one or more houses? | 3 | <input type="checkbox"/> |
| d) a building for two families?                       | 4 | <input type="checkbox"/> |
| e) a building for three or four families?             | 5 | <input type="checkbox"/> |
| f) a building for five or more families?              | 6 | <input type="checkbox"/> |
| g) a boat, tent or van                                | 7 | <input type="checkbox"/> |
| e) other: _____                                       | 8 | <input type="checkbox"/> |

38. Does your home have any of the following?

- |  | NO                       | YES                      |
|--|--------------------------|--------------------------|
| 38.1 central heating                         | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.2 ducted air heating (forced air heating) | <input type="checkbox"/> | <input type="checkbox"/> |
| 38.3 air conditioning                        | <input type="checkbox"/> | <input type="checkbox"/> |

39. Which of the following appliances do you use for heating or for hot water?

- |                                   | NO                       | YES                      |
|-----------------------------------|--------------------------|--------------------------|
| 39.1 open coal, coke or wood fire | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.2 open gas fire                | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.3 electric heater              | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.4 paraffin heater              | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.5 gas-fired boiler             | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.6 oil-fired boiler             | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.7 portable gas heater          | <input type="checkbox"/> | <input type="checkbox"/> |
| 39.8 other: _____                 | <input type="checkbox"/> | <input type="checkbox"/> |

40. What kind of stove do you **mostly** use for cooking?

- |   | <b>TICK ONE BOX ONLY</b> |                          |
|---|--------------------------|--------------------------|
| a) coal, coke or wood (solid fuel)?                 | 1                        | <input type="checkbox"/> |
| b) gas (gas from the mains)?                        | 2                        | <input type="checkbox"/> |
| c) electric?  | 3                        | <input type="checkbox"/> |
| d) paraffin (kerosene)?                             | 4                        | <input type="checkbox"/> |
| e) microwave  | 5                        | <input type="checkbox"/> |
| f) gas (gas from bottles or other non-mains source) | 6                        | <input type="checkbox"/> |
| g) other: _____                                     | 7                        | <input type="checkbox"/> |

**40.1 IF YOU USE GAS FOR COOKING** Which of the following do you have?

- |                 | NO                       | YES                      |
|-----------------|--------------------------|--------------------------|
| 40.1.1 gas hob  | <input type="checkbox"/> | <input type="checkbox"/> |
| 40.1.2 gas oven | <input type="checkbox"/> | <input type="checkbox"/> |

41. What kind of stove was mostly used for cooking in the home you lived in when you were five years old?

- |   | <b>TICK ONE BOX ONLY</b> |                          |
|---|--------------------------|--------------------------|
| a) coal, coke or wood (solid fuel)?                 | 1                        | <input type="checkbox"/> |
| b) gas (gas from the mains)?                        | 2                        | <input type="checkbox"/> |
| c) electric?  | 3                        | <input type="checkbox"/> |
| d) paraffin?  | 4                        | <input type="checkbox"/> |
| e) gas (gas from bottles or other non-mains source) | 5                        | <input type="checkbox"/> |
| f) don't know                                       | 6                        | <input type="checkbox"/> |
| g) other: _____                                     | 7                        | <input type="checkbox"/> |

**Appendix B 1 – ECRHS II Main Questionnaire**

MINUTES

42. *On average* how long have you spent cooking with your stove each day over the *last four weeks*?

43. *Over the last four weeks* when you were cooking did you have a door or window to the outside air open TICK ONE BOX ONLY

- a) most of the time 1
- b) some of time 2
- c) rarely (or only occasionally) 3
- d) I do not have a door or window that opens to the outside in my kitchen 4

44. Do you have an extractor fan over the cooker? NO YES DK

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 45, IF 'YES':**

44.1 When cooking, do you use the fan TICK ONE BOX ONLY

- a) all of the time? 1
- b) some of the time? 2
- c) none of the time? 3

44.2 Does the fan take the fumes outside the house? NO YES DK

45. Does the room which you use most at home during the day NO YES  
 45.1 have fitted carpets covering the whole floor?    
 45.2 contain rugs?    
 45.3 have double glazing?

46 How old is the oldest carpet or rug in the room which you use most at home during the day? , TICK ONE BOX ONLY

- a) less than one year 1
- b) 1-5 years old 2
- c) more than 5 years old 3

47 On what floor is the room which you use most at home during the day?    
*(The lowest floor of a building is 00)*

48. Does your bedroom NO YES  
 48.1 have fitted carpets covering the whole floor?    
 48.2 contain rugs?    
 48.3 have double glazing?

49 How old is the oldest carpet or rug in your bedroom TICK ONE BOX ONLY  
 a) less than one year   
 b) 1-5 years old   
 c) more than 5 years old

TICK ONE BOX ONLY

50 How old is your mattress

a) less than one year	<input type="checkbox"/>
b) 1-5 years old	<input type="checkbox"/>
c) more than 5 years old	<input type="checkbox"/>

51 What floor of the building is your bedroom on? (lowest=00)

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

NO YES

52. Do you sleep with the windows open at night during winter?

<input type="checkbox"/>	<input type="checkbox"/>
--------------------------	--------------------------

**IF 'NO' GO TO QUESTION 53, IF 'YES':**

52.1 Do you sleep with the windows open

a) all of the time?	TICK ONE BOX ONLY	1	<input type="checkbox"/>
b) sometimes?		2	<input type="checkbox"/>
c) only occasionally?		3	<input type="checkbox"/>

53. Has there been any water damage to the building or its contents, for example, from broken pipes, leaks or floods?

NO	YES	DK
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**IF YES**

53.1 Has there been any water damage in the last 12 months

NO	YES	DK
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

54. **Within the last 12 months** have you had wet or damp spots on surfaces inside your home other than in the basement (for example on walls, wall paper, ceilings or carpets)?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

55. Has there ever been any mould or mildew on any surface, other than food, inside the home?

NO	YES	DK
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 56, IF 'YES':**

55.1.1-6 Which rooms have been affected?

55.1.1 bathroom(s)	NO	YES
55.1.2 bedroom(s)	<input type="checkbox"/>	<input type="checkbox"/>
55.1.3 living area(s)	<input type="checkbox"/>	<input type="checkbox"/>
55.1.4 kitchen	<input type="checkbox"/>	<input type="checkbox"/>
55.1.5 basement or attic	<input type="checkbox"/>	<input type="checkbox"/>
55.1.6 other: _____	<input type="checkbox"/>	<input type="checkbox"/>

55.2 Has there been mould or mildew on any surfaces inside the home in the last **12 months**?

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

**Appendix B 1 – ECRHS II Main Questionnaire**

‘This scale looks like a thermometer; it allows you to rate your personal opinion regarding the following question on annoyance from air pollution. You can indicate your level of annoyance on this scale between 0 and 10 where 0 mean does not annoy at all' and 10 means intolerable annoyance.'

56 . How much are you annoyed by outdoor air pollution (from traffic, industry, etc.) if you keep the windows open?

	10	intolerable annoyance
	9	
	8	
	7	
	6	
	5	
	4	
	3	
	2	
	1	
	0	doesn't annoy at all

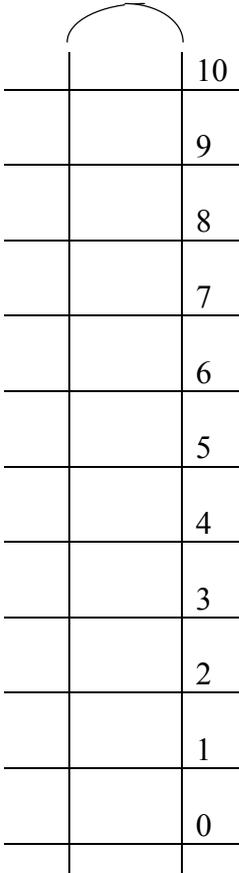
--	--

THOSE WHO HAVE NOT MOVED HOME SINCE LAST SURVEY (Check with response to question 37)

**GO TO QUESTION 58**

**THOSE WHO HAVE MOVED SINCE LAST SURVEY** – answer 57

57. How much were you annoyed by outdoor air pollution (from traffic, industry, etc.) in your previous home, if you kept the windows open?

	10	intolerable annoyance
	9	
	8	
	7	
	6	
	5	
	4	
	3	
	2	
	1	
	0	doesn't annoy at all

--	--

58. How often do cars pass your house?

- a) constantly
- b) frequently
- c) seldom
- d) never

TICK ONE BOX ONLY

1	
2	
3	
4	

59. How often do heavy vehicles (e.g. trucks/buses) pass your house? TICK ONE BOX ONLY

a) constantly		1	<input type="checkbox"/>
b) frequently		2	<input type="checkbox"/>
c) seldom		3	<input type="checkbox"/>
d) never		4	<input type="checkbox"/>

60. Have you taken any of the following measures to reduce allergen or exposure to allergen in your home since the last survey?		NO	YES
60.1 changed from carpet to a wooden or other smooth surface on floor of the room you use most		<input type="checkbox"/>	<input type="checkbox"/>
60.2 changed from carpet to a wooden or to a smooth surface on floor of your bedroom		<input type="checkbox"/>	<input type="checkbox"/>
60.3 bought a new carpet for the room you use most		<input type="checkbox"/>	<input type="checkbox"/>
60.4 bought a new carpet for your bedroom		<input type="checkbox"/>	<input type="checkbox"/>
60.5 used antidust-mite sprays		<input type="checkbox"/>	<input type="checkbox"/>
60.6 put an allergy-proof cover on your mattress		<input type="checkbox"/>	<input type="checkbox"/>
60.7 sold, given away or destroyed a pet dog or cat		<input type="checkbox"/>	<input type="checkbox"/>

61. Do you keep a cat?		NO	YES
		<input type="checkbox"/>	<input type="checkbox"/>
<b><i>IF 'NO' GO TO QUESTION 62, IF 'YES'</i></b>			
61.1 Is your cat (are your cats) allowed inside the house?		<input type="checkbox"/>	<input type="checkbox"/>
61.2 Is your cat (are your cats) allowed in the bedroom?		<input type="checkbox"/>	<input type="checkbox"/>

62. Do you keep a dog?		NO	YES
		<input type="checkbox"/>	<input type="checkbox"/>
<b><i>IF 'NO' GO TO QUESTION 63, IF 'YES':</i></b>			
62.1 Is your dog (are your dogs) allowed inside the house?		<input type="checkbox"/>	<input type="checkbox"/>
62.2 Is your dog (are your dogs) allowed in your bedroom?		<input type="checkbox"/>	<input type="checkbox"/>

63. Do you keep any birds?		NO	YES
		<input type="checkbox"/>	<input type="checkbox"/>
<b><i>IF 'NO' GO TO QUESTION 64, IF 'YES':</i></b>			
63.1 Are any of these birds kept inside the house?		<input type="checkbox"/>	<input type="checkbox"/>

64. Was there a cat in your home?		NO	YES	DK
64.1 during your first year of life		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
64.2 when you were aged 1 to 4 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
64.3 when you were aged 5-15 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

65. Was there a dog in your home?		NO	YES	DK
65.1 during your first year of life		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
65.2 when you were aged 1 to 4 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
65.3 when you were aged 5-15 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

66. Was there a bird in your home?		NO	YES	DK
66.1. during your first year of life		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
66.2 when you were aged 1 to 4 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
66.3 when you were aged 5-15 years		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

67. What term best describes the place you lived most of the time when you were under the age of five years?

TICK ONE BOX ONLY

- |                            |   |                          |
|----------------------------|---|--------------------------|
| a) farm                    | 1 | <input type="checkbox"/> |
| b) village in a rural area | 2 | <input type="checkbox"/> |
| c) small town              | 3 | <input type="checkbox"/> |
| d) suburb of a city        | 4 | <input type="checkbox"/> |
| e) inner city              | 5 | <input type="checkbox"/> |

68. When you are near animals, such as cats, dogs or horses, do you *ever*

NO YES

- |   |                          |                          |
|---|--------------------------|--------------------------|
| 68.1 start to cough?                                | <input type="checkbox"/> | <input type="checkbox"/> |
| 68.2 start to wheeze?                               | <input type="checkbox"/> | <input type="checkbox"/> |
| 68.3 get a feeling of tightness in your chest?      | <input type="checkbox"/> | <input type="checkbox"/> |
| 68.4 start to feel short of breath?                 | <input type="checkbox"/> | <input type="checkbox"/> |
| 68.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 68.6 get itchy or watering eyes?                    | <input type="checkbox"/> | <input type="checkbox"/> |

69. When you are in a dusty part of the house, or near pillows or duvets do you *ever*

NO YES

- |   |                          |                          |
|---|--------------------------|--------------------------|
| 69.1 start to cough?                                | <input type="checkbox"/> | <input type="checkbox"/> |
| 69.2 start to wheeze?                               | <input type="checkbox"/> | <input type="checkbox"/> |
| 69.3 get a feeling of tightness in your chest?      | <input type="checkbox"/> | <input type="checkbox"/> |
| 69.4 start to feel short of breath?                 | <input type="checkbox"/> | <input type="checkbox"/> |
| 69.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 69.6 get itchy or watering eyes?                    | <input type="checkbox"/> | <input type="checkbox"/> |

70. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you *ever*

NO YES

- |   |                          |                          |
|---|--------------------------|--------------------------|
| 70.1 start to cough?                                | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.2 start to wheeze?                               | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.3 get a feeling of tightness in your chest?      | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.4 start to feel short of breath?                 | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.5 get a runny or stuffy nose or start to sneeze? | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.6 get itchy or watering eyes?                    | <input type="checkbox"/> | <input type="checkbox"/> |

**IF 'YES' TO ANY OF THE ABOVE:**

70.7.1-4 Which time of year does this happen?

NO YES

- |               |                          |                          |
|---------------|--------------------------|--------------------------|
| 70.7.1 winter | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.7.2 spring | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.7.3 summer | <input type="checkbox"/> | <input type="checkbox"/> |
| 70.7.4 autumn | <input type="checkbox"/> | <input type="checkbox"/> |

71. How often do you eat pre-packaged food, such as tinned food or pre-prepared frozen meals?

TICK ONE BOX ONLY

- |                           |   |                          |
|---------------------------|---|--------------------------|
| a) every day or most days | 1 | <input type="checkbox"/> |
| b) at least once a week   | 2 | <input type="checkbox"/> |
| c) less than once a week  | 3 | <input type="checkbox"/> |

72 Do you take snacks between meals? NO YES

**IF 'NO' GO TO QUESTION 73, IF 'YES':**

72.1.1-3 Which of the following would you have as a snack at least **once a week?**

	NO	YES
72.1.1 savoury biscuits or crisps	<input type="checkbox"/>	<input type="checkbox"/>
72.1.2 sweets, chocolates or sweet biscuits	<input type="checkbox"/>	<input type="checkbox"/>
72.1.3 fruit or vegetables	<input type="checkbox"/>	<input type="checkbox"/>

73. Have you ever had an illness or trouble caused by eating a **particular** food or foods? NO YES

**IF 'NO' GO TO QUESTION 74, IF 'YES':**

73.1 Have you nearly always had the same illness or trouble after eating this type of food? NO YES

**IF 'NO' GO TO QUESTION 74, IF 'YES':**

73.1.1 What type of food was this? [List up to 3]

	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>

73.1.2.1-6 Did this illness or trouble include

	NO	YES
73.1.2.1 a rash or itchy skin?	<input type="checkbox"/>	<input type="checkbox"/>
73.1.2.2 diarrhoea or vomiting?	<input type="checkbox"/>	<input type="checkbox"/>
73.1.2.3 runny or stuffy nose?	<input type="checkbox"/>	<input type="checkbox"/>
73.1.2.4 severe headaches?	<input type="checkbox"/>	<input type="checkbox"/>
73.1.2.5 breathlessness?	<input type="checkbox"/>	<input type="checkbox"/>
73.1.2.6 other: _____	<input type="checkbox"/>	<input type="checkbox"/>

74. Have you ever smoked for as long as a year? NO YES

**['YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year]**

**IF 'NO' GO TO QUESTION 75, IF 'YES':**

74.1 How old were you when you started smoking? YEARS

74.2 Do you **now** smoke, as of **one month ago?** NO YES

**IF 'NO' GO TO QUESTION 74.3, IF 'YES':**

74.2.1-4 How much do you **now** smoke on average? NUMBER

74.2.1 number of cigarettes per day	<input type="checkbox"/>	<input type="checkbox"/>
74.2.2 number of cigarillos per day	<input type="checkbox"/>	<input type="checkbox"/>
74.2.3 number of cigars a week	<input type="checkbox"/>	<input type="checkbox"/>
74.2.4 pipe tobacco in a) ounces / week	<input type="checkbox"/>	<input type="checkbox"/>
b) grams / week	<input type="checkbox"/>	<input type="checkbox"/>

74.3 Have you stopped or cut down smoking? NO YES

**IF 'NO' GO TO QUESTION 74.4, IF 'YES':**

74.3.1 how old were you when you stopped or cut down smoking?

YEARS  

--	--

74.3.2.1-4 **on average** of the entire time you smoked, before you stopped or cut down, how much did you smoke?

NUMBER

74.3.2.1 number of cigarettes per day

--	--

74.3.2.2 number of cigarillos per day

--	--

74.3.2.3 number of cigars a week

--	--

74.3.2.4 pipe tobacco in a) ounces / week

--	--

b) grams / week

--	--	--

NO YES

74.4 Do you or did you inhale the smoke?

--	--

75. Have you been **regularly** exposed to tobacco smoke in the last **12 months**? ['Regularly' means on most days or nights]

NO YES

--	--

**IF 'NO' GO TO QUESTION 76, IF 'YES':**

75.1. Not counting yourself, how many people in your household smoke regularly?

NUMBER

--	--

75.2 Do people smoke regularly in the room where you work?

NO YES

--	--

75.3 How many hours per day are you exposed to **other people's** tobacco smoke?

HOURS

--	--

75.4 Please provide more information.

How many hours per day, are you exposed to other peoples tobacco smoke in the following locations?

HOURS

at home

--	--

at workplace

--	--

in bars, restaurants, cinemas or similar social settings

--	--

elsewhere

--	--

76. Have you used any **inhaled** medicines to help your breathing at any time in the last **12 months**?

NO YES

--	--

**IF NO' GO TO QUESTION 77, IF 'YES':**

Which of the following have you used in the last **12 months**?

NO YES

76.1 short acting **beta-2-agonist inhalers**

--	--

(Please include combinations that include beta 2 and steroids in section 76.5)

76.1.1 If used, which one? \_\_\_\_\_

--	--

76.1.2 What type of inhaler do you use?

--

76.1.3. What is the dose per puff (in micrograms)?

NUMBER

--	--	--	--

76.1.4. In the last 3 months, how have you used them:

TICK ONE BOX ONLY

a) when needed

--

b) in short courses

--

c) continuously

--

d) not at all

--

**If answer to 76.1.4 is when needed:**

76.1.5 Number of puffs per month

NUMBER

--	--

**If answer to 76.1.4 is in short courses**

- 76.1.6 number of courses
- 76.1.7 number of puffs per day
- 76.1.8 average number of days per month

NUMBER


**If answer to 76.1.4 is continuously**

- 76.1.9 number of puffs per day

NUMBER

--	--

**76.2 long acting beta-2-agonist inhalers**

(Please include combinations that include beta 2 and steroids in section 76.5)

NO YES

--	--

76.2.1 If used, which one? \_\_\_\_\_

76.2.2 What type of inhaler do you use?


NUMBER

76.2.3. What is the dose per puff (in micrograms)?

--	--

76.2.4. In the last 3 months, how have you used them:

- a) when needed
- b) in short courses
- c) continuously
- d) not at all

TICK ONE BOX ONLY

1	
2	
3	
4	

***If answer to 76.2.4 is when needed:***

76.2.5 Number of puffs per month

NUMBER

--	--

**If answer to 76.2.4 is in short courses**

- 76.2.6 number of courses
- 76.2.7 number of puffs per day
- 76.2.8 average number of days per month

NUMBER


**If answer to 76.2.4 is continuously**

76.2.9 number of puffs per day

NUMBER

--	--

**76.3 non-specific adrenoreceptor agonist inhalers**

NO YES

--	--

76.3.1 If used, which one? \_\_\_\_\_

--	--

**76.4 anti-muscarinic inhalers**

NO YES

--	--

76.4.1 If used, which one? \_\_\_\_\_

76.4.2 What type of inhaler do you use?


NUMBER

76.4.3. What is the dose per puff (in micrograms)?

--	--	--

**Appendix B 1 – ECRHS II Main Questionnaire**

76.4.4. In the last 3 months, how have you used them: **TICK ONE BOX ONLY**

a) when needed 1   
 b) in short courses 2   
 c) continuously 3   
 d) not at all 4

***If answer to 76.4.4 is when needed:*** **NUMBER**  
 76.4.5 Number of puffs per month

***If answer to 76.4.4 is in short courses*** **NUMBER**

76.4.6 number of courses    
 76.4.7 number of puffs per day    
 76.4.8 average number of days per month

***If answer to 76.4.4 is continuously*** **NUMBER**  
 76.4.9 number of puffs per day

**76.5 inhaled steroids**  
*(if combined B2 and steroid please insert inhaled steroid dose)*

**NO YES**

76.5.1 If used, which one? \_\_\_\_\_    
 76.5.2 What type of inhaler do you use?

76.5.3. What is the dose per puff (in micrograms)? **NUMBER**

76.5.4. In the last 3 months, how have you used them: **TICK ONE BOX ONLY**

a) when needed 1   
 b) in short courses 2   
 c) continuously 3   
 d) not at all 4

***If answer to 76.5.4 is when needed:*** **NUMBER**  
 76.5.5 Number of puffs per month

***If answer to 76.5.4 is in short courses*** **NUMBER**

76.5.6 number of courses    
 76.5.7 number of puffs per day    
 76.5.8 average number of days per month

***If answer to 76.5.4 is continuously*** **NUMBER**  
 76.5.9 number of puffs per day

**76.6 inhaled cromoglycate/nedocromil** **NO YES**

76.6.1 If used, which one? \_\_\_\_\_    
**NUMBER**

76.6.2. What is the dose per puff (in milligrams)?

**Appendix B 1 – ECRHS II Main Questionnaire**

76.6.3. In the last 3 months, how have you used them: **TICK ONE BOX ONLY**

a) when needed 1   
 b) in short courses 2   
 c) continuously 3   
 d) not at all 4

***If answer to 76.6.3 is when needed:*** **NUMBER**  
 76.6.4 Number of puffs per month

***If answer to 76.6.3 is in short courses*** **NUMBER**

76.6.5 number of courses    
 76.6.6 number of puffs per day    
 76.6.7 average number of days per month

***If answer to 76.6.3 is continuously*** **NUMBER**  
 76.6.8 number of puffs per day

**76.7 inhaled compounds**

76.7.1 If used, which one? \_\_\_\_\_    
 76.7.2 What type of inhaler do you use?

76.7.3. What is the dose per puff (in micrograms)? **NUMBER**

77. Have you used any **pills, capsules, tablets** or **medicines**, other than inhaled medicines, to help your breathing at any time in the last **12 months**? **NO YES**

**IF 'NO' GO TO QUESTION 78, IF 'YES':**

Which of the following have you used in the last **12 months**?

**77.1 oral beta-2-agonists** **NO YES**

77.1.1 If used, which one? \_\_\_\_\_    
 77.1.2 what dose of tablet

77.1.3. In the last 3 months, how have you used them: **TICK ONE BOX ONLY**

a) when needed 1   
 b) in short courses 2   
 c) continuously 3   
 d) not at all 4

***If answer to 77.1.3 is when needed:*** **NUMBER**  
 77.1.4 number of tablets per month

***If answer to 77.1.3 is in short courses*** **NUMBER**

77.1.5 number of courses    
 77.1.6 tablets per day    
 77.1.7 average number of days per month

**If answer to 77.1.3 is continuously**

77.1.8 tablets per day

NUMBER

--	--

NO YES

**77.2 oral methylxanthines**

--	--

77.2.1 if used, which one? \_\_\_\_\_

77.2.2 what dose of tablet

--	--	--

77.2.3. In the last 3 months, how have you used them:

TICK ONE BOX ONLY

a) when needed

1

b) in short courses

2

c) continuously

3

d) not at all

4

***If answer to 77.2.3 is when needed:***

77.2.4 number of tablets per month

NUMBER

--	--

**If answer to 77.2.3 is in short courses**

77.2.5 number of courses

77.2.6 tablets per day

77.2.7 average number of days per month

NUMBER


**If answer to 77.2.3 is continuously**

77.2.8 tablets per day

NUMBER

--	--

**77.3 oral steroids**

NO YES

--	--

77.3.1 If used, which one? \_\_\_\_\_

77.3.2 what dose of tablet

--	--	--

77.3.3. In the last 12 months, how have you used them:

TICK ONE BOX ONLY

a) when needed

1

b) in short courses

2

c) continuously

3

***If answer to 77.3.3 is when needed:***

77.3.4 number of tablets per month

NUMBER

--	--

**If answer to 77.3.3 is in short courses**

77.3.5 number of courses

77.3.6 tablets per day

77.3.7 average number of days per month

NUMBER


**If answer to 77.3.3 is continuously**

77.3.8 tablets per day

NUMBER

--	--

NO YES

--	--

77.3.9. Have you used them in the last **3 months**?

**77.4 oral anti-leukotrienes**

NO YES

77.4.1 If used, which one? \_\_\_\_\_  
 77.4.2 what dose of tablet


77.4.3. In the last 3 months, how have you used them:  
 a) when needed  
 b) in short courses  
 c) continuously  
 d) not at all

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

***If answer to 77.4.3 is when needed:***

77.4.4 number of tablets per month

NUMBER  

--	--

***If answer to 77.4.3 is in short courses***

77.4.5 number of courses  
 77.4.6 tablets per day  
 77.4.7 average number of days per month

NUMBER  


***If answer to 77.4.3 is continuously***

77.4.8 tablets per day

NUMBER  

--	--

**77.5 ketotifen**

NO YES

77.5.1 If used, which one? \_\_\_\_\_  
 77.5.2 what dose of tablet


77.5.3. In the last 3 months, how have you used them:  
 a) when needed  
 b) in short courses  
 c) continuously  
 d) not at all

TICK ONE BOX ONLY

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

***If answer to 77.5.3 is when needed:***

77.5.4 number of tablets per month

NUMBER  

--	--

***If answer to 77.5.3 is in short courses***

77.5.5 number of courses  
 77.5.6 tablets per day  
 77.5.7 average number of days per month

NUMBER  


***If answer to 77.5.3 is continuously***

77.5.8 tablets per day

NUMBER  

--	--

78. Since the last survey have you ever used inhaled steroids (show list)?

***IF NO GO TO QUESTION 79***

78.1. How old were you when you first started to use inhaled steroids?

NO YES  
   
 YEARS  

--	--

**Appendix B 1 – ECRHS II Main Questionnaire**

78.2. Have you used inhaled steroids *every year* since the last survey? NO YES  
   
**IF NO GO TO QUESTION 78.3, IF YES**

78.2.1. On average how many months each year have you taken them? MONTHS  
   
**NOW GO TO Q79**

78.3 How many of the years since the last survey have you taken inhaled steroids? YEARS

78.4. On average how many months of each of these years have you taken them? MONTHS

79. Have you been vaccinated for allergy since the last survey? NO YES DK  
    
**IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 80, IF 'YES':**

79.1 Have you been vaccinated for allergy in the last *12 months*? NO YES

80. Have you had any other **injections** to help your breathing at any time in the last *12 months*? NO YES  
   
**IF 'NO' GO TO QUESTION 81, IF 'YES':**

80.1 What injections? \_\_\_\_\_

81. Have you had any suppositories to help your breathing at any time in the last *12 months*? NO YES  
   
**IF 'NO' GO TO QUESTION 82, IF 'YES':**

81.1 What suppositories? \_\_\_\_\_

82 . Have you used any other **remedies** to help your breathing at any time in the last *12 months*? NO YES  
   
**IF 'NO' GO TO QUESTION 83 IF 'YES':**

82.1. What remedies? \_\_\_\_\_

83. Has your doctor ever prescribed medicines, including inhalers, for your breathing? NO YES

**IF 'NO' GO TO QUESTION 84, IF 'YES':**

83.1 If you are prescribed medicines for your breathing, do you *normally* take TICK ONE BOX ONLY

- a) all of the medicine? 1
- b) most of the medicine? 2
- c) some of the medicine? 3
- d) none of the medicine? 4

**Appendix B 1 – ECRHS II Main Questionnaire**

83.2 *When your breathing gets worse*, and you are prescribed medicines for your breathing, do you normally take TICK ONE BOX ONLY

a) all of the medicine? 1

b) most of the medicine? 2

c) some of the medicine? 3

d) none of the medicine? 4

83.3 Do you think it is bad for you to take medicines all the time to help your breathing? NO YES

83.4 Do you think you should take as much medicine as you need to get rid of *all* your breathing problems? NO YES

84. Since the last survey have you visited a hospital casualty department or emergency room because of breathing problems? NO YES

**IF NO GO TO Q85, IF YES**

84.1 Have you visited a hospital casualty department or emergency room because of breathing problems in the *last 12 months*? NO YES

**IF NO GO TO 85, IF YES**

84.1.1 Was this due to asthma, shortness of breath or wheezing? NO YES

84.1.2 How many times *in the last 12 months*? TIMES

85. Since the last survey have you spent a night in hospital because of breathing problems? NO YES

**IF NO GO TO Q86 IF YES**

85.1 Have you spent a night in hospital because of breathing problems in the *last 12 months*? NO YES

**IF NO GO TO Q86, IF YES**

85.1.1 Was this due to asthma, shortness of breath or wheezing? NO YES

85.1.2 How many nights have you spent on each of the following types of ward in *the last 12 months*? NUMBER

General	<input type="text"/>	<input type="text"/>
Chest medicine	<input type="text"/>	<input type="text"/>
Rehabilitation	<input type="text"/>	<input type="text"/>
Intensive care unit	<input type="text"/>	<input type="text"/>
Other	<input type="text"/>	<input type="text"/>

86. Since the last survey have you been seen by a doctor because of breathing problems or because of shortness of breath? NO YES

**IF NO GO TO Q87, IF YES**

86.1 Have you been seen by a general practitioner because of breathing problems or shortness of breath in the *last 12 months*? NO YES

**IF NO GO TO Q86.4, IF YES**

86.2. Was this due to asthma, shortness of breath or wheezing? NO YES

**Appendix B 1 – ECRHS II Main Questionnaire**

86.3 How many times have you been seen by your general practitioner because of breathing problems or shortness of breath in each of these locations over the last 12 months?

at home (excluding emergency visits)

in his surgery

at home in an emergency

at another location

NUMBER


86.4 Have you seen a specialist (chest physician, allergy specialist, internal medicine specialist, ENT doctor) because of your breathing problems or shortness of breath *in the last 12 months*? **IF NO GO TO Q87 IF YES**

86.4.1 How many times?

NO YES

--	--

NUMBER

--	--

87. Are you given regular appointments to be seen by a doctor (or nurse) for your asthma, wheezing or shortness of breath?

NO YES

--	--

**IF NO GO TO Q88 IF YES**

87.1. Are you given regular appointments with a hospital doctor?

NO YES

--	--

87.2 Are you given regular appointments with your general practitioner?

NO YES

--	--

87.3. Are you given regular appointments with a nurse?

NO YES

--	--

88. How many times have you visited the following because of breathing problems or shortness of breath *in the last 12 months*?

88.1 nurse

88.2 physiotherapist

88.3 practitioner of ‘alternative’ medicine

NUMBER


89. Have you had any clinical or laboratory tests because of asthma wheezing or shortness of breath *in the last 12 months*?

NO YES

--	--

**IF NO GOT Q90 ,IF YES**

89.1. How many times have you had the following *in the last 12 months*?

Breathing test in a laboratory specially for lung function measures

Skin test for allergy

Blood test for allergy

x-rays

NUMBER


90. Are you currently working?

NO YES

--	--

**IF NO GO TO Q90.2 IF YES**

90.1. How many days of work have you lost because of asthma, shortness of breath or wheezing in the last **12 months**?

NUMBER

--	--	--

90.2. Were you forced to **give up working** because of asthma, wheezing or shortness of breath in the last **12 months**?

NO YES

--	--

**Appendix B 1 – ECRHS II Main Questionnaire**

**IF NO GO TO 91. IF YES**

91.2.1. When?

DAY	MONTH	YEAR
<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>

91. Have there been any days when you have had to **give up activities other than work**

(e.g. looking after children, the house, studying) because of your asthma, wheezing or shortness of breath in the **last 12 months?**

NO	YES
<input type="checkbox"/>	<input type="checkbox"/>

**IF NO YOU HAVE FINISHED THE QUESTIONNAIRE IF YES**

91.2. How many days on average each month?

Subjects Gender

M	F
<input type="checkbox"/>	<input type="checkbox"/>

Subjects Date of Birth

DAY	MONTH	YEAR
<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/>

**INTERVIEW TYPE?**

**TICK ONE BOX ONLY**

- a) At centre face to face
- b) At home face to face
- c) By telephone
- d) Self completed at home

1	<input type="checkbox"/>
2	<input type="checkbox"/>
3	<input type="checkbox"/>
4	<input type="checkbox"/>

**END**

FIELDWORKER NUMBER

# Appendix F

## Paper I



# Inhaled steroids are associated with reduced lung function decline in subjects with asthma with elevated total IgE

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**Background:** Few studies have investigated the long-term association between inhaled corticosteroids (ICSs) and lung function decline in asthma.

**Objective:** To evaluate whether prolonged treatment with ICSs is associated with FEV<sub>1</sub> decline in adults with asthma.

**Methods:** An international cohort of 667 subjects with asthma (20-44 years old) was identified in the European Community Respiratory Health Survey (1991-1993) and followed up from 1999 to 2002. Spirometry was performed on both occasions. FEV<sub>1</sub> decline was analyzed according to age, sex, height, body mass index, total IgE, time of ICS use, and smoking, while adjusting for potential confounders.

**Results:** As ICS use increased, the decline in FEV<sub>1</sub> was lower ( $P$  trend = .025): on average, decline passed from 34 mL/y in nonusers (half of the sample) to 20 mL/y in subjects treated for 48 months or more (18%). When adjusting for all covariates, there was an interaction ( $P$  = .02) between ICS use and total IgE: in subjects with high (>100 kU/L) IgE, ICS use for 4 years or more was associated with a lower FEV<sub>1</sub> decline (23 mL/y;

95% CI, 8-38 compared with nonusers). This association was not seen in those with lower IgE.

**Conclusion:** Although confirming a beneficial long-term association between ICSs and lung function in asthma, our study suggests that subjects with high IgE could maximally benefit from a prolonged ICS treatment.

**Clinical implications:** This study adds further evidence to the beneficial effect of inhaled steroids on lung function in asthma; future studies will clarify whether calibrating the corticosteroid dose according to the level of total IgE is a feasible approach in asthma management. (*J Allergy Clin Immunol* 2007;119:611-7.)

**Key words:** Asthma, lung function decline, inhaled corticosteroids, total IgE, eosinophils, prospective cohort study, FEV<sub>1</sub> decline, European Community Respiratory Health Survey, ECRHS

In patients with asthma, the decline in lung function is accelerated compared with subjects without asthma<sup>1</sup> and, in more severe asthma, it may result in an irreversible airflow obstruction.<sup>2,3</sup> The rapid decline in lung function and the airflow obstruction may stem from structural changes (airway remodeling) or functional changes that may or may not be associated with the underlying chronic inflammation.<sup>4-8</sup>

Inhaled corticosteroids (ICSs) are the mainstay of asthma treatment,<sup>9</sup> and many short-term clinical trials have demonstrated their efficacy in decreasing airway inflammation, improving lung function, and reducing symptoms and airway hyperresponsiveness.<sup>10-13</sup> However, there is limited evidence that ICSs are able to slow down the progressive loss of lung function over time in asthma, and also that ICSs can prevent or revert structural changes in the lungs.<sup>14-16</sup> This is mainly because of the difficulty in following up patients in clinical trials over many years. Therefore, observational studies make an important contribution to assess the effectiveness of the long-term use of ICSs.

Recently, 2 observational follow-up studies have been published,<sup>17,18</sup> both reporting a beneficial effect of the long-term use of ICSs on lung function decline. Lange et al<sup>18</sup> reported that treatment with ICSs was associated with an 18 mL/y reduction in the rate of decline in FEV<sub>1</sub> compared with no use of ICSs, and Dijkstra et al<sup>17</sup> found

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**Abbreviations used**

ATS: American Thoracic Society
BMI: Body mass index
ECRHS: European Community Respiratory Health Survey
ED: Emergency department
ICS: Inhaled corticosteroid
IQR: Interquartile range
LABA: Long-acting $\beta_2$ -agonist

a similar effect only in nonsmoking men. However, both surveys investigated relatively small samples of selected individuals.

The aim of this study was to evaluate whether prolonged use of ICSs is associated with a reduced long-term decline in lung function in a large, international, population-based cohort of subjects with asthma, followed up for 9 years in the European Community Respiratory Health Survey (ECRHS).

**METHODS****Study design**

The ECRHS is an international multicenter study of asthma. The first survey<sup>19</sup> was performed from 1991 to 1993 on random community-based samples of adults age 20 to 44 years. Each participant was sent a brief questionnaire (stage 1) and, from those who responded, a 20% random sample was invited to undergo a more detailed clinical examination (stage 2). In addition, a symptomatic sample consisting of those who reported symptoms of waking with shortness of breath or asthma attacks in the last 12 months or who were using asthma medication in stage 1 was also studied. The ECRHS II<sup>20</sup> was a follow-up study of all participants in stage 2 of the ECRHS I, performed from 1999 to 2002 (the full protocol can be found at [www.ecrhs.org](http://www.ecrhs.org)). Subjects answered a standardized questionnaire administered by trained interviewers and underwent lung function and blood tests. Quality control procedures are fully described in the study protocols.<sup>19,20</sup> The current study includes data from 26 centers that took part in the ECRHS II. Ethical approval was obtained for each center from the appropriate institutional or regional ethics committee, and written consent was obtained for each participant.

**Subjects and definitions**

All individuals with current asthma identified in the ECRHS I (1991-1993) who had performed spirometry according to the American Thoracic Society (ATS) criteria for reproducibility<sup>21</sup> and who participated in the ECRHS II were eligible for this analysis. Current asthma was defined as having reported, in the ECRHS I, asthma confirmed by a doctor and having had asthmalike symptoms (wheeze; nocturnal chest tightness; attacks of breathlessness after activity, at rest, or at nighttime; asthma attacks), and/or having used inhaled/oral medicines because of breathing problems during the last 12 months.

In the ECRHS I, 1348 subjects with current asthma had their lung function measured according to the ATS criteria (700 from the random and 648 from the symptomatic sample). Although there were no differences in age, sex, smoking habits, FEV<sub>1</sub>, and IgE levels at baseline (ECRHS I) in random and symptomatic subjects, the former had a slightly longer duration of the disease (17.8 vs 16.2 years) and a

lower percentage of manual workers (25% vs 35%) and of people reporting exposure to vapors, gas, dust, or fumes in the workplace (44% vs 50%). Of these 1348 individuals, 860 (64%) attended the second study (1999-2002) and were therefore eligible for the analysis.

In the ECRHS II (1999-2002), some eligible subjects did not repeat spirometry (135 subjects) or had their lung function measured in disagreement with the ATS criteria (32 subjects) and were excluded from the analysis. Among those who had performed the lung function test, some had used inhaled long-acting  $\beta_2$ -agonists (LABAs) in the 12 hours before the test. Because the bronchodilating effect of LABAs can persist for 8 to 12 hours after use, these subjects were also excluded (26 subjects). Finally, 667 subjects were included in the analysis (311 from the random and 356 from the symptomatic sample).

**Decline in lung function**

The maximum FEV<sub>1</sub> of as many as 5 technically acceptable blows was recorded, both at baseline and at the end of the follow-up, according to the ATS criteria for reproducibility.<sup>21</sup> The predicted value of FEV<sub>1</sub> was calculated on the basis of sex, age, and height,<sup>22</sup> and the FEV<sub>1</sub> % predicted was obtained (100 \* measured FEV<sub>1</sub>/predicted FEV<sub>1</sub>).

For each subject, the average change in lung function during the follow-up (in mL/y) was computed as the difference between FEV<sub>1</sub> measured in the ECRHS I and II, divided by the individual duration of the follow-up (ie, a positive value represents decline).

**Clinical and questionnaire data**

In both surveys, the height, weight, and serum total IgE level of the participants were measured. For each subject, detailed information was collected by questionnaire about sex, age, smoking habits (smoking status, number of cigarettes smoked per day, age at which they started, age at which they gave up if they did), occupation, exposure to occupational risk (if a subject had ever been exposed to vapors, dust, gas, or fumes in the working environment), age at first asthma attack, family asthma (if a subject reported that his/her mother or father had ever had asthma), and hospitalizations and/or emergency department (ED) visits (if a subject had ever spent 1 night in the hospital, and/or visited a casualty department or ED) because of breathing problems.

Asthma duration was estimated as the difference between the age of the subject at the ECRHS I interview and the age when the first asthma attack occurred. Body mass index (BMI) was computed dividing weight by height squared (kg/m<sup>2</sup>). Lifetime pack-years were calculated combining information on smoking habits obtained in the ECRHS I and II.<sup>23</sup>

At baseline, subjects performed a methacholine challenge test<sup>24</sup>; however, bronchial hyperresponsiveness was not considered in the main analysis because about 20% of the subjects had not performed the challenge test, and the exclusion criteria for the test were associated with baseline FEV<sub>1</sub> (eg, FEV<sub>1</sub> < 70% predicted, FEV<sub>1</sub> < 1.5 L).

**Use of inhaled steroids**

In both surveys, the participants were asked whether they had used ICSs in the last 12 months, and the type/brand of steroid and the type of inhaler employed over the last year were recorded. In the ECRHS II, quantitative information was collected about ICS use during the follow-up (how many months per year, how many years since the last survey a subject had been on ICSs). The data on ICSs were combined to calculate the cumulative time of treatment during the follow-up. Subjects with asthma were stratified according to the time of steroid use into (1) nonusers, (2) people who had used ICSs for <8.7 months (1st tertile of time of ICS use distribution among users), (3)  $\geq 8.7$  months but <48 months (2nd tertile), and (4)  $\geq 48$  months.

**TABLE I.** Baseline (ECRHS I) characteristics of excluded and included subjects; data provided as means (SDs) or percentages (%) unless stated otherwise\*

	Excluded	Included	P value
Subjects (N)	681	667	—
Sex (% female)	54.6	55.0	.88
Age (y)	32.3 (7.2)	33.9 (7.2)	.0001
BMI (kg/m <sup>2</sup> )	23.9 (4.3)	24.2 (4.5)	.31
Smoking habits* (pack-years)	2.0 (10.0)	0.5 (9.0)	.046
Occupation			
(% nonmanual)	36.4	48.0	<.0001
(% manual)	31.3	29.1	
(% other)	32.3	22.9	
Occupational exposure (%)	46.6	47.6	.71
Duration of asthma* (y)	16.2 (16.5)	15.6 (19.1)	.60
Family asthma (%)	28.5	26.2	.36
Total IgE (log transformation)	1.98 (0.71)	1.91 (0.68)	.053
Hospitalizations/ED visits (%)	36.3	35.9	.88
ICS† (% users)	41.6	43.4	.60
FEV <sub>1</sub> (L)	3.4 (0.9)	3.4 (0.8)	.50

\*Median (interquartile range); P values of nonparametric test reported.

†Over the period of the last 12 months.

## Statistics

Data were summarized as percentages or means (with SDs), with 95% CIs. Median with interquartile range (IQR) was used for asymmetrical variables.  $\chi^2$  Test, Student *t* test, ANOVA, and Wilcoxon test were used to test differences, where appropriate, using a significance level of .05.

To assess the association between FEV<sub>1</sub> decline (dependent variable) and the cumulative time of ICS use, a 2-level random intercept regression model was fitted to the data, with level 1 units (subjects) nested into level 2 units (centers crossed by type of sample: random or symptomatic). The independent variables included in the model were sex, age, height, baseline BMI, and smoking habits (lifetime pack-years); the model also adjusted for a set of potential confounders measured at baseline—that is, occupation (in a manual job, nonmanual job, or other), occupational risk, duration of asthma, family asthma, total IgE, and previous hospitalizations/ED visits for breathing problems. When evaluating the interaction between time of ICS use and total IgE, the latter was used as a dichotomous variable ( $\leq 100$  kU/L;  $> 100$  kU/L).<sup>25</sup>

The statistical analysis was performed using STATA software, release 8.2 (Stata Corp, College Station, Tex).

## RESULTS

### Comparison between included and excluded subjects

Table I describes the main baseline features of the subjects included and not included in the analysis. The latter are either nonparticipants in the ECRHS II, or participants whose FEV<sub>1</sub> was unavailable, not in agreement with the ATS criteria, or measured within 12 hours after the last administration of a LABA. Subjects included in the analysis were slightly older and had a lower percentage of current smokers (30% vs 39%) and of manual workers than subjects who were excluded. On average, among the individuals included in the analysis, the follow-up time was 9 years (range, 6–11 years).

**TABLE II.** Baseline (ECRHS I) characteristics (and lifetime pack-years smoked) of the subjects with asthma, stratified according to the use of inhaled corticosteroids during the follow-up; data provided as means (SDs) or percentages (%) unless stated otherwise\*

	ICSs		P value
	Nonusers	Users	
Subjects, N (%)	297 (46.7)	339 (53.3)	—
Sex (% female)	45.4	63.7	<.001
Age (y)	33.7 (7.1)	34.1 (7.3)	.44
BMI (kg/m <sup>2</sup> )	23.6 (3.6)	24.5 (4.9)	.001
Smoking habits* (lifetime pack-years)	1.6 (15.6)	0 (10.0)	.013
Occupation			
(% nonmanual)	47.5	49.0	.48
(% manual)	28.0	30.4	
(% other)	24.5	20.6	
Occupational exposure (%)	47.6	48.4	.85
Duration of asthma* (y)	18.7 (19.1)	13.5 (17.9)	.001
Family asthma (%)	19.3	31.1	.001
Total IgE‡ (log transformation)	1.84 (0.66)	1.96 (0.68)	.033
Hospitalizations/ED visits (%)	28.6	42.9	<.0001
FEV <sub>1</sub> (L)	3.7 (0.8)	3.2 (0.8)	<.0001
FEV <sub>1</sub> % predicted†	101.6 (12.6)	95.0 (17.9)	<.0001

\*Median (interquartile range); P values of nonparametric test reported.

†Predicted values of FEV<sub>1</sub> were calculated on the basis of sex, age, and height.

‡Corresponding to a geometric mean of 69.6 kU/L in nonusers and of 91.9 kU/L in users.

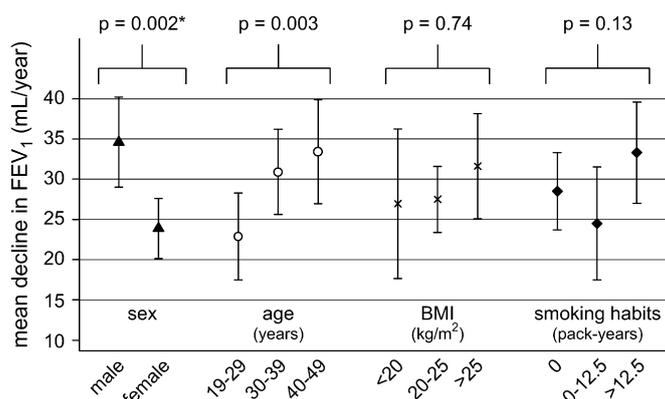
### Use of inhaled steroids

The cumulative time of ICS use could not be evaluated for 31 individuals (<5%) because some data were missing. Out of the remaining 636 subjects, 297 (47%) had never been on ICSs, whereas 339 (53%) had used ICSs during the follow-up. Among ICS users, the median cumulative time of treatment was 1.4 (IQR, 6.3) years, with no significant difference between men and women (*P* = .33). Among people who had used ICSs for  $\geq 4$  years, the median time of use was 8.2 (IQR, 2.0) years.

The steroid that was most commonly used during the year before the follow-up visit (ECRHS II) was budesonide (51%), followed by beclomethasone (27%), and fluticasone (20%); the majority of subjects used dry-powder inhalers (65%),  $\frac{1}{3}$  used metered-dose inhalers, and only 1% used nebulizers.

### Characteristics of steroid users and nonsteroid users

At baseline, on average, subjects with asthma treated with ICSs during the follow-up (Table II) had a worse lung function, higher levels of total IgE, a shorter duration of asthma, and a greater prevalence of family asthma and of hospitalizations/ED visits than nonsteroid users. Steroid users were more likely to be women and had a higher baseline BMI than nonsteroid users, and they also



**FIG 1.** Unadjusted mean annual decline in FEV<sub>1</sub> (with 95% CIs and *P* value for trend) from 1991-1993 to 1999-2002, according to sex, age, and BMI at baseline, and lifetime pack-years smoked. \*Student *t* test.

**TABLE III.** Multiple regression coefficients\* with 95% CIs and related *P* values for the association between the mean decline in FEV<sub>1</sub> and sex, age, and BMI at baseline and smoking habits and ICS use during the follow-up

Covariate	Coefficient (mL/y)	95% CI	<i>P</i> value
Sex† (female)	-9.5	-20.4; 1.4	.09
Age‡ (y)	0.8	0.2; 1.4	.005
BMI‡ (kg/m <sup>2</sup> )	-0.4	-1.3; 0.5	.40
Smoking habits‡ (lifetime pack-years)	0.04	-0.2; 0.3	.76
Use of ICSs†			
<8.7 months	-3.9	-14.4; 6.5	.46
8.7 months to 4 years	-4.8	-15.5; 6.0	.38
>4 years	-10.7	-21.3; -0.1	.048

\*Adjusted for height, occupation, exposure to occupational risk, duration of asthma, familiarity of asthma, total IgE, previous hospitalizations/ED visits; obtained through a 2-level random intercept regression model fitted on subjects with complete information (*n* = 511).

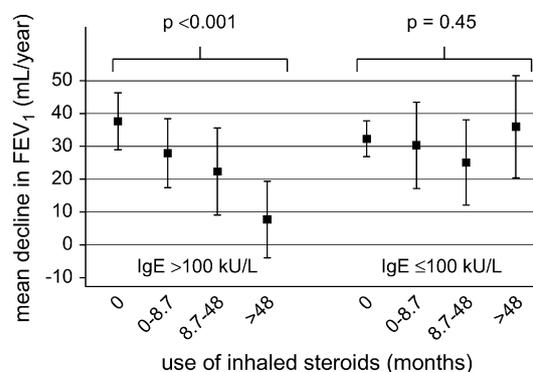
†The coefficient represents the difference in the mean decline in FEV<sub>1</sub> decline (mL/y) between subjects with the characteristic and subjects belonging to the reference category (ie, "male" for variable "sex" and "no ICSs" for variable "use of ICSs").

‡The coefficient represents the change in FEV<sub>1</sub> decline (mL/y) for a unit change in the covariate.

had a lower percentage of current smokers at baseline (24% vs 36%).

### Decline in lung function

In subjects with asthma, the average decline in FEV<sub>1</sub> was 35 (95% CI, 29-40) mL/y for men and 24 (95% CI, 20-28) mL/y for women (Fig 1). FEV<sub>1</sub> decline was positively associated with age (*P* trend = .003), but not with baseline BMI and with lifetime exposure to active smoking (pack-years). FEV<sub>1</sub> decline was 32 (95% CI, 27-36) mL/y and 25 (95% CI, 21-30) mL/y for individuals exposed and not exposed to occupational risk, respectively. FEV<sub>1</sub> decline was unrelated to occupation, hospitalizations/ED visits, total IgE, family asthma, and asthma duration at baseline.



**FIG 2.** Unadjusted mean annual decline in FEV<sub>1</sub> (with 95% CIs and *P* value for trend), according to the level of total IgE and to the time of ICS use during the follow-up (nonusers, 1st, 2nd, and 3rd tertile). Elevated (>100 kU/L) total IgE levels were present in 47% of the subjects included in the analysis.

FEV<sub>1</sub> decline was lower in subjects who had used ICSs for a longer time (*P* trend = .025); it was 34 mL/y in nonusers, 28 mL/year in subjects treated for <8.7 months, 24 mL/y in subjects treated for ≥8.7 months but <48 months, and 20 mL/y in subjects treated for ≥48 months.

When all the variables were considered simultaneously (Table III), the use of ICSs for ≥48 months was statistically significantly associated with a lower decline (11 mL/y; 95% CI, 0.1-21 compared with nonusers). A shorter therapy with steroids (<48 months) was also associated with a lower decline (about 4 mL/y), but it was not statistically significant. FEV<sub>1</sub> decline was greater for older subjects (0.8 mL/y; 95% CI, 0.2-1.4 for every additional year in subject age). None of the other covariates considered in the analysis was significantly associated with FEV<sub>1</sub> decline. There was no statistically significant interaction between sex and ICS use, nor between lifetime pack-years smoked and ICS use, but a significant (*P* = .02) interaction was found between having an elevated (>100 kU/L) level of total IgE and ICS use (Fig 2). After adjusting for all the potential confounders, in subjects having total IgE > 100

kU/L (47% of the subjects included in the analysis), the use of ICSs for  $\geq 4$  years was associated with a lower FEV<sub>1</sub> decline (23 mL/y; 95% CI, 8-38 compared with nonusers). In ICS users with elevated total IgE, the Pearson correlation coefficient between FEV<sub>1</sub> decline and total IgE was 0.07 (95% CI, -0.09 to 0.22).

When the analysis was repeated adjusting for bronchial hyperresponsiveness, coded as present (if a subject had PD<sub>20</sub> < 1000  $\mu$ g), absent, or test not performed, the results were fully consistent with those presented (data not shown).

## DISCUSSION

In a large community-based sample of subjects with asthma from the general population, followed up for 9 years, we have shown that a lower decline in FEV<sub>1</sub> was associated with a prolonged use of inhaled steroids. This association was only observed in those with elevated total IgE levels. There was a dose-response relationship, with lower decline in those who had used inhaled steroids for the longest periods. The association was found in men and in women, as well as in smokers and in nonsmokers.

In the whole cohort, the average FEV<sub>1</sub> decline was 11 mL/y lower in subjects with asthma who reported that they had used ICSs for 4 years or more during the follow-up, compared with nonusers. The current finding is in line both with clinical trials,<sup>12,13,26-28</sup> and with 2 recent observational studies.<sup>17,18</sup> Our estimate of the prevented FEV<sub>1</sub> decline that was potentially attributable to ICSs (11 mL/y; 95% CI, 0.1-21) is somewhat smaller than that reported in the study by Lange et al<sup>18</sup> (18 mL/y). This may be a result of the relatively young age of our cohort. In fact, the rate of lung function decline increases with age, as supported by our results (Table III) and as previously reported<sup>29,30</sup>; accordingly, one could expect a greater benefit from ICSs in patients whose FEV<sub>1</sub> is declining more steeply.

An intriguing and original finding of our analysis was that a lower decline in lung function in long-term steroid users was observed only in subjects with asthma with elevated (>100 kU/L) total serum IgE levels, whereas subjects with low IgE seemed to be less responsive. There was no correlation between lung function decline and total IgE in ICS users with elevated total IgE, supporting the idea that the association between lower FEV<sub>1</sub> decline and ICS use was an all-or-none association.

High serum total IgE levels are strongly associated with asthma, independently of specific allergic sensitization,<sup>31</sup> with disease severity,<sup>32</sup> and are a major contributing factor for the development of bronchial hyperresponsiveness in both allergic and nonallergic asthma.<sup>31</sup> Elevated total serum IgE levels reflect the nature of the underlying airway inflammation, and are associated with a higher eosinophil count and higher eosinophil cationic protein (a marker of eosinophil activation) in the blood.<sup>33</sup> Recently, anti-IgE treatment has been shown to be associated with significant and profound reductions in tissue eosinophils and in mast cells, as well as T cells and B cells, suggesting that

blocking IgE may inhibit more chronic aspects of allergic inflammation.<sup>34,35</sup> Furthermore, total serum IgE is an independent predictor of immediate treatment response to ICSs,<sup>36</sup> and a decrease in total serum IgE is significantly correlated with an improvement in asthma symptom scores.<sup>37</sup>

We speculate that the lower decline of lung function in long-term ICS users with asthma is mainly a result of the effect of steroids on the eosinophils. These cells produce cytokines, chemokines, lipid mediators, and growth factors, and can also cause an increase in mucus production, resulting in subepithelial fibrosis and in an alteration of airway smooth muscle contractility.<sup>38</sup> Eosinophils are thought to be key effector cells contributing to both airway inflammation and remodeling in asthma.<sup>38</sup> Steroids are able to inhibit the mediator release from eosinophils, to decrease the eosinophil count by inducing apoptosis,<sup>39,40</sup> and to attenuate the levels of eosinophil progenitors in peripheral blood and, to some extent, in bone marrow.<sup>38,39,41-43</sup>

Furthermore, in subjects having low IgE levels, inflammation could be predominantly neutrophilic,<sup>42</sup> and it has been shown that neutrophils are not sensitive to the effect of steroids. Indeed, systemic steroids increase peripheral neutrophil counts, a fact that may reflect an increased survival time because of an inhibitory action on neutrophil apoptosis.<sup>44</sup>

In agreement with Lange et al,<sup>18</sup> but not with Dijkstra et al,<sup>17</sup> we found no evidence that the association between ICS use and lung function decline was different in men and in women.

The lack of association between smoking and lung function deterioration was somewhat surprising. One possible explanation could be the young age of our cohort. Also, one could speculate that this finding could reflect the healthy smoker effect (only subjects with asthma with relatively good lung function at baseline smoke or start smoking, whereas subjects with more severe asthma refrain from smoking).

Previous studies have found that smoking interferes with the long-term beneficial effects of steroids.<sup>17,26,45,46</sup> The lack of interaction between smoking habits and ICS use found in our study probably reflects that the group of subjects selected for our analysis does not allow a full appreciation of the effect of smoking on lung function decline, and hence on its potential modification on the effect of ICSs.

In evaluating the association between the long-term use of ICSs and the average decline in FEV<sub>1</sub>, we adjusted for different characteristics of the subjects (age, height, BMI, different hazardous exposures), as well as for variables related to asthma (duration and familiarity) and its severity (previous hospitalizations and/or ED visits, total IgE levels). Nevertheless, our results may be affected by some bias, given that the allocation of subjects to ICS treatment was not based on randomization but rather on indication. ICS users may differ from nonusers in some aspects that have not been measured. For example, subjects who get access to and regularly take prescribed medications may be more likely to adopt generally healthier

lifestyles (such as healthy diet and exercise). High dietary intake of antioxidants has been associated with lung function,<sup>47</sup> but we have no information on this for our sample. On the other hand, one might expect subjects with more severe disease to be prescribed more medication. At baseline, the treated group had poorer lung function, higher rates of hospitalization for respiratory diseases, and higher prevalence of family asthma than the untreated group. Therefore, we might expect them to have a steeper decrease in lung function than subjects who did not receive treatment (the horse-racing effect).<sup>48,49</sup> If this is true, we have underestimated the association between ICS use and FEV<sub>1</sub> decline.

A potential limitation of the current study is that a self-reported doctor diagnosis of asthma was used to identify individuals with asthma. Although this definition may be open to some degree of misclassification, it has been proven to be highly specific,<sup>50</sup> so that only milder or undiagnosed asthmatics would not have been included in our study.

Compared with other recent longitudinal studies investigating the association between ICSs and lung function decline,<sup>17,18</sup> our study has some points of strength. First, the ECRHS sample was selected from the general population in an international setting. Second, in the current study, a cumulative time of ICS use was estimated for each individual in the follow-up period. Finally, the individuals who had used inhaled LABAs in the 12 hours before lung function testing were excluded, thus reducing the risk of a bias because of the residual bronchodilating effect of these drugs.

In conclusion, the longitudinal analysis of the decline of lung function in a large, international, population-based sample of subjects with asthma supports the hypothesis that the long-term use of ICSs might prevent the deterioration of lung function in individuals having elevated levels of total serum IgE, whereas lung function could be less influenced by steroids in subjects with lower levels of IgE. This finding is not to be interpreted as an advise to prescribe ICSs only in subjects with asthma and elevated IgE levels. In fact, we investigated only the effect of a prolonged use of ICSs on lung function deterioration, whereas ICSs have been clearly shown to have favorable effects on several short-term outcomes. Moreover, a long-term use of ICSs has been demonstrated to reduce the risk of both asthma-related death<sup>51</sup> and hospitalization.<sup>52</sup> Our findings underline the importance of total IgE as a feature of asthma, not only because it helps to predict its severity<sup>32</sup> and prognosis, but also because it might influence decisions on long-term anti-inflammatory treatment. Further investigations are needed to clarify whether calibrating the corticosteroid dose according to the level of IgE is a feasible approach in asthma management.

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# Appendix G

## Paper II



# Body mass index, weight gain, and other determinants of lung function decline in adult asthma

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**Background:** Little is known about factors associated with lung function decline in asthma.

**Objective:** To identify the determinants of FEV<sub>1</sub> decline in adults with asthma with and without airflow obstruction at baseline.

**Methods:** An international cohort of 638 subjects with asthma (20-44 years old) was identified in the European Community Respiratory Health Survey (1991-1993) and followed up from 1998 to 2002. Spirometry was performed on both occasions. FEV<sub>1</sub> decline was related to potential determinants evaluated at baseline and during the follow-up by random intercept linear regression models. The analyses were stratified by the presence of airflow obstruction (FEV<sub>1</sub>/forced vital capacity < 0.70) at baseline.

**Results:** In the group of individuals without airflow obstruction (n = 544), a faster FEV<sub>1</sub> decline was observed for subjects with intermediate body mass index (BMI) than for lean and obese subjects. FEV<sub>1</sub> decline was associated with weight gain independently of baseline BMI, and this association was stronger in men (20; 95% CI, 10-30, mL/y/kg gained) than in women (6; 95% CI, 1-11, mL/y). In the group of individuals with

airflow obstruction (n = 94), the absence of allergen sensitization and a low BMI at baseline were associated with a faster FEV<sub>1</sub> decline, whereas weight gain was not associated with decline.

**Conclusions:** The detrimental effect of weight gain on FEV<sub>1</sub> decline is particularly relevant in subjects with asthma who still do not have an established airflow obstruction. Our findings support the importance of weight management in asthma and recommend weight loss in overweight or obese individuals with asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

**Key words:** Asthma, lung function decline, airflow obstruction, body mass index, weight gain, obesity, FEV<sub>1</sub>, allergen sensitization, prospective cohort study, predictors

Impaired pulmonary function, as measured by a low FEV<sub>1</sub>, is a predictor of mortality in the general<sup>1</sup> as well as in the asthmatic population.<sup>2</sup> Subjects with asthma have a faster FEV<sub>1</sub> decline than subjects without asthma,<sup>3</sup> and FEV<sub>1</sub> decline leads to persistent airflow obstruction for some individuals with asthma. Compared with subjects without obstruction, subjects with airflow obstruction have a more severe form of asthma, they may be on an inadequate asthma treatment, and their airways may already have been remodeled by chronic inflammation.<sup>4</sup> Subjects in the 2 groups may have different individual characteristics and past exposures, a different history of asthma, and different determinants of lung function change over time. Some recent articles have suggested that long-term treatment with inhaled steroids may slow down the decline in FEV<sub>1</sub>,<sup>5-7</sup> particularly in subjects with elevated total IgE levels.<sup>7</sup> However, little is known about other factors associated with rapid FEV<sub>1</sub> decline in adult asthma. We aimed at investigating the determinants of FEV<sub>1</sub> decline in a large, international, population-based cohort of subjects with asthma with or without airflow obstruction at baseline, who were followed up for 9 years in the European Community Respiratory Health Survey (ECRHS).

## METHODS

### Study design

The ECRHS is an international multicentre study of asthma. The first survey (ECRHS I)<sup>8</sup> was carried out from 1991 to 1993 on random community-based samples of adults age 20 to 44 years. Each participant was sent a brief questionnaire (stage 1), and from those who responded, a 20% random sample was invited to undergo a more detailed clinical examination (stage 2). In addition, a sample (called "symptomatic sample" in the article) consisting of

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**Abbreviations used**

ATS:	American Thoracic Society
BMI:	Body mass index
ECRHS:	European Community Respiratory Health Survey
ETS:	Environmental tobacco smoke
FVC:	Forced vital capacity
Q <sub>1</sub> :	1st quartile
Q <sub>3</sub> :	3rd quartile

subjects not already included in the random sample who reported asthmalike symptoms in the last 12 months, or who were using asthma medication in stage 1, was also studied. Subjects from the random and from the symptomatic samples were examined at the same time and with the same methods and accuracy. The ECRHS II<sup>9</sup> was a follow-up study of all the participants in stage 2 of the ECRHS I, performed between 1999 and 2002 (the full protocol can be found at <http://www.ecrhs.org>). In both studies, subjects answered a standardized questionnaire administered by trained interviewers and they underwent lung function and blood tests. The current study includes data from 26 centers that took part in the ECRHS II. Ethical approval was obtained for each center from the appropriate institutional or regional ethics committee, and written consent was obtained from each participant.

**Subjects and definitions**

All individuals with current asthma identified in the ECRHS I (1991-1993) who had performed spirometry complying with the American Thoracic Society (ATS) criteria for reproducibility<sup>10</sup> and who had participated in the ECRHS II were eligible for these analyses. Current asthma was defined as having reported, in the ECRHS I, asthma confirmed by a doctor, and having had asthmalike symptoms (wheeze; nocturnal chest tightness; attacks of breathlessness after activity, at rest, or at night time; asthma attacks), and/or having used inhaled/oral medicines because of breathing problems during the last 12 months. In the ECRHS I, 1348 subjects with current asthma had their lung function measured according to the ATS criteria. Compared with subjects from the symptomatic sample (n = 648), subjects from the random sample (n = 700) had a longer duration of the disease (median, 17 vs 15 years), had a lower body mass index (BMI; 23 vs 24 kg/m<sup>2</sup>) and included a smaller percentage of individuals with a low educational level (9% vs 15%;  $P < .01$ ). Of these 1348 individuals, 860 (64%) participated in the second study (1999-2002) and were therefore eligible for the analyses. In the ECRHS II (1999-2002), some eligible subjects did not repeat spirometry (135 subjects), or their lung function was not measured in compliance with the ATS criteria (32 subjects), or they had used inhaled long-acting  $\beta_2$ -agonists in the 12 hours before the test (26 patients), and were therefore excluded from the analyses.

**Lung function**

The maximum FEV<sub>1</sub> and forced vital capacity (FVC) of at least 2 and as many as 5 technically acceptable maneuvers were recorded, according to the ATS criteria for reproducibility.<sup>10</sup> FEV<sub>1</sub> % predicted and FVC % predicted were calculated on the basis of the equations by Quanjer et al.<sup>11</sup> The average decline in FEV<sub>1</sub> (or FVC) during the follow-up was computed as the difference between FEV<sub>1</sub> (or FVC) measured in the ECRHS I and II, divided by the duration of the follow-up (expressed in mL/y). %FEV<sub>1</sub>/FVC decline was also computed as the difference between %FEV<sub>1</sub>/FVC in the ECRHS I and II, divided by the duration of the follow-up (expressed in %/y). Baseline airflow obstruction was defined as FEV<sub>1</sub>/FVC < 0.70<sup>12</sup> at the first visit (ECRHS I). Because a bronchodilator challenge test was not part of the ECRHS core protocol, the follow-up spirometry was used to confirm baseline airflow obstruction. Accordingly, 29 subjects with baseline airflow obstruction, who had FEV<sub>1</sub>/FVC  $\geq$  0.70 at the follow-up, were excluded from the analyses to reduce the number of false-positives results.<sup>13</sup> A sensitivity analysis was performed to confirm that this did not affect our results (data not shown).

**Clinical and questionnaire data**

The clinical interview included questions on asthma and respiratory symptoms, smoking habits (smoking status, number of cigarettes smoked per day, age when subjects started smoking, age when they gave it up if they did), environmental tobacco smoke (ETS; ie, regular exposure to other people's smoke for 1 h/d or more), educational level (low if a subject had completed full-time education before the age of 16 years), age at first asthma attack, family asthma (mother/father ever had asthma), and use of inhaled steroids during the follow-up. Pack-years smoked were calculated for the period before the ECRHS I and for the follow-up period.<sup>14</sup> Asthma duration was estimated as the difference between the age of the subject at the ECRHS I interview and the age when the first asthma attack occurred. The subject's weight (kg) and height (m) were measured, and BMI was calculated (weight/height<sup>2</sup>). BMI gain (kg/[m<sup>2</sup>·y]) or weight gain (kg/y) during the follow-up was computed as the difference between BMI/weight, measured in the ECRHS II and I, divided by the duration of the follow-up.

At baseline (ECRHS I), total IgE and specific IgE against house dust mite, cat dander, timothy grass, *Cladosporium* species, and a local allergen were measured. A subject was considered sensitized to any allergen if the assay result for at least 1 allergen was higher than 0.35 kU/L. Subjects performed a methacholine challenge test,<sup>15</sup> but bronchial hyperresponsiveness was not considered in the analyses because about 20% of the subjects had not performed the challenge test, and the exclusion criteria for the test were associated with baseline FEV<sub>1</sub> (eg, FEV<sub>1</sub> < 70% predicted; FEV<sub>1</sub> < 1.5 L).

**Statistics**

Overall, 638 subjects were included in the analyses. All the analyses were stratified by the presence of airflow obstruction (FEV<sub>1</sub>/FVC < 0.70) at baseline. Data were summarized as percentages, or medians with interquartile ranges (1st quartile-3rd quartile [Q<sub>1</sub>-Q<sub>3</sub>]). The  $\chi^2$  test, the Fisher exact test, and the Wilcoxon test were used to test differences, where appropriate ( $\alpha = 0.05$ ). To assess the association between FEV<sub>1</sub> decline and the potential determinants, 2-level (with level-1 units, subjects, nested into level-2 units, centers, crossed by type of sample: random/symptomatic) random intercept multiple linear regression models<sup>16</sup> were fitted to the data. The independent variables included in the models were BMI, educational level, pack-years, ETS exposure, sensitization to allergens, asthma duration, and family asthma measured at baseline; and BMI/weight gain, pack-years, and change in ETS exposure measured during the follow-up. The models were also adjusted for sex, age, height, and the interaction between inhaled steroid use during the follow-up (<4 years vs  $\geq$  4 years) and baseline total IgE ( $\leq$  100 kU/L vs > 100 kU/L).<sup>7</sup> In the group of subjects without airflow obstruction, BMI squared was also included in the model, because it significantly ( $P = .0009$ ) improved the model fitting. Statistical interactions were evaluated through likelihood ratio tests. The statistical analyses were performed using STATA software, release 9.2 (Stata Corp, College Station, Tex).

**RESULTS****Comparison between included and excluded subjects**

Subjects excluded from the analyses (n = 710) were younger (median age [Q<sub>1</sub>-Q<sub>3</sub>], 32 [26, 38] years vs 34 [28, 40] years;  $P < .001$ ), and they were more likely to be current smokers (38% vs 30%;  $P = .01$ ) than subjects who were included (n = 638). However, the 2 groups were similar in regard to all the other baseline covariates (including BMI: median, 23 kg/m<sup>2</sup> in both groups), as well as baseline FEV<sub>1</sub> and FVC.

**Characteristics and lung function of the subjects included in the analyses**

Subjects in the random (n = 299) and subjects in the symptomatic (n = 339) samples were similar for baseline FEV<sub>1</sub>

**TABLE I.** Baseline and follow-up characteristics of the subjects with asthma, stratified by the presence of airflow obstruction at baseline

	Airflow obstruction at baseline		P value
	No	Yes	
No. of subjects (%)	544 (85)	94 (15)	—
Sex (% female)	<b>59</b>	<b>34</b>	<b>&lt;.001</b>
Baseline covariates			
Age (y)	<b>33 (28-40)</b>	<b>36 (30-43)</b>	<b>.004</b>
BMI (kg/m <sup>2</sup> )	23 (21-26)	23 (21-26)	.57
Low educational level (%)	11	14	.50
Smoking habits			
Nonsmokers (%)	49	40	.25
Ex-smokers (%)	21	26	
Current smokers (%)	30	34	
Pack-years	0 (0-9)	2 (0-10)	.16
ETS (%)	48	44	.45
Sensitization to any allergen (%)	67	75	.12
Duration of asthma (y)	<b>15 (6-25)</b>	<b>22 (9-29)</b>	<b>&lt;.001</b>
Family asthma (%)	26	26	.92
Follow-up covariates			
Weight gain (%)	80	73	.12
<b>BMI gain (kg/[m<sup>2</sup>·y])</b>	<b>0.18 (0.04-0.35)</b>	<b>0.15 (0-0.24)</b>	<b>.01</b>
Pack-years between surveys	0 (0-2)	0 (0-4)	.24
Change in ETS (%)			
Unchanged	69	70	.74
Worsened	6	7	
Improved	25	23	

Data are provided as percentages or medians (Q<sub>1</sub>-Q<sub>3</sub>). Statistically significant results ( $P < .05$ ) are in *boldface*.

(median [Q<sub>1</sub>-Q<sub>3</sub>], 3.4 [2.9, 4.0] L vs 3.3 [2.8, 4.1] L;  $P = .15$ ), as well as for FEV<sub>1</sub> decline during the follow-up (median [Q<sub>1</sub>-Q<sub>3</sub>], 29 [7, 50] mL/y vs 29 [10, 52] mL/y;  $P = .75$ ). As shown in **Table I**, subjects with airflow obstruction at baseline ( $n = 94$ ; 15%) were more likely to be men, to be older, and to have had asthma for a longer time than subjects without airflow obstruction ( $n = 544$ ; 85%). Moreover, they had a lower BMI gain during the follow-up. In subjects without baseline airflow obstruction, a high baseline BMI was associated with a lower baseline FEV<sub>1</sub>, both in men and in women (**Fig 1**). Obese subjects (BMI range, 30-47 kg/m<sup>2</sup>) had a lower median FEV<sub>1</sub> than nonobese subjects (3.0 vs 3.5 L;  $P < .001$ ), a lower median FVC (3.8 vs 4.2;  $P < .001$ ) and FEV<sub>1</sub>/FVC (0.80 vs 0.82;  $P = .10$ ).

### Determinants of lung function decline

FEV<sub>1</sub> decline and %FEV<sub>1</sub>/FVC decline were greater for subjects without airflow obstruction than for subjects with airflow obstruction (**Table II**).

In subjects with asthma without airflow obstruction at baseline (also see univariate analyses in this article's **Table E1** of the Online Repository at [www.jacionline.org](http://www.jacionline.org)), FEV<sub>1</sub> decline was associated with baseline BMI in a quadratic way (**Table III, column 1**). In other words, men and women with an intermediate BMI had the greatest FEV<sub>1</sub> decline (**Fig 2**). Subjects with a high educational level had a faster FEV<sub>1</sub> decline than subjects with a low educational level ( $P = .003$ ; **Table III, column 1**). Independently of baseline BMI, FEV<sub>1</sub> decline was 28 mL/y greater for every BMI unit (1 kg/m<sup>2</sup>) gained during 1 year of follow-up ( $P < .001$ ). Moreover, there was an interaction between sex and BMI

gain ( $P = .003$ ): FEV<sub>1</sub> decline was 61.8 (95% CI, 32.0-91.7;  $P < .001$ ) mL/y greater per BMI unit gained during 1 year of follow-up in men, and 20.2 (95% CI, 7.9-32.6;  $P = .001$ ) mL/y greater per BMI unit gained in women (**Fig 3**). This corresponds to a FEV<sub>1</sub> decline of 20.0 (95% CI, 10.4-29.5;  $P < .001$ ) mL/y/kg gained during 1 year of follow-up in men, and to 6.9 (95% CI, 2.5-11.4;  $P = .002$ ) mL/y/kg gained in women.

In subjects with asthma with airflow obstruction at baseline (see this article's **Table E2** of the Online Repository at [www.jacionline.org](http://www.jacionline.org)), the absence of allergen sensitization ( $P = .035$ ) and a lower BMI ( $P = .008$ ) at baseline were both associated with a faster FEV<sub>1</sub> decline (**Table III, column 2**).

No significant association was found between exposure to active or passive smoking and FEV<sub>1</sub> decline, either in subjects with or in subjects without airflow obstruction.

### DISCUSSION

A large community-based sample of subjects with asthma was surveyed in 1991/93 and again in 1999/2002. In the group of individuals without airflow obstruction at baseline, a faster FEV<sub>1</sub> decline was observed in subjects whose BMI was intermediate than for lean and obese subjects. FEV<sub>1</sub> decline was associated with BMI gain independently of baseline BMI, and this association was stronger in men than in women. In the group of individuals with airflow obstruction at baseline, weight gain was not associated with decline.

The analyses were stratified by the presence of airflow obstruction at baseline to account for the heterogeneity of asthma phenotypes.<sup>4</sup> Because a bronchodilator challenge test was not part of the ECRHS core protocol, we used the follow-up spirometry to confirm baseline airflow obstruction.

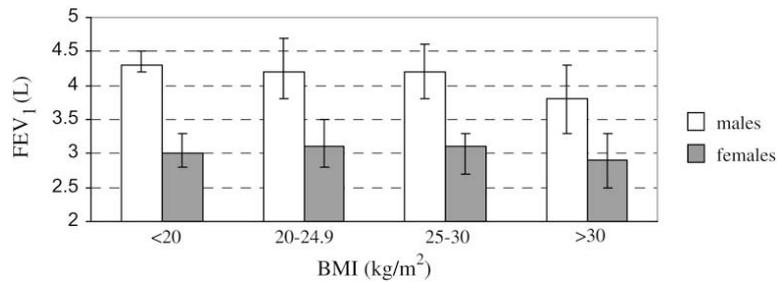
The stratification by airflow obstruction at baseline seems to be supported *a posteriori* by our findings, because the role played by BMI and weight gain in FEV<sub>1</sub> decline was different in the 2 groups. Furthermore, a joint *post hoc* analysis (data not reported) showed a significant interaction between BMI gain and the presence of airflow obstruction ( $P = .001$ ).

Compared with subjects with airflow obstruction, subjects without airflow obstruction had a faster FEV<sub>1</sub> decline and %FEV<sub>1</sub>/FVC decline during the follow-up. This may be because a steep decline had already occurred before the first visit in subjects with airflow obstruction, as suggested by their lower baseline FEV<sub>1</sub>.

### Baseline BMI and FEV<sub>1</sub> decline in subjects with asthma without airflow obstruction at baseline

A high baseline BMI was associated with a lower baseline FEV<sub>1</sub>, but not with a faster FEV<sub>1</sub> decline during the follow-up. Several studies have reported that obesity decreases lung volumes. In fact, the adipose tissue increases chest wall loading.<sup>17-19</sup> This would also explain the concomitant FVC decline observed in the obese (BMI > 30 kg/m<sup>2</sup>). However, no evident restrictive pattern (defined by BMI  $\geq 30$  kg/m<sup>2</sup> and FEV<sub>1</sub>/FVC >0.70 and FVC <80% predicted)<sup>20</sup> was found at baseline in any of the subjects.

In the absence of a weight reduction, there is no evident reason why the lung function decline in obese subjects should be blunted with respect to nonobese subjects. However, asthma in the obese may be different from asthma in normal-weight subjects. Obesity causes lower than normal functional residual capacity, with the



**FIG 1.** Baseline FEV<sub>1</sub> by categories of baseline BMI for men and women without airflow obstruction at baseline. Medians with Q<sub>1</sub>-Q<sub>3</sub> are reported. The number of subjects in the BMI categories <20, 20-24.9, 25-30, and >30 kg/m<sup>2</sup> are 12, 134, 71, and 7 (men) and 51, 182, 52, and 35 (women), respectively.

**TABLE II.** Baseline lung function and lung function decline of the subjects with asthma, stratified by the presence of airflow obstruction at baseline

	Airflow obstruction at baseline		P value
	No	Yes	
FEV <sub>1</sub> % predicted (%)	<b>102 (101-103)</b>	<b>78 (75-82)</b>	<.001
FVC % predicted (%)	107 (106-108)	106 (102-110)	.97
FEV <sub>1</sub> (L)*	<b>3.6 (3.5-3.6)</b>	<b>2.7 (2.6-2.8)</b>	<.001
FVC (L)*	4.4 (4.4-4.5)	4.4 (4.3-4.5)	.65
FEV <sub>1</sub> /FVC†	0.82	0.63	—
FEV <sub>1</sub> decline (mL/y)*	<b>33 (28-37)</b>	<b>23 (14-32)</b>	.04
FVC decline (mL/y)*	22 (15-29)	26 (15-38)	.43
%FEV <sub>1</sub> /FVC decline (%/y)†	<b>0.35 (0.29-0.41)</b>	<b>0.16 (0.03-0.29)</b>	.008

Data are provided as means (with 95% CIs). Statistically significant results ( $P < .05$ ) are in *boldface*.

\*Adjusted for sex, age, and height.

†Adjusted for sex and age.

consequence of unloading the airway smooth muscle and allowing it to shorten excessively when activated,<sup>19,21</sup> and a decrease in operational lung volume.<sup>22</sup> Moreover, breathing at lower tidal volumes inhibits the bronchodilating effect of tidal strain on the airway smooth muscle.<sup>19,23</sup> These mechanical and functional changes within the respiratory system may explain the observed link between obesity and bronchial asthma.<sup>24</sup> Our data support the hypothesis that asthma in the obese is less determined by inflammation than in the nonobese, in agreement with the reversible nature of lung function decline observed with weight loss.<sup>25</sup> This hypothesis, if true, could lead to the implication that, in obese subjects with uncontrolled asthma, weight loss should be primarily targeted, and that it may be worth assessing the level of bronchial inflammation before increasing inhaled steroid doses. However, the faster FEV<sub>1</sub> decline seen in the nonobese compared with the obese could be a result of the fact that obese subjects had a lower baseline FEV<sub>1</sub>, and that the process of decline may no longer be progressive after the FEV<sub>1</sub> had previously dropped to a considerable extent.

Obese subjects tend to report doctor-diagnosed asthma, as well as asthmalike symptoms and use of bronchodilators, more frequently than nonobese subjects, even if they do not have an objective impairment of lung function.<sup>26</sup> In view of our epidemiologic definition of asthma, one could hypothesize that asthma has been overdiagnosed in the obese subgroup. However, for subjects with BMI <20, BMI 20 to 24.9, BMI 25 to 30, and BMI >30 kg/m<sup>2</sup>, the prevalences of family asthma were 15%, 26%, 29%,

**TABLE III.** Multiple regression coefficients\* with 95% CIs for the association between FEV<sub>1</sub> decline and the covariates indicated in subjects with asthma stratified by the presence of airflow obstruction at baseline

	Airflow obstruction at baseline	
	No (n = 371)†	Yes (n = 76)†
	Estimate   (95% CI) (mL/y)	Estimate   (95% CI) (mL/y)
Baseline covariates		
BMI (kg/m <sup>2</sup> )	<b>11.8 (5.4, 18.2)‡</b>	-4.6 (-8.1, -1.2) ‡
BMI <sup>2</sup> ([kg/m <sup>2</sup> ] <sup>2</sup> )	-0.2 (-0.3, -0.09) ‡	—
Low educational level	-18.7 (-30.8, -6.5) ‡	20.2 (-15.2, 55.6)
Pack-years (1-U increase)	-0.2 (-0.6, 0.3)	-0.7 (-2.2, 0.8)
Exposure to ETS	5.9 (-4.4, 16.2)	-29.3 (-62.2, 3.6)
Sensitization to any allergen	-4.0 (-13.1, 5.0)	-29.2 (-56.3, -2.1)
Duration of asthma (y)	-0.3 (-0.7, 0.01)	-0.4 (-1.5, 0.7)
Family asthma	-0.8 (-9.1, 7.6)	-21.4 (-47.8, 5.0)
Follow-up covariates		
BMI gain(kg/[m <sup>2</sup> -y])	<b>28.1 (15.1, 41.1)‡</b>	-7.4 (-51.4, 36.6)
Pack-years between surveys (1-U increase)	-0.2 (-1.3, 1.0)	3.7 (-0.6, 8.0)
Change in ETS		
Worsened vs unchanged	-3.9 (-20.4, 12.6)	26.2 (-23.5, 75.8)
Improved vs unchanged	-6.8 (-17.9, 4.2)	13.7 (-22.6, 50.0)
Intercept§	38.9 (27.2, 50.6)‡	77.1 (44.9, 109.3)‡

Statistically significant results ( $P < .05$ ) are in *boldface*.

\*Adjusted also for sex, age, height, and the interaction between total IgE and inhaled steroid use.

†Number of subjects with complete information.

‡ $P < .01$ .

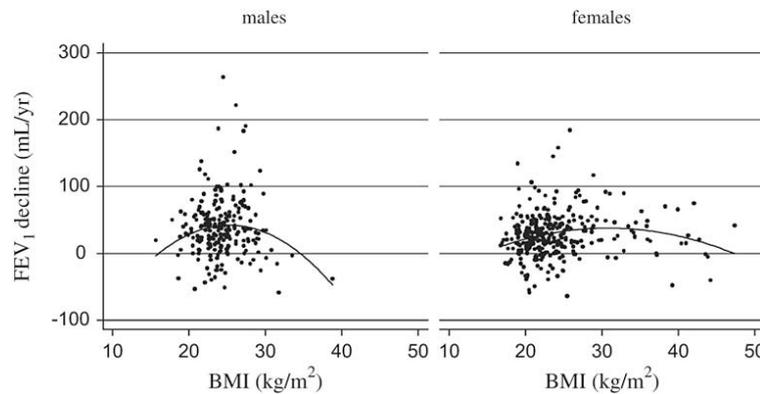
§Quantitative variables were centered around their mean to calculate the intercepts; the intercept represents the value of FEV<sub>1</sub> decline when all categorical variables are set to the reference category (eg, sex = male) and all the quantitative variables are set to their mean.

||The estimate for different variables represents the difference in the annual decline in FEV<sub>1</sub> between those with and those without these characteristics (eg, subjects without airflow obstruction at baseline who gained 1 BMI unit per year of follow-up had an additional decline in FEV<sub>1</sub> of 28.1 mL/y compared with those who did not gain weight).

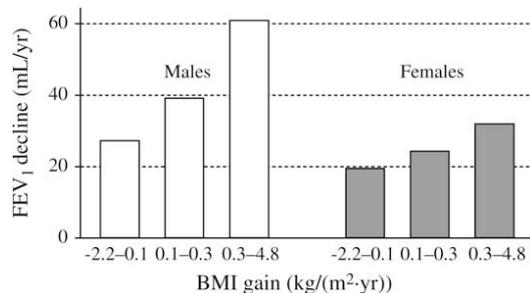
and 35% ( $P = .11$ ), respectively. Thus, this explanation seems unconvincing.

### BMI gain and FEV<sub>1</sub> decline in subjects with asthma without airflow obstruction at baseline

In subjects without airflow obstruction, we found that BMI gain during the follow-up was associated with FEV<sub>1</sub> decline, and this



**FIG 2.** Plot of FEV<sub>1</sub> decline vs baseline BMI, and of the curve representing the FEV<sub>1</sub> decline predicted on the basis of a linear regression on BMI and BMI squared, in men and women without airflow obstruction at baseline.



**FIG 3.** Mean FEV<sub>1</sub> decline (adjusted for all the covariates considered in the multivariate analysis) by categories of BMI gain (according to tertiles) for men and women without airflow obstruction at baseline. *P* value for trend: *P* < .001 (men) and *P* = .001 (women).

association was stronger in men than in women. In men, FEV<sub>1</sub> dropped by 20 (95% CI, 10-30) mL/y for every kilogram gained during the follow-up, whereas in women, FEV<sub>1</sub> decreased by 6 (95% CI, 1-11) mL/y for every kilogram gained. This might be explained by the fact that men who gain weight tend to accumulate abdominal and visceral fat, whereas women who gain weight tend to accumulate peripheral fat.<sup>27</sup> Chinn et al<sup>14</sup> found that the FEV<sub>1</sub> decline in the general population of the ECRHS was about 40% lower than the decline we found in the subjects with asthma. Therefore, one could speculate that the negative effect of weight gain on lung function is greater in subjects with asthma than in people from the general population.

The observed association between BMI gain and FEV<sub>1</sub> decline was independent of baseline BMI. This is also confirmed when we consider the group of subjects who were obese at baseline (*n* = 42). Among them, 31 individuals gained weight during the follow-up (median BMI gain [range], 0.5 [0.04, 3.0] kg/m<sup>2</sup>), and 11 individuals decreased their weight (median BMI gain [range], -0.2 [-2.2, -0.05] kg/m<sup>2</sup>). FEV<sub>1</sub> decline was faster in the obese subjects who gained weight (median [Q<sub>1</sub>-Q<sub>3</sub>], 26 [-1, 47] mL/y) than in the obese subjects who decreased their weight (median [Q<sub>1</sub>-Q<sub>3</sub>], 5 [-38, 34] mL/y; *P* = .13).

### Educational level and FEV<sub>1</sub> decline in subjects with asthma without airflow obstruction at baseline

In the population without asthma, a low socioeconomic level is almost universally associated with worse health outcomes,

including lung function.<sup>28</sup> In our data, subjects with asthma without airflow obstruction who had a high educational level had a faster FEV<sub>1</sub> decline than those with a lower educational level. However, the latter had a low FEV<sub>1</sub> already at baseline (Table E1). It is possible that a low educational level was associated with an early drop of FEV<sub>1</sub> and that, subsequently, the process of FEV<sub>1</sub> decline was no longer progressive.

### Active smoking, passive smoking, and FEV<sub>1</sub> decline in subjects with or without airflow obstruction at baseline

Many<sup>29-31</sup> but not all<sup>32</sup> studies have found that smokers with asthma have a steeper decline in lung function than nonsmokers with asthma. Several factors could explain the lack of an association that was observed in our study. First, our subjects were relatively young, and the detrimental effect of smoking could be greater at older ages.<sup>30</sup> Second, the exposure to active smoking of our cohort was relatively low. In the Copenhagen City Heart Study, about 70% of the subjects with asthma were smokers,<sup>29</sup> and they had even been exposed to smoking for more than 30 years on average.<sup>33</sup> In our study, only 30% were current smokers, and the exposure to active smoking tended to diminish significantly during the follow-up.<sup>34</sup> Finally, our finding could reflect the healthy smoker effect.

### Determinants of FEV<sub>1</sub> decline in subjects with asthma with airflow obstruction at baseline

Among subjects with airflow obstruction at baseline, those who were not sensitized to allergens had on average 29 mL/y more decline than those who were not. This is in line with the finding that the rate of lung function decline is greater in subjects with intrinsic asthma than in those with extrinsic asthma,<sup>32</sup> and with previous observations.<sup>7</sup>

A 1-U decrease in baseline BMI was associated with a 5 mL/y decrease in FEV<sub>1</sub> during the follow-up. In this group of subjects with airflow obstruction at baseline, leanness itself may be a marker of an early form of chronic obstructive pulmonary disease.<sup>35</sup> No association was found between FEV<sub>1</sub> decline and BMI gain, probably because mechanisms that are typical of milder asthma (including the effect of weight gain) could be less important in severe asthma, whereas a serious long-lasting inflammation

may play a crucial role. Moreover, subjects with airflow obstruction gained on average less weight than those without.

## Conclusion

In European subjects with asthma without airflow obstruction at baseline, individuals with a high BMI had the lowest FEV<sub>1</sub> at baseline. Weight gain was positively associated with FEV<sub>1</sub> decline, independently of baseline BMI, and this association was stronger in men than in women. The detrimental effect of BMI gain on lung function might be greater for subjects with asthma than for subjects without asthma, but studies are needed to clarify this. Among subjects with asthma with airflow obstruction at baseline, lean subjects without sensitization to allergens had the greatest FEV<sub>1</sub> decline, whereas weight gain was not associated with FEV<sub>1</sub> decline.

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**Clinical implications: Weight management or weight loss should be encouraged in patients with asthma. Asthma in the obese may be different from asthma in the nonobese, possibly because respiratory mechanical factors have a crucial role in the obese.**

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