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INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA- LIKE SYMPTOMS

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PAPER I-VI		

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Sue-Chu M, Larsson L, Bjermer L. Prevalence of asthma in young cross-country skiers in central Scandinavia: differences between Norway and Sweden. Respir Med 1996; 90: 99-105.

II Sue-Chu M, Karjalainen E-M, Altraja A, Laitinen A, Laitinen L A, Næss A-B, Larsson L, Bjermer L. Lymphoid aggregates in endobronchial biopsies from young elite cross-country skiers. Am J Resp Crit Care Med 1998; 158: 597-601.

III Sue-Chu M, Larsson L, Moen T, Rennard S I , Bjermer L. Bronchoscopy and bronchoalveolar lavage findings in cross- country skiers with "ski-asthma". Eur Respir J 1999; 3: 626-633

IV Karjalainen E-M, Laitinen A , Sue-Chu M, Altraja A, Bjermer L, Laitinen L A. Evidence of airway inflammation and remodelling in ski athletes with and without bronchial hyperresponsiveness to methacholine.
Submitted Am J Resp Crit Care Med 1999

V Sue-Chu M, Karjalainen E-M, Laitinen A, Larsson L, Laitinen L A, Bjermer L. Placebo-controlled study of inhaled budesonide on indices of airways inflammation in bronchoalveolar lavage fluid and bronchial biopsies in cross country skiers.
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VI Sue-Chu M, Henriksen AH, Bjermer L. Non-invasive Evaluation of lower airway inflammation in hyperresponsive Elite Cross Country Skiers and Asthmatics.
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ABBREVIATIONS

AMP	Adenosine 5´-monophosphate
APAAP	Alkaline phosphatase anti-alkaline phosphatase
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BALT	Bronchus associated lymphoid tissue
BR	Bronchial responsiveness
BHR	Bronchial hyperresponsiveness
BM	Basement membrane
ECP	Eosinophil cationic protein
EIB	Exercise-induced bronchoconstriction
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
mAb(s)	Mouse monoclonal antibody (antibodies)
NO	Nitric oxide
OCT	Ornithyl carbamyl transferase
PEF	Peak expiratory flow rate
PBS	Phosphate buffered saline.
Tn	Tenascin

SUMMARY

Bronchial asthma is a chronic inflammatory disorder of the airways which is manifested clinically by symptoms of airway obstruction and bronchial hyperresponsiveness to a variety of stimuli, including exercise and methacholine. Airway inflammation can be assessed directly by invasive methods involving fibreoptic bronchoscopy, bronchoalveolar lavage and endobronchial biopsy, and indirectly by non-invasive methods such as measurement of hyperresponsiveness to adenosine 5´monophosphate and exhaled NO concentration. Studies within the past two decades have suggested a dual nature to the relationship between asthma and sport. Elite, competitive, cross-country skiers have an apparent increased problem with asthma-like symptoms and hyperresponsiveness to methacholine. This was addressed in the present study with the aim of achieving a better understanding of the underlying pathogenetic mechanisms and pathophysiology of this entity.

The prevalence of asthma-like symptoms, as assessed by a self-completed questionnaire, and bronchial hyperresponsiveness to methacholine were compared in young adult competitive cross-country skiers in Trøndelag in central Norway and Jämtland in Central Sweden, areas with a coastal and an inland winter climate, respectively. The presence of asthma-like symptoms, defined as the presence of wheeze and abnormal breathlessness or chest tightness within the previous 12 months, and bronchial hyperresponsiveness, defined as a $PD_{20}FEV_1$ of < 9.1 µmol methacholine, was chosen as the operational definition of ski asthma. Bronchial responsiveness to AMP in non-steroid treated ski asthmatics was compared to that in nonsteroid- and steroid-treated asthmatics, and exhaled NO levels were compared in skiers, asthmatics and healthy controls. Bronchial inflammation was assessed macroscopically, in the BAL fluid, and in endobronchial biopsies by immunohistochemistry and electron microscopy, and compared with asthmatics and healthy controls. Finally, the effect of intervention with inhaled budesonide over a competitive season on inflammatory indices was also investigated.

Of a study population of 118 Norwegian and 53 Swedish skiers, 46% and 51% reported asthma-like symptoms, respectively. Although the prevalence of hyperresponsivenesness with asthma-like symptoms and of respiratory allergy was three-fold greater in Swedish than in Norwegian skiers, there was no association of hyperresponsiveness with self-reported respiratory allergy in skiers from the region with a colder winter climate. In ski asthmatics, responsiveness to adenosine 5´-monophosphate and levels of exhaled NO were lower than in asthmatics, in spite of a similar degree of responsiveness to methacholine. Exhaled NO concentration was not significantly different than in healthy, nonatopic controls. The airways were visibly inflamed at bronchoscopy, and there was an absence of eosinophilia and ECP in the bronchoalveolar lavage fluid profile, which differed in several respects from healthy controls. The bronchial mucosa was infiltrated with inflammatory cells, predominantly lymphocytes and neutrophils, and a high frequency of lymphoid aggregates with features suggestive of bronchus associated lymphoid tissue was observed. There was increased expression of tenascin in the subepithelial basement membrane, suggestive of airway remodelling. The presence of these mucosal findings in skiers was irrespective of the presence of asthma-like symptoms, hyperresponsiveness or respiratory allergy.

Inhaled budesonide in a daily dose of 800 µg over a competitive season significantly improved FEV_1 but did not have any apparent beneficial effect on asthma-like symptoms or airway inflammation assessed at bronchoscopy, in the BAL fluid or in biopsies, compared to placebo. Skiers had a spontaneous improvement in bronchial responsiveness to methacholine and decreased lymphocyte activation at the end of a competitive season.

Competitive cross-country skiing may be a risk factor for the development of asthma-like symptoms and bronchial hyperresponsiveness to methacholine. Hyperpnea of cold, dry air represent a significant environmental

stress to the airways of these athletes, leading to bronchial inflammation with remodelling in skiers with and without “ski asthma”. Unlike asthmatic inflammation, the role of the eosinophil appears to be limited, while that of the lymphocyte and the neutrophil appears to be of more significance in the inflammatory process in the skiers. This process does not appear to be influenced by daily treatment with inhaled budesonide over a competitive season. The inflammatory condition in competitive skiers may best be described as cold air- and hyperpnea-induced bronchitis; thus reduction of the environmental stress may be more important than pharmacological intervention in the management of this entity.

INTRODUCTION

BRONCHIAL ASTHMA –DEFINITION

Asthma has traditionally been functionally defined as "a disease characterised by an increased responsiveness of the trachea and bronchi to various stimuli and manifested by a widespread narrowing of the airways that changes in severity either spontaneously or as a result of therapy " [1]. Studies over the past four decades, initially in post-mortem material, later of bronchoalveolar lavage fluid and endobronchial biopsies and recently in induced sputum, have consistently highlighted the inflammatory changes in the airways in asthmatics[2, 3]. This has resulted in a shift of emphasis away from airway smooth muscle dysfunction to a T-cell modulated chronic desquamative eosinophilic bronchitis as the primary abnormality in asthma [4, 5, 6]. This is emphasised in the current operational definition of asthma, which states that

"*Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils, and T-lymphocytes. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and /or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli "* [7].

EXERCISE-INDUCED BRONCHOCONSTRICTION– DEFINITION AND PATHOPHYSIOLOGY

Aretaeus of Cappodoccia described the presence of breathing difficulties on physical exercise as early as 2000 years ago. At that time, he wrote " if from running, gymnastics or exercise, or any other work the breathing becomes difficult, it is called Asthma" [8]. This observation was repeated by Sir John Floyer, a physician who also suffered from asthma, in his "Treatise of the asthma ", published in 1698 - " All exercise makes the asthmatic to breathe short.... and if the Exercise be continued it occasions a Fit "[9]. He also appreciated that the exercise activity that induced the maximum degree of ventilation also precipitated the most severe symptoms of asthma.

Exercise-induced asthma and exercise-induced bronchoconstriction (EIB) are synonymous terms used to define the airway narrowing that follows vigorous exercise. A fall in FEV_1 of 10% or more after a standardised bicycle ergometer or treadmill exercise protocol in the laboratory is regarded as diagnostic of EIB [10], whereas a fall of 15% or more in PEF has been used when exercise testing is conducted outside of the laboratory [11]. In addition to the nature, duration and intensity of the exercise, the environmental conditions under which exercise is performed also influences the airway response to exercise. Such factors include the levels of allergens and environmental pollutants and the humidity and temperature of the inspired air, with an additive effect of inhalation of subfreezing air [12, 13].

EIB is a sensitive indicator of asthma, as it is present in 80% asthmatics not treated with inhaled corticosteroids [14] and in 50 % asthmatics despite current treatment with inhaled steroids [15] and is absent in those asthmatics who become symptom free [16].

Full conditioning of the inspired air occurs in the nose and the upper airway under resting conditions. During exercise, an increase in ventilation and gas exchange is necessary in addition to an increase in skeletal muscle activity and cardiac output to meet the increased aerobic demands. The increase in minute ventilation is facilitated in part by an increase in the diameter of the lumen of the lower airways [17], and results in a shift in ventilation from nasal to oronasal route, which forces the task of conditioning of the inspired air on to the lower

airways. In normal individuals, a transitory increase in airway resistance may accompany vigorous exercise with a maximum post- exercise fall in FEV_1 of less than 10%. In other individuals, resistance to airflow is increased as early as four minutes after the start of prolonged strenuous exercise [18,19]. They commonly complain of wheeze, chest tightness, difficult breathing or cough during or within the first 30 minutes after exercise, may have difficulty in continuing or resuming exercise, and the post-exercise fall in FEV_1 exceeds 10%. The increased resistance to airflow is due to airway narrowing, which develops predominantly in the large airways. In severe cases, the small airways are involved [20], leading to pulmonary hyperinflation and arterial hypoxemia [21]. Spontaneous resolution of asthmatic symptoms and airway obstruction occurs within 60 minutes in most persons, and in approximately 40- 50% of subjects, is accompanied by a refractory period of two to four hours duration [22]. During this period, significantly less (less than 50%) bronchoconstriction is evoked on performance of an identical exercise task. The degree of this protection is independent of the severity of the preceding bronchoconstriction [23]. Moreover, absence of bronchoconstriction does not protect against bronchoconstriction in a subsequent exercise challenge [24]. In some patients, the initial episode may be followed three to ten hours later by a second episode of bronchoconstriction [25], which is associated with increased neutrophil chemotactic activity [26] and is localised to the peripheral airways [27]. However, the existence of this late response has been disputed by others [28].

The stimulus for acute airway narrowing in sensitive subjects is the loss of water from the airways, while the mechanism is thought to be due to the osmotic and thermal effects associated with water loss. According to the osmotic theory [29], airway narrowing is due bronchial smooth muscle contraction in response to mediators released from mast cells, airway epithelial cells and nerve cells, secondary to the development of a hyperosmolar environment in the airways. In contrast, the thermal theory [30] proposes that airway cooling and bronchoconstriction occurs during exercise and that the increase in airways resistance after exercise is due to the reactive hyperaemia in the bronchial vasculature and subsequent oedema.

BRONCHIAL RESPONSIVENESS AND HYPERRESPONSIVENESS

Bronchial responsiveness (BR) is the tendency of the airways to constrict to a variety of physical or chemical stimuli. Bronchial hyperresponsiveness (BHR) is present when the degree of bronchial responsiveness is greater than that observed in normal subjects. The airways are more sensitive in response to the constricting stimulus (hypersensitivity) or are more reactive in the degree to which they will constrict, with elevation or disappearance of a maximal response plateau (hyperreactivity), or both. In man as well as in other species, the distinction between normal and heightened BR is not sharp.

An increased responsiveness to a variety of physical and chemical stimuli can be demonstrated more readily in asthmatic than in nonasthmatic subjects. This has been recognised as an important and cardinal feature of asthma. These stimuli can be classified into direct and indirect stimuli, which reflect the presumed mechanisms of bronchoconstriction. Of the direct stimuli, histamine acts on airway H_1-receptors, while the synthetic muscarinic agonist, methacholine acts on muscarinic M_3 receptors on the airway smooth muscle. Histamine also has indirect effects as it increases vascular permeability. Indirect stimuli act through the release of constrictor mediators from cells within the airways or via neural pathways. Hyperresponsiveness to indirect stimuli should correlate better with naturally occurring symptoms of variable airflow limitation, which are chiefly induced by indirect mechanisms[31]. Such stimuli include exercise, isocapnic hyperventilation with room or cold air, hypo- or hypertonic aerosols and adenosine 5´-monophosphate (AMP). After inhalation, AMP is rapidly converted to adenosine, which reacts with

purine receptors located on the surface of pre-activated mast cells with an increased release of mediators such as histamine, prostanoids and leukotrienes [32,33,34,35]. Hyperresponsiveness to AMP is considered to be specific for asthmatic subjects [36].

The mechanisms of bronchial hyperresponsiveness in asthma are not known. Twin studies have suggested that environmental factors are more important than a genetic predisposition for the development of hyperresponsiveness [37, 38]. Bronchial hyperresponsiveness is associated with airway inflammation. A transient increase in hyperresponsiveness is associated temporally with an influx of inflammatory cells into the airways [39]. Significant positive correlations of airway responsiveness with counts of mast cells and eosinophils [40] and neutrophils [41] in BAL fluid and of activated eosinophil count in bronchial biopsies [42] have been reported. On the other hand, stable hyperresponsiveness in asthmatic patients may not be associated with persistent airway inflammation in bronchial biopsies. Persistent bronchial hyperresponsiveness to methacholine was present in asthmatics who had no evidence of cellular inflammation in the bronchial mucosa after ten years of treatment with inhaled corticosteroids [43]. Persistent inflammation may cause airway remodelling with structural changes such as hypertrophy / hyperplasia of smooth muscle, increased vascularity and a thickening of the reticular basement membrane from increased deposition of fibrous proteins like collagens and tenascin may be present in asthmatic airways. This remodelling of the airway may have an effect on clinical indices of asthma, including bronchial responsiveness and may to a limited degree be influenced by pharmacological treatment[44, 45]. The deposition of tenascin is increased in both seasonal allergic and chronic asthmatics, and is decreased by inhaled corticosteroid therapy [46].

NITRIC OXIDE

Nitric oxide (NO) is produced from the enzymatic conversion of the amino acid L-arginine to L-citrulline by the NO synthase enzyme, which has three isoforms. The isoforms are based on the three different genes that have been cloned and sequenced, cell or organ localisation and sensitivity to calcium stimulation. The constitutive NO synthase (cNOS) has two isoforms isolated from brain and endothelial cells, is calcium dependent with the generation of picomolar quantities of NO and are expressed in health. The inducible NO synthase (iNOS) is calcium independent, induced by proinflammatory cytokines with the production of nanomolar quantities of NO and is increasingly found in disease states [47]. With the exception of iNOS in the paranasal sinuses that is constitutively expressed [48], this isoform is sensitive to corticosteroids.

Minute concentrations of NO of endogenous origin are detectable in the exhaled air in man [49]. Although the paranasal sinuses are the principal source of nitric oxide in the upper airway and NO is autoinhaled during respiration with air with absorption of about 50-70% of the autoinhaled dose by the lungs [50], a significant proportion of exhaled nitric oxide arises in the large airways of normal subjects [51]. The precise cell source in the lower airways of NO in normal subjects is not known with certainty, but the airway epithelium, inflammatory cells, sensory nerves, endothelial cells and vascular and airway smooth muscle cells are believed to be principal sources of NO[52]. Increased levels of exhaled NO have been described in bronchial asthma [53], the late asthmatic response after allergen challenge[54], upper and lower respiratory tract infections [55] and bronchiectasis [56], and in allergic rhinitis [57]. A decrease in the level of exhaled NO together with clinical improvement is seen after inhaled corticosteroid therapy in asthma [58]. Moreover, reducing the dose of inhaled steroids in asthmatics was accompanied by an increase in exhaled NO levels [59]. The measurement of exhaled NO has been suggested to

be a promising simple and completely non-invasive, indirect marker for monitoring airway inflammation and the effects of anti-inflammatory treatment in asthma [60].

ASTHMA AND SPORT

Since the disqualification of an asthmatic gold medallist in the 1972 Olympic Games for the use of a banned drug [61], considerable attention has been focused on asthma in sports athletes. The relationship between sport and asthma appears to be dual in nature. The frequency of self-reported asthma can vary from 4.3 to 9% % in Olympic summer athletes and up to 12% of players in an American football team [62, 63, 64]. On the other hand, highly trained athletes without a known history of asthma commonly report respiratory symptoms, such as chest tightness, cough, wheezing, or prolonged shortness of breath after exercise. The frequency of such symptoms varies from 6.9% to 19% in the highly trained summer athletes [62, 64]. A number of studies have specifically examined exercise testing in athletes, and produced a ten-fold range of frequencies (2.8% -28%) for the finding of exercise-induced bronchoconstriction [65, 66, 67, 68]. Underestimation of the frequency of exercise-induced bronchoconstriction cannot be excluded, as in some studies only athletes with respiratory symptoms were tested and exercise-induced bronchoconstriction may be asymptomatic [68].

The frequency of asthma symptoms is greater in winter sports athletes. Interest in this problem was kindled in May 1992 following the observation that 37% of cross-country skiers finishing in the top 15 places in short distance competitive events in the 1991 world championship used anti-asthmatic medication [Videman Tapio, American College of Sports Medicine meeting, May 1992]. This initiated a discussion in the Medical Committee of the International Ski Federation and the Northern European mass media as to the possible explanation for this phenomenon. In a subsequent study, Larsson L and co-workers reported prevalences of 24% for asthma symptoms and 18% for use of anti-asthmatic medication in young skiers [69]. Moreover, the cumulative prevalence of asthma was 15%, compared to 6% in age-matched nonathletic controls, and the common use of anti-asthmatic medication was a reflection of the high prevalence of self-reported asthma and asthma-associated symptoms. An even higher prevalence of asthmatic symptoms was found in adult skiers, being reported by 74% of subjects, while a combination of asthma symptoms and hyperresponsive airways or physician diagnosed asthma, or both was present in 49% of subjects. Further, bronchial hyperresponsiveness to methacholine was persistent in both the winter and summer months, and Larsson K and co-workers hypothesised that strenuous training at low temperatures may be pathogenetic for asthma, possibly due to repeated breathing of cold air in large amounts [70].

AIMS OF THE PRESENT STUDY

The overall aim of the study was to explore the apparent increased problem of asthma related to competitive cross-country skiing. By the use of a questionnaire, non-invasive and invasive methods, we hoped to achieve a better understanding of pathogenetic mechanisms and underlying pathophysiology.

This was addressed by

- comparing the prevalence of asthma-like symptoms and bronchial hyperresponsiveness in young adult cross country skiers from two regions with different winter climatic conditions in Central Scandinavia.
- characterising the phenomenon of bronchial hyperresponsiveness to methacholine in skiers and asthmatics by the use of inhalation provocation to AMP and measurement of nitric oxide in the exhaled air.
- assessing macroscopic inflammatory changes in the proximal airways at bronchoscopy, analysing the BAL fluid for evidence of inflammatory change and comparing the changes with those seen in healthy control subjects.
- assessing and comparing the morphological changes in endobronchial biopsies in skiers and asthmatic subjects with bronchial hyperresponsiveness, compared to healthy controls
- assessing the effect of inhaled budesonide over the competitive season on macroscopic inflammation, and inflammatory indices in the BAL fluid and endobronchial biopsies.

MATERIALS AND METHODS

SUBJECTS

Three groups of subjects were investigated. Elite cross country skiing athletes were students attending senior secondary schools with integrated curricula for educational and athletic requirements in regions of Trøndelag, Central Norway (Heimdal and Meråker) and of Jämtland, Central Sweden (Järpen), (Paper I-VI) and conscripted soldiers in the Swedish military ski platoon based in Östersund (Paper I -V). Asthmatic subjects were patients attending the outpatient clinic of the Department of Lung Medicine in Trondheim, Norway, secondary school students in the county of North Trøndelag (Paper VI) and the University Lung Hospital in Tartu, Estonia (Paper IV). Healthy control subjects were medical students from Trondheim (Paper II, III), students in Tartu (Paper IV) and North Trøndelag (Paper VI). All subjects were non-smokers.

The study population in Paper I consisted of 171 skiers (Norway - 118 skiers, Sweden - 38 skiers and 15 conscripted skiers). From this population, subsets were investigated in Paper II-V. In Paper VI, additional skiers from Norway were recruited to supplement the study population from Paper I. Asthmatic subjects were categorised into subsets of mild intermittent asthma (Paper VI) and mild to moderate persistent asthma (Paper IV, VI). The number of subjects from each group is detailed in the methods section in each paper.

All subjects, as well as the parents of those subjects less than 18 years of age, gave written informed consent, and the study was approved by the Regional Ethics Committee in Trondheim, Norway and the ethical committee in Tartu, Estonia.

QUESTIONNAIRE

The questionnaire used in Paper I was in the native language of the subjects and was based on that used by Larsson and co-workers in an earlier study of skiers [69]. Each skier was asked to estimate the time spent in training during the previous year and the number of years of competition. There were also questions about known respiratory allergy, cigarette smoking, medical consultations within the last 5 yr for respiratory symptoms, a physician diagnosis of asthma, use of anti-asthmatic medication and the presence within the last year of respiratory symptoms, such as cough, attacks of shortness of breath, chest tightness and wheezing. Enquiry was made about the presence of these symptoms at rest, on exercise, or on exposure to cold conditions and irritants.

LUNG FUNCTION TESTS

Lung function parameters (FEV_1 and FVC) was measured with a turbine flowmeter, the Microlab 3300 Mk2 spirometer (Micro Medical Ltd, Gillingham, Kent, UK) in Paper 1-V, or with a pneumotachograph, the Jaeger Flowscreen or Masterscope spirometer (Erich Jaeger Laboratories, Würzburg, Germany) in paper IV and VI. The better of two measurements of FEV_1 with less than 5% variation was recorded at baseline and during bronchial provocation testing. Predicted normal values were based on European Coal and Steel Community reference values for those subjects over 18 years of age and Zapletal reference values for those under 18 years of age.

BRONCHIAL PROVOCATION TESTS

Bronchial provocation testing was performed with a controlled tidal volume breathing technique and an inhalation synchronised dosimetric jet nebuliser system. The Spira Elektro 2 nebuliser system (Respiratory Care Centre,

Hameenlinna, Finland) was used in Paper 1-VI and the MedicAid jet nebuliser supplied with the Aerosol Provocation System (APS, Jæger) was used in the exhaled NO part of Paper VI.

The Spira system, which incorporates a turbine flow sensor for monitoring tidal volume and inspiratory flow, was used for provocation testing with methacholine, AMP and histamine. The characteristics of the system and the good reproducibility of provocation testing have been documented previously [71, 72]. The particle size from the Spira Elektra 2 nebuliser with a flow rate of 7.5L/min has a mass median diameter of 1.6µm [73]. The inhalation technique was practised prior to provocation testing so that an inspired tidal volume of 0.5 L was easily and consistently achieved at an inspiratory flow rate of less than 0.5 L /s. The protocol for methacholine provocation consisted of a cumulative dose of 1800 µg (9.1μ mol) methacholine administered in four stages - four and 11 inhalations of methacholine 2.5 mg ml $^{-1}$, followed by 3 and 10 inhalations of methacholine 25 mg ml $^{-1}$. For AMP provocation, the protocol consisted of a cumulative dose of 145.4 µmol (50500 µg) delivered in six increments. These consisted of inhalation of four and ten breaths of 25 mg·ml $^{-1}$ and three, six, ten and 20 breaths of mg.ml $^{-1}$ solution of AMP (Sigma Chemicals, St. Louis, MO, USA), freshly prepared in saline. For histamine provocation in Estonia (Paper IV), buffered histamine diphosphate in saline solution in concentrations of 4 and 16 mg/ml were administered in successive doses of 25, 100, 400 and 1600 µg [72].

For tests performed with this system on subjects in Norway and Sweden, the driving pressure was 1.3 bar with onset of nebulisation occurring after 100 ml of inspired tidal volume and an aerosol delivery time of 0.5 s. With these settings, the mean (± SD) output of the dosimeter was 5.0 ±0.5 µL per inhalation, determined by 5 weighings on a gravimetric balance. In Estonia, the driving pressure was 2 bar with a nebulisation time of 0.4 s, which gave a mean (± SD) output of 6.5 ± 0.3 µl per inhalation [72]

The APS system was used for bronchial provocation with methacholine in asthmatic and control subjects in the exhaled NO study in Paper VI. The particle size delivered by the MedicAid nebuliser with a driving pressure of 1.5-1.6 bar has a mass median diameter of 3.2 µm. A cumulative dose of 10.1 µmol (2000 µg) methacholine was delivered in five increments of 50 and 100 µg by nebulisation of 5mg/ml solution and 300, 600 and 950 µg by nebulisation of 50 mg/ ml solution.

Prior to bronchial provocation testing, inhaled steroids were not discontinued, but short-acting β_2 agonists were withheld for at least 8 hours and long-acting β_2 agonists, chromones and theophylline were withheld for at least 24 hours. Short-acting systemic antihistamines were withheld for at least 24 hours prior to AMP provocation. FEV_1 was measured at baseline after the inhalation of normal saline, at 90 s after the completion of each stage and 180 s after the final stage. The better of two measurements with less than 5% variation was recorded. For provocation with methacholine and AMP, the test was stopped if the FEV_1 fell by 20% or more, relative to baseline FEV_1. For histamine provocation, the test was stopped if the FEV_1 decreased by 15% or more from baseline.

Bronchial hyperresponsiveness was defined as the cumulative dose of methacholine and AMP, which caused a decline in FEV_1 by 20% or more (PD_{20} FEV_1), relative to baseline. For histamine, the PD_{15} FEV_1 was used. The PD_{15} FEV_1 and PD_{20} FEV_1 was determined by interpolation of the last two points on the log dose-response plot. The PD_{20} FEV_1 for methacholine was defined as ≤ 9.1μmol (1800 μg) with the Spira system and ≤ 10.1 µmol (2000 µg) with the APS system. For AMP, the PD_{20} FEV_1 was ≤ 145.4 µmol (50500 µg), and the PD_{15} FEV_1 for histamine was defined as 1.6 mg.

Bronchial responsiveness was expressed as the dose response ratio or slope (% fall in FEV_1 · µmol $^{-1}$), as proposed by O'Connor [74].

DEFINITIONS

In skiers, positive asthma symptomatology was defined as the presence within the last year of wheeze together with abnormal breathlessness or chest tightness either on exertion, at rest, or on exposure to irritants. Current asthma or ski asthma was defined as positive asthma symptomatology with BHR to methacholine at the time of investigation. Total asthma was defined as current asthma in addition to physician diagnosed asthma currently treated with steroids.

In asthmatic subjects, intermittent asthma was defined as a history of asthma symptoms and bronchial hyperresponsiveness to methacholine. Mild asthma was defined as a history of asthmatic symptoms controlled by β2 agonists and or theophylline, bronchial hyperresponsiveness to histamine, positive bronchodilator test and an FEV_1 greater than 80 % of predicted. Moderate asthma was defined as asthma requiring daily treatment with inhaled corticosteroids for adequate control of symptoms and normal lung function.

SCREENING FOR ALLERGIC SENSITISATION

Allergy screening was performed with serological testing of subjects in Norway and Sweden. The presence of serum immunoglobulin (Ig) E to a panel of eight aeroallergens (house dust mite, cat, dog, horse, timothy grass and birch pollens, mugwort and cladosporium) was screened for with the Phadiotop CAP™ test (Pharmacia Diagnostics, Lund, Sweden) in Paper III –V. In Paper VI, the AlaTOP microtitre plate method (Diagnostics Products Corp., Los Angeles, Ca., USA) was employed. For asthmatic and control subjects in Estonia, a skin prick test was used to investigate for sensitisation to a panel of twelve common aeroallergens (Allergologisk Laboratorium A/S, Hørsholm, Denmark).

EXHALED NO MEASUREMENT (Paper VI)

NO concentration in the expired air was measured immediately before spirometry and bronchial provocation by the chemiluminescence method and in accordance with the recommendations of the ERS Task Force Report on standardisation of equipment specification and respiratory manoeuvres [75]. The LR 2000 nitric oxide gas analyser (Logan Research Ltd., Rochester, UK) was calibrated with a certified reference calibration gas mixture of a known concentration of NO in nitrogen ("SpectraSeal", BOC Specialty Gases, Guildford, UK) at a gas sample flow rate of 250 ml / minute. Auto zero was performed prior to the acquisition of each measurement. NO levels were measured in duplicate with the subject in the seated position. A nose clip was applied immediately prior to oral inspiration to total lung capacity. With the help of a biofeedback monitor, the subject immediately commenced controlled exhalation to residual volume against a target resistance of 4-5 cm H_2O and at an expiratory flow rate of 250 ml / sec. Expired air was sampled by the analyser at a rate of 250 ml / min by sidestream sampling. Levels of exhaled NO were based on analysis of the plateau portion of the exhaled NO curves. The average value for each subject was used for analysis. The coefficient of variation (CV) for exhaled NO were 11.4% and the agreement between 2 independent readers was excellent (r = 0.99, CV 7.1 %). All NO curves were subjected to a thorough quality control, and non- optimal NO curves were not included in the study.

BRONCHOSCOPY, MACROSCOPIC INFLAMMATORY INDEX, ENDOBRONCHIAL BIOPSIES AND BRONCHOALVEOLAR LAVAGE

Bronchoscopy was performed with the flexible fibreoptic bronchoscope in accordance with published international guidelines [76]. The procedure in healthy control subjects in Paper II-III and skiers in Paper II-V was performed in the autumn and/or late winter months at the University Hospital, Trondheim, Norway, and in asthmatic patients and healthy control subjects in Paper IV was performed at Tartu University Lung Hospital, Estonia. Subjects were not investigated within four weeks of an upper respiratory tract infection. Skiers and healthy control subjects in Trondheim were premedicated with nebulized salbutamol (1 mg / ml, 2.5 ml) and ipratropium bromide (0.25 mg / ml, 1 ml) at 15 minutes prior to, and intravenous glycopyrronium (0.2 mg / ml, 1.5 - 2.5 ml), midazolam (1 mg / ml, 1 - 2 ml) and alfentanil (0.5 mg / ml, 0.5 ml), immediately prior to bronchoscopy. Asthmatic patients and control subjects in Tartu were premedicated with intramuscular atropine (0.5-1.0 mg**)** and oral diazepam (5-10mg). Local anaesthesia was achieved with xylocaine hydrochloride, administered as a spray to the oropharynx and as 2-4% solution instilled by indirect laryngoscopy and through the working channel of the bronchoscope into the tracheobronchial tree, to a total dose of 400mg. Supplementary oxygen (2l / min) was administered via a nasal cannula, and oxygenation status was monitored by pulse oxymetry during the procedure. The bronchoscopes used were Olympus BF -XT 20, BF -IT 30 or BT-IT 20D bronchoscopes (Olympus Optical Co., Tokyo, Japan).

The macroscopic inflammatory index was based on friability, vascularity and oedema of the bronchial mucosa, and the amount of secretions in the proximal airways. Friability was the susceptibility of the mucosa to bleed on contact with the bronchoscope. Each parameter was assessed on a 5- point scale (0 - 4) by an experienced bronchoscopist (LB) who was blinded for the clinical status of the skiers. A maximum score of 16 indicated the presence of severe macroscopic inflammatory changes. The index was recorded in all control subjects, 11 nonasthmatic and 13 hyperresponsive skiers in study III, and in 9 skiers at baseline and after intervention in the budesonide and placebo groups in Paper V.

The biopsies were taken from the second and third generation carinae of the right and left lobes with the Olympus FB19C, FB20C or 35C biopsy forceps, gently extracted and immediately snap-frozen in liquid nitrogen and stored at -70 ^{o} C. In two skiers, biopsies were fixed in 4% buffered formaldehyde solution for routine histopathologic studies.

With the bronchoscope in the wedged position, bronchoalveolar lavage (BAL) of the medial segment of the middle lobe was performed with 2 x 60 ml aliquots of prewarmed (37^{o} C) phosphate buffered saline (PBS). The bronchoalveolar lavage fluid (BAL fluid) was recovered into siliconised plastic containers and stored in an icebath (4^{o} C) until processed.

Variations in bronchoscope and forceps models and premedication were due to the routine practices that were current in the two centres at that time. The procedure was well tolerated by all subjects, and no complications or adverse events were noted.

PROCESSING OF BRONCHIAL BIOPSIES (PAPER II, IV, V)

The stored biopsy specimens were embedded in Tissue Tek ornithyl carbamyl transferase (OCT) medium (Miles Inc., Elkart, IN, U.S.A.). Serial cryosections of 5 µm thickness were cut on a Leitz 1720 Digital Cryostat (Ernst Leitz GmbH, Wetzlar, Germany). Microscope slides containing three sections per slide were made of each biopsy

specimen, air dried, and stored at –70 °C. Sections were fixed in precooled acetone -20 °C for 10 min prior to immunohistochemistry.

Monoclonal antibodies

Commercially available mouse monoclonal antibodies (mAbs) were used to identify specific markers of the inflammatory cells. Eosinophils were identified with the EG2 mAb (dilution 1:50, Kabi Pharmacia Diagnostic AB, Uppsala, Sweden), which recognises the cleaved form of eosinophil cationic protein (ECP) [77]. The AA1 mast cell tryptase clone mAb (1:500), BerMAC3 mAb (1:25), anti-CD3 (1:1000) mAb and neutrophil elastase clone NP57 mAb (1:2000) were used for the detection of mast cells, macrophages, T-lymphocytes, and neutrophils, respectively (all from Dako AS, Glostrup, Denmark). In Study II, the T-cytotoxic and T-helper subsets of T-lymphocytes were detected with anti-CD8 (1:1000) and anti-CD4 (1:100) mAbs, and B lymphocytes were detected with anti-CD20 (1:10 to 1:25) mAb (Dako AS). The extracellular matrix protein, Tenascin (Tn) was detected with the 100EB2 mAb, which recognises the fourth and fifth fibronectin-like domains in the Tn-C molecule [78].

Immunohistochemistry

Inflammatory cells

Antibody binding to the specific antigens on the inflammatory cells was visualised with the alkaline phosphatase anti-alkaline phosphatase (APAAP) method (Dako A/S). Briefly, acetone-fixed specimens were washed in Tris-buffered saline (TBS, pH 7.4) for 10 min, exposed to the primary mAb for 30 min in a moist chamber at room temperature and washed in TBS. This was followed by application of rabbit anti-mouse immunoglobulin (dilution 1:25) for 30 min, washing in TBS, application of mouse monoclonal APAAP for 30 min, and a final wash in TBS. The colour reaction was developed with a New Fuchsin- based substrate system (Sigma, St. Louis, MO, USA). Endogenous alkaline phosphatase activity was blocked by 1M levamisole (Sigma). The sections were counterstained with Mayer's hemalan (dilution 1:5, 5s, E Merck, Darmstadt, Germany) before mounting in prewarmed (50°C) Glycergel (Dako).

Tenascin

Tn distribution was detected by an indirect immunofluorescence method [Laitinen tenascin]. Briefly, acetone-fixed specimens were successively incubated in a moist chamber at room temperature for 30 min with the primary mAb and with fluorescein isothiocyanate (FITC)-conjugated sheep anti-mouse IgG (dilution 1:150 - Jackson Immunosearch Laboratories, West Grove, PA, USA). They were mounted in Veronal-glycerol buffer (1:1, pH 8.4). Washing with phosphate buffered saline (PBS) was performed at each stage.

Routine immunohistopathology

Formaldehyde-fixed biopsies were embedded in paraffin and 4 µm thick sections were prepared and stained with the routine haematoxylin-eosin-saffran method and immunostaining for T-lymphocytes with anti-CD3 (1:200) mAb and

B-lymphocytes with anti-CD20 (1:900) mAb. Specific antibody binding was visualised by the labelled streptavidin-biotin- peroxidase method.

Electronmicroscopy

Some of the specimens were fixed and prepared for electron microscopy as described before [79] to create a more detailed view of the inflammatory cells and their relationship with other structural components such as the blood vessels in the mucosa.

QUANTITATIVE ANALYSES ON BRONCHIAL BIOPSIES

Inflammatory cell counts

Slide preparations were examined with Leitz Dialux 22 EB light microscope. (Ernst Leitz). EG2-positive eosinophils, mast cells, macrophages, neutrophils and T-lymphocytes were quantified over the entire section area of the biopsy sample. For each cell type, the section was photographed at an original magnification of x16 on to Kodak Ektachrome ™ EPN 100 colour slide film. The slides were projected onto a 42 x 60-inch digitising tablet (Kurta IS/THREE, Kurta Corp., Phoenix, USA) to reach a final magnification of x 695. The tablet was calibrated with a microscopic scale, which was photographed and reproduced at similar magnifications. The bronchial epithelium and subepithelial connective tissue or lamina propria were delineated and charted into the computer. Damaged, stretched, squeezed or double-folded areas, together with areas with smooth muscle and lymphoid aggregates were not charted. The cell density in the epithelium and the lamina propria was computed separately with the AutoCad program, version 10.1 (Autodesk Inc., Sausolito, Ca, USA) and then pooled to give the cell density as cells per mm^2 of total mucosa analysed.

Tenascin immunoreactivity

The thickness of the tenascin immunoreactivity was measured in the subepithelial basement membrane (BM) zone [80]. Slides were examined with a Leitz Aristoplan fluorescence microscope (Ernst Leitz GmbH) equipped with an appropriate filter for FITC fluorescence. The areas that contained cross-sections of the positively stained BM area were photographed at a preliminary magnification of x 80 on to Kodak T-MAX 400/800 black and white film (Eastman Kodak Company, Rochester, NY, USA). Paper photocopies with a final magnification of x 643 were made. The thickness of the tenascin immunoreactive area in the BM zone in the photocopies was semiautomatically computed using a calibrated digitising table (Kurta IS/THREE) and a pointing device to chart the area of stained basement membrane into a computer. Areas where the BM zone were not cut clearly crosswise were avoided. The AutoCad program, version 10.1 (Autodesk Inc.) was used to calculate the minimal distance between each point on the superficial and deep borders. The mean distance, expressed in micrometres, was computed and considered to be the thickness of the analysed staining in each subject.

Lymphoid aggregates

A lymphoid aggregate was defined as a follicle-like cluster of more than 50 cells, which was closely related to the bronchial epithelium or to the bronchial gland or ducts in the submucosa. The presence of the CD8 and CD4 T-lymphocyte subsets, B-lymphocytes and macrophages within the aggregates was recorded.

Slides and photocopies were coded before analysis so that their origin remained unknown to the observer. Only slide preparations that contained cryosections from biopsies with bronchial epithelium, basement membrane,

lamina propria and submucosa were evaluated. The same researcher performed the inflammatory cell counts. Another researcher assessed lymphoid aggregates. All measurements were performed at the Institute of Biomedicine, Department of Anatomy, Helsinki University, Finland.

PROCESSING AND ANALYSIS OF BAL FLUID (PAPER III, V)

BAL fluid was processed within two hours of recovery. The fluid was pooled and filtered through a nylon filter with a pore diameter of 100 µm (Sintab Product AB, Malmö, Sweden).

Cellular components (Paper III, V)

The total cell count was performed within 2 hours of lavage with an automated cell counter (Technicon H1, Technicon Instruments, Tarrytown, NY., USA). Cytospin preparations were prepared by centrifugation of 400 µl aliquots of BAL fluid at 500 x g for 5 minutes at 4^{o} C, air dried and stained with May-Grünewald-Giemsa. Differential cell counting of macrophages, lymphocytes, neutrophils and eosinophils in 300 cells, excluding epithelial cells was performed Mast cell counts were performed on slides stained with acid toluidine blue and counterstained with Mayer's haematoxylin [81]. Differential cell counts were expressed as a percentage of the total cell count.

T-lymphocytes subtypes and the activation status was determined by flow cytometry FACSCAN flow cytometer (Becton- Dickinson Immunocytometry Systems). Surface markers of mature T cells (CD3), T-helper cells (CD4), T-suppressor cells (CD8), primary T-helper cells (CD4+ CD45RA), memory T-helper cells (CD4+ CD45RO), natural killer cells (CD16+56) and lymphocyte activation markers HLA-DR (DR) and IL2- receptor (CD25) in cell pellets were stained for with mAbs conjugated with fluorescein isothiocyanate and phycoerythrin. The method is described in detail in Paper III.

Soluble components (Paper III)

Concentrations of markers of inflammation, inflammatory cell activation and fibroblastic activity were measured in the unconcentrated supernatant BAL fluid. Albumin was measured by immunoturbidimetry (Unimate 3 ALB, F Hoffmann-La Roche, Basel, Switzerland) Tumour necrosis factor α (TNFα) was measured with indirect enzyme-linked immunosorbent assay (ELISA) with a lower limit of detection of 0.5 ng / l. Myeloperoxidase (MPO) and eosinophil cationic protein (ECP) were measured with radioimmunoassay (RIA) (Pharmacia MPO RIA, detection limit 8 µg / l and Pharmacia ECP RIA, detection limit 2 µg / l, respectively, Kabi Pharmacia Diagnostics, Uppsala, Sweden). The concentrations of hyaluronan and fibronectin were determined by a radiometric assay (Pharmacia HA test, detection limit 5 µg / l) [82] and ELISA (detection limit of 10 µg / l) [83], respectively.

STATISTICS

Unpaired data on lung function and continuous demographic parameters were analysed for statistical significance with the independent sample *t*-test or analysis of variance (ANOVA) with Neuman-Kuels' or Bonferroni's multiple comparison tests. Other unpaired continuous data were not normally distributed and were analysed with the Mann-Whitney U-test or Kruskal-Wallis H-test with Dunn's correction for multiple comparisons. Paired continuous normally and non-normally distributed data were analysed by a paired sample *t*-test and Wilcoxon rank sum *W* test,

respectively. All tests were two-tailed. Associations between categorical parameters were assessed with the *chi*-square test and Fisher's exact test, when appropriate. Correlation coefficients were calculated using Spearman's rank method. A *P* value of ≤ 0.05 was considered to be statistically significant.

GENERAL SUMMARY

Prevalence of asthma in young cross-country skiers in central Scandinavia: differences between Norway and Sweden. Sue-Chu M, Larsson L, Bjermer L.

Paper I is a study of the prevalences of asthma-like symptoms, bronchial hyperresponsiveness to methacholine and asthma in 171 young, elite cross-country skiers in Trøndelag, Norway (N=113) and the inland region of Jämtland, Sweden (N= 58). The former has a coastal type of winter climate, while the latter has a colder and drier winter climate.

Asthma-like symptoms, such as wheeze, chest tightness and abnormal shortness of breath on exercise and on exposure to airway irritants, were reported by 46% and 51% of skiers in Trøndelag and Jämtland, respectively. When compared to Norwegian skiers, Swedish skiers reported more often cough on winter training (64% Vs 42%, $P < 0.01$). Moreover, they had a three-fold greater prevalence of exacerbation of symptoms on exposure to cold (45% vs. 14%, $P<0.001$), BHR to methacholine (43 Vs 14%, $P < 0.001$), current asthma (28% vs. 9%, $P < 0.01$) and clinically diagnosed asthma (42 Vs 12%, $P < 0.001$). They were more likely to consult their physician, be diagnosed as asthmatic and treated with β_2 agonist and inhaled corticosteroid medication than their Norwegian counterparts. Further, although three-fold greater in Sweden, self-reported allergy was not associated with asthma symptomatology, BHR and current asthma. Finally, every third skier with BHR to methacholine was asymptomatic.

This study suggests that inhalation of cold, dry air may be a significant pathogenic factor for the development of asthma symptoms and hyperresponsiveness to methacholine.

Lymphoid aggregates in endobronchial biopsies from young elite cross-country skiers.

Sue-Chu M, Karjalainen E-M, Altraja A, Laitinen A, Laitinen L A, Næss A-B, Larsson L, Bjermer L.

Paper II describes the first reported observation of lymphoid aggregates in endobronchial biopsies of the subcarinae of second and third generation bronchi. Of 44 skiers (26 with ski asthma) and 12 healthy control subjects, aggregates were detected in 28 (64%) skiers and 3 (25%) controls. The aggregates consisted of a follicle-like cluster of more than 50 cells, which were located in the lamina propria, commonly closely below the basement membrane and thus closely related to the bronchial epithelium, bronchial glands or ducts in the submucosa. Zonal localisation of T- and B-lymphocytes, as well as the presence of both T-helper and T-suppressor subsets and macrophages on immunohistochemistry suggest that these aggregates share some resemblance to what is usually as defined as bronchus associated lymphoid tissue (BALT).

The occurrence of BALT-like aggregates indicates that ski asthma is associated with a certain degree of inflammation within the airways. The exact nature and function of these aggregates and whether these findings represent a unique feature of "ski asthma" require further clarification.

Bronchoscopy and bronchoalveolar lavage findings in cross- country skiers with "ski-asthma". Sue-Chu M, Larsson L, Moen T, Rennard S I , Bjermer L.

Paper III is a study of the macroscopic changes seen in the proximal airways at bronchoscopy and of the cellular and selected non-cellular changes in the BAL fluid of 30 skiers (12 with ski asthma) and 10 nonatopic, healthy controls. In skiers, the proximal airways were macroscopically more inflamed, and the BAL fluid profile differed

in several respects from healthy controls. There was an increase in total cell count and % counts of lymphocytes and mast cells and a decrease in % macrophage count, but neutrophil and eosinophil counts were not different. Of the lymphocyte subtypes, T-helper lymphocytes, the ratio of T-helper to T-suppressor lymphocytes and chronic activation of T-suppressor lymphocytes were greater in skiers. The pro-inflammatory cytokine TNF alpha was above the detection threshold of 0.4
$ng \cdot L^{-1}$ in 40% of skiers. Further, myeloperoxidase was detected in 20% of skiers, suggesting activation of neutrophils in the BAL fluid. In contrast, there was no suggestion of eosinophil activation, as ECP was below the detection threshold of $2\mu g \, .L^{-1}$. On subgroup analysis, skiers with ski asthma tend to show higher degrees of macroscopic inflammation.
Even though airway inflammation was obvious on visual inspection, the inflammatory changes in the BAL fluid were of a minor degree. The profile of the BAL findings, including the absence of eosinophilic inflammation, was in contrast with that reported in other studies of asthmatics.

Evidence of airway inflammation and remodelling in ski athletes with and without bronchial hyperresponsiveness to methacholine.
Karjalainen E-M, Laitinen A , Sue-Chu M, Altraja A, Bjermer L, Laitinen L A.
Paper IV is an immunohistochemical study of endobronchial biopsies of the proximal airways of 40 skiers (30 with hyperresponsiveness to methacholine), 12 mild asthmatics with hyperresponsiveness to histamine and 12 healthy controls. The density of inflammatory cells in the bronchial mucosa and expression of the extracellular matrix protein, tenascin in the subepithelial basement membrane were quantitated. The density of T-lymphocytes, macrophages and eosinophils were respectively, 43-fold ($P < 0.001$), 26-fold ($P < 0.001$) and two-fold ($P < 0.001$) greater in skiers than in controls. Mast cell count in skiers was not significantly different than in controls. Inflammatory changes were present irrespective of asthma-like symptoms, hyperresponsiveness, or atopy. Tenascin expression was significantly greater in skiers than in controls [median (IQR) 6.7 (5.3-8.5) Vs 0.8 (0-3.1) μm, $P < 0.001$], and was not correlated with inflammatory cell counts. With the exception of a two-fold greater density of neutrophils, the degree of the inflammatory reaction in skiers was lower than that in asthmatics.
Hyperpnea associated with competitive skiing appears to influence the airways. There is evidence that is suggestive of airway remodelling. The increased thickness of tenascin immunoreactivity and infiltration of inflammatory cells seen in both hyperresponsive and nonhyperresponsive skiers suggests that all skiers react to a certain degree to the environmental stress on the airways. The usefulness of the criteria of bronchial hyperresponsiveness and asthma symptoms to differentiate healthy skiers from " asthmatic" skiers may thus be of limited value.

P**lacebo-controlled study of inhaled budesonide on indices of airways inflammation in bronchoalveolar lavage fluid and bronchial biopsies in cross country skiers**.
Sue-Chu M, Karjalainen E-M, Laitinen A, Larsson L, Laitinen L A, Bjermer L.
Paper V is a randomised double-blind placebo-controlled parallel group study of the effect of inhaled budesonide, 400 μg twice daily for a mean (range) treatment period of 22 (10-32) weeks over the competitive season, on airways inflammation and tenascin expression in 25 skiers with ski asthma. There was a spontaneous improvement in bronchial responsiveness and decreased activation of T-suppressor (CD8) lymphocytes in both groups. Despite this, no changes were seen with regard to tenascin expression or cellular inflammation in the bronchial mucosa,

and asthma-like symptoms were unchanged in 17 (68%) skiers. Within the budesonide group, there was a decrease in IL2-receptor positive T-helper lymphocytes and an improvement in FEV_1.
We were not able to show any clear beneficial effect of budesonide in ski asthma. The spontaneous improvement seen in bronchial responsiveness suggests that changes in environmental stress to the airways may be more beneficial than pharmacological intervention.

Non-invasive Evaluation of lower airway inflammation in hyperresponsive Elite Cross Country Skiers and Asthmatics. Sue-Chu M, Henriksen AH, Bjermer L.
In Paper VI , we investigated bronchial responsiveness to adenosine 5'- monophosphate (AMP) and nitric oxide (NO) concentration in the exhaled air, both indirect markers of asthmatic airway inflammation, in two study populations of skiers and asthmatics. Although the groups of 18 skiers with ski asthma, 15 non-steroid and 14 steroid-treated asthmatics were not significantly different in responsiveness to methacholine, responsiveness to AMP increased in order of magnitude from ski asthma < nonsteroid-treated < steroid-treated asthma. BHR to AMP was present in 5 (28%) skiers, 6 (40%) non-steroid and 10 (71%) steroid-treated asthmatic subjects.
Exhaled NO was measured in 44 (9 with ski asthma) skiers, 29 subjects with mild intermittent asthma and 82 healthy nonatopic controls. There was no significant difference in exhaled NO in skiers and controls [median (IQR) 6.5 (4.1-9.9) Vs 5.2 (4.2- 6.5) ppb]. Compared to skiers, exhaled NO in mild intermittent asthmatics was three-fold greater [median (IQR) 19.2 (5.1-25.6) ppb, $P < 0.01$]. Exhaled NO was not elevated in ski asthma and was two- and four-fold greater in atopic than nonatopic subjects in the skier ($P < 0.001$) and asthmatic ($P < 0.01$) groups, respectively. A significant correlation to methacholine responsiveness was observed in atopic asthmatics ($N=22$, $rho = 0.55$, $P< 0.01$).
Despite a similar degree of responsiveness to methacholine, ski asthmatics were generally less responsive to AMP and had lower levels of exhaled NO, compared to asthmatics. If hyperresponsiveness to AMP is indicative of pre-activated mucosal mast cells, this study suggests this is not a predominant feature in ski asthma. NO does not seem to reflect the presence of inflammation in ski asthma, but is merely an indicator of the presence of atopy.

DISCUSSION

In this thesis, we found an increased prevalence of asthma symptoms and BHR in skiers from the region that had a climate with extreme cold and dry air. Moreover, both invasive and non-invasive studies suggest that long term exposure to cold dry air in athletes leads to changes in the lower airways with some of the features of the inflammatory process and ongoing airway remodelling that are seen in asthmatics. However, on looking more carefully at the inflammation changes, it is obvious on comparison of these two entities that the differences are greater than the similarities.

One special difference was the presence of lymphoid aggregates. These have seldom been observed in biopsy studies of asthmatics. In one study, Trigg and co-workers described that lymphocytes were often seen in clusters in some asthmatics [84], while the presence of lymphocyte aggregates containing T-as well as B-lymphocytes was noted by Sont and co-workers [85]. The reasons for the increased prevalence in skiers are unknown. Serological screening of the skiers with lymphoid aggregates for current infection with common respiratory viruses was negative (unpublished observation). Interestingly, similar aggregates are present in the subconjunctival mucosa of contact-lens wearers [86]. The aetiology of this phenomenon is unknown, but may be a response to an assumed chronic irritation.

Another difference was the lack of eosinophilia and ECP in the BAL fluid. Eosinophilia and ECP are consistently found in asthmatics [40,87, 88, 89, 90, 91] and considered to be characteristic features of asthmatic inflammation. In addition, the role of the neutrophil appears to be more prominent in the inflammatory process in skiers than in asthmatics. This was the only cell type with a greater degree of infiltration of the mucosa in the skiers, compared to asthmatics. Because of a lack of sections, we were not able to quantify neutrophils in control subjects. However, with the exception of one study which reported an increase during natural pollen exposure [92], other studies of both extrinsic and intrinsic asthmatics have consistently reported that neutrophil infiltration in biopsies is not different or is even lesser than in healthy controls [93, 94, 95, 96]. Moreover, skiers had evidence of neutrophil activation in the BAL fluid. This has not been reported in subjects with stable asthma, but has been observed in subjects with chronic bronchitis [97].

Although observed in previous studies of asthmatics [98, 99], an increase in tenascin expression has not so far been reported in other airway diseases. In the present study, we have demonstrated an increased expression of tenascin in the subepithelial reticular layer of the basement membrane in these skiers, compared to control subjects. Moreover, the expression of this extracellular matrix protein was similar to what was seen in the asthmatic subjects, while we did not find any difference in skiers with and without hyperresponsiveness. In adults, an increased expression is due either to increased production or a decreased degradation of tenascin. An increased production occurs in association with healing and repair process after tissue injury [100], and may be the reason for the increased expression in skiers. The airways of these athletes are repeatedly exposed to poorly conditioned air during the hyperpnea of exercise. While difficult to assess in bronchial biopsies in man, epithelial damage has been demonstrated in dogs after a five minute period of hyperventilation with poorly conditioned air [101]. Thus it is plausible to believe that chronic environmental stress may be a major cause of increased tenascin expression. Interestingly, 40% of skiers had detectable amounts of the pro-inflammatory cytokine TNF alpha in the BAL fluid (Paper III), which can stimulate human airway epithelial cells to produce tenascin *in vitro* [102].

Bronchial responsiveness to AMP was lower in ski asthmatics than in mild to moderate asthmatics with a similar degree of methacholine responsiveness. As AMP is believed to act indirectly through release of

bronchospastic mediators from pre-activated mast cells and methacholine acting directly on bronchial smooth muscle, different mechanisms may be responsible for the bronchial hyperresponsiveness in skiers and asthmatics. In normal human subjects, TNF alpha can induce an increase in airway responsiveness and is associated with neutrophil infiltration [103]. Whether this occurs in skiers is unknown, but we were able to detect this pro-inflammatory cytokine in the BAL fluid in 40% of skiers, and the neutrophil count in the BAL fluid was positively correlated with the index of macroscopic inflammation in the proximal airways.

Ski asthmatics did not have an increase in exhaled NO levels. Although suggested as a potential indirect marker of airway inflammation and greater in asthmatics than in ski asthmatics, exhaled NO may be more indicative of the atopic status and may not always reflect the degree of inflammation in the lower airways in nonatopic individuals. Similar observations that exhaled NO may be a good indicator of allergic inflammation have been reported in other studies of subjects with asthma and allergic rhinitis without asthma [104, 57]. Thus the measurement of exhaled NO may be of limited value in the monitoring of airways inflammation in competitive skiers.

The inflammation in the airways of skiers was not apparently improved by inhaled budesonide treatment. This is in contrast to studies in asthmatics which have shown that the inhaled steroids consistently reduce the number of mast cells, eosinophil and T-lymphocyte [84, 105, 106,107, 108] in the bronchial mucosa and downregulate tenascin expression in seasonal allergic asthmatics during the birch pollen season [98]. The apparent lack of effect suggest that the mechanisms involved in the mucosal infiltration of inflammatory cells in skiers may be different from those in asthmatics and may be less responsive to corticosteroids. Further, placebo-treated skiers had rather surprisingly, a spontaneous improvement in bronchial responsiveness that was accompanied by an improvement in macroscopic inflammation but without a concomitant change in the degree of cellular inflammation. Together with the presence of inflammatory changes seen in nonhyperresponsive skiers in the autumn season, this indicates that bronchial inflammation in competitive skiers does not appear to be direcetly related to hyperresponsiveness to methacholine. Moreover, the presence of inflammatory changes in our skiers is in contrast to the total absence of any evidence of inflammation in a previous study of clinically healthy subjects regularly participating in sporting activities and investigated in the winter months and with hyperresponsiveness to histamine [109].

In summary, cross-country skiing with the inhalation of cold dry air represents a significant environmental stress to the airways. This stress leads to a certain degree of inflammation and remodelling of the airways. Even though there was a tendency for more pronounced changes in skiers with asthma-like symptoms and bronchial hyperresponsiveness, these changes were seen in all skiers with no clear difference in those with and without "clinical ski asthma", compared to controls. The inflammatory condition in competitive skiers may best be described as cold air- and hyperpnea-induced bronchitis with and without bronchial hyperresponsiveness to methacholine. Our attempt and failure to treat this condition with inhaled corticosteroids indicate that reduction of the environmental stress may be more important than pharmacological intervention. We believe that the knowledge gained from these ski asthmatics provides a broader understanding not only of "ski asthma", but also a better understanding of asthma pathophysiology per se.

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Malcolm Sue-Chu

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