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Asthma and Exercise

Climatic effects upon Exersice-Induced Bronchoconstriction and Exercise Capacity in asthmatic subjects

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Summary

The present thesis consists of five studies with the main objective to investigate the effect of different climatic conditions upon exercise capacity and exercise-induced bronchoconstriction (EIB) in subjects with diagnosed EIB (reduction in forced expiratory volume in 1 second (FEV_1) ≥10% from before to after exercise). In order to assess exercise capacity measured by peak oxygen uptake ($\dot{V}O_{2\,peak}$) and peak minute ventilation ($\dot{V}E_{peak}$), two different exercise protocols were evaluated. The usefulness to diagnose EIB in medium altitude by a competitive cross-country skiing field test was compared to assessment of methacholine induced bronchial hyperresponsiveness (BHR) in elite cross-country skiers.

In the first study (paper 1) the possibility of measuring $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$ with an EIB-test protocol was evaluated. A common stepwise protocol (designed for assessing $\dot{V}O_{2\,peak}$) and an EIB-test protocol (designed for provoking EIB) were compared for determination of $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$ in 40 healthy subjects. No difference in $\dot{V}O_{2\,peak}$ or $\dot{V}E_{peak}$ was found between the protocols, and we concluded that an EIB-test protocol may thus be used for both provoking EIB and determine aerobic capacity.
The second, third and fourth study were carried out to assess the effect of a hypobaric environment (2500 meters above sea level (masl)) (paper 2), a humid environment (95% relative humidity) (paper 3) and a cold environment (-18^{o}C) (paper 4) upon EIB and exercise capacity performed by the use of an EIB-test protocol. Twenty subjects with diagnosed EIB were included in each of the studies. The three different climatic conditions were compared to a normobaric, regular indoor environment (200 masl, 20 ^{o}C and 40% relative humidity).
The hypobaric environment had no effect upon EIB, whereas $\dot{V}O_{2\,peak}$ was reduced by 10.1%. EIB was reduced to the half in the humid environment and $\dot{V}O_{2\,peak}$ increased 4.5%. In the cold environment EIB was increased and $\dot{V}O_{2\,peak}$ reduced by 6.5%.
$\dot{V}E_{peak}$ did not differ in any of the climatic conditions but the running speed was significantly reduced in the hypobaric environment and in the cold environment while significantly increased in the humid environment.
We concluded that a hypobaric environment did not influence EIB but reduced exercise capacity. A humid environment improved both EIB and exercise capacity and a cold environment increased EIB and reduced exercise capacity in subjects with EIB.

In the fifth study (paper 5) a methacholine bronchial provocation test, $PD_{20\,methacholine}$ (the dose of inhaled methacholine causing a reduction in FEV_1 of 20%), was compared to an exercise-field test consisting of a cross-country skiing competition in the assessment of asthma and bronchial hyperresponsiveness (BHR) among 24 elite cross-country skiers.
Nine out of 24 (37.5 %) athletes experienced a positive methacholine test (PD_{20}<8μmol) whereas only two of the 24 subjects (8.3%) had a reduction in FEV ≥10% from before to after the exercise-field test. A significant negative correlation was found between age and bronchial responsiveness, r = - 0.47, p=0.02.
It was concluded that the measurement of BHR to methacholine is more sensitive than exercise-field testing in confirming the diagnosis of BHR or asthma in elite cross-country skiers.

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2. Berntsen S., Stensrud T., Ingjer F., Vilberg A., Carlsen K-H. Asthma in medium altitude - exercise-induced bronchoconstriction in hypobaric environment in subjects with asthma. Allergy 2005 Oct; 60(10):1308-11.

3. Stensrud T., Berntsen S., Carlsen K-H. Humidity influences exercise capacity in subjects with exercise-induced bronchoconstriction (EIB). Respir Med 2006 Sep; 100(9):1633-41.

4. Stensrud T., Berntsen S., Carlsen K-H. Exercise capacity and exercise-induced bronchoconstriction in a cold environment. Respir Med 2007 Jul; 101(7):1529-36.

5. Stensrud T., Mykland K.V., Gabrielsen K. and Carlsen K-H. Bronchial hyperresponsiveness in skiers: field test versus methacholine provocation? Medicine and Science in Sports and Exercise Med Sci Sports Exerc 2007 Oct; 39(10):1681-6.

Abbreviations

EIB:	exercise-induced bronchoconstriction
EIA:	exercise-induced asthma
BHR	bronchial hyperresponsiveness
FEV_1:	forced expiratory volume in one second (L)
FVC:	forced vital capacity (L)
FEF_{50}:	forced expiratory flow at 50% of FVC ($L \cdot second^{-1}$)
PEF:	peak expiratory flow ($L \cdot second^{-1}$)
$PD_{20methacholine}$:	the dose of inhaled methacholine causing a reduction in FEV_1 of 20%
SPT:	skin prick test
$\dot{V}O_2$:	oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$)
$\dot{V}O_{2\ peak}$:	highest recorded oxygen uptake during the exercise tests ($mL \cdot kg^{-1} \cdot min^{-1}$)
$\dot{V}E$:	ventilation ($L \cdot min^{-1}$)
$\dot{V}E_{peak}$:	highest recorded minute ventilation during the exercise tests ($L \cdot min^{-1}$)
V_{tpeak} :	peak tidal volume (L)
RER:	respiratory exchange ratio ($VCO_2 \cdot \dot{V}O_2^{-1}$)
RER_{peak} :	peak respiratory exchange ratio ($VCO_{2peak} \cdot \dot{V}O_{2\ peak}^{-1}$)
BF:	breathing frequency ($breath \cdot min^{-1}$)
BF_{peak} :	peak breathing frequency ($breath \cdot min^{-1}$)
HR:	heart rate ($beats \cdot min^{-1}$)
HR_{peak} :	peak heart rate ($beats \cdot min^{-1}$)
SaO_2	oxygen saturation in arterial blood (%)
SpO_2:	oxygen saturation in arterial blood measured by pulse oximetry (%)
AUC:	area under the curve

1. Introduction

"If from running, gymnastic exercises, or any other work, the breathing become difficult, it is called *ASTHMA (αμθσα)"*

The extant work of Aretaeus, the Cappadocian (100 A.D.)

Adams F, London, The Sydenham Society 1856: 316-31).

The above quotation shows that exercise-induced asthma (EIA) has been well known for two thousand years. EIA is common in asthmatic subjects and may occur in 70-90% of untreated patients (1;2). The term EIA is used to denote symptoms and signs of asthma provoked by physical exercise, whereas exercise-induced bronchoconstriction (EIB) is used to denote the measured decrease in lung function after an exercise test as defined by the joint Task Force on Sports and Asthma of the Euroepan Respiratory Society and the European Academy of Allergy and Clinical Immunology (3).

It is known that reduced physical fitness and physical activity are important for the development of chronic disorders, including asthma (4;5). An individual's pattern of physical activity and future activity level is often founded during childhood and adolescence, and is considered to be useful determinants for the quality of life in later years. Assessment of physical fitness may thus give important information about physical functioning in patients with chronic lung disorders, including asthma.

EIB influences daily life activities and sports activities in children, adolescents and adults. To enable optimal choice of treatment, an accurate assessment of EIB is therefore important. EIB consists of bronchoconstriction occurring immediately or soon after physical exertion (6-8), and is best assessed by a standardised exercise test. Commonly used is running on a treadmill for 6-8 minutes at a submaximal work load

(9;10). Lately it has been maintained that an exercise load corresponding to 95% of maximum heart rate (HR_{max}) is preferable to obtain a high sensitivity (8). Until now, assessment of EIB and peak oxygen uptake ($\dot{V}O_{2\ peak}$) have been performed with two different test protocols on separate days. Obtaining information about both EIB and $\dot{V}O_{2\ peak}$ with one single exercise test would be useful both in a clinical practice and in research, and the possibility of combining assessment of EIB and $\dot{V}O_{2\ peak}$ into one exercise test would reduce the burden for the patient and the costs for the health system. However, before the EIB-test protocol can be used for assessment of exercise capacity in clinical studies as well as epidemiologic studies, a comparison between the EIB-test protocol and a common stepwise protocol for determination of $\dot{V}O_{2\ peak}$ and peak minute ventilation ($\dot{V}E_{peak}$) is required.

More knowledge about different climatic conditions in relation to EIB and exercise capacity is needed for giving optimal training advice and treatment to asthmatic athletes. Elite athletes are often practising altitude training in unfavourable environments, especially athletes within endurance sports. More knowledge is also needed in relationship to regular physical training of asthmatic subjects, especially in the Scandinavian countries and in other countries with sub arctic climate where the winter season can be quite cold. Furthermore it is not unusual for children and adults to take part in activities like mountain-climbing, skiing and tracking in medium or higher altitudes where the barometric pressure is lower than at sea level.

It is known that cold, dry air increases EIB, and that humid air reduces EIB in subjects with asthma (11-14). However, to our knowledge only few studies concerning the effect of different climatic conditions upon exercise capacity in subjects suffering from

EIB, and these studies have given different results (15-17). Hypoxic gas inhalation has been reported to enhance bronchial hyperresponsiveness (BHR) and result in bronchoconstriction in some animal models, and in humans with asthma (18;19). The data on humans have so far been conflicting (20;21). As far as we know, there are no other studies of the effect of a hypobaric environment upon exercise capacity in subjects with EIB. Several authors have on the other hand reported reduced exercise capacity in healthy, trained and untrained subjects in a hypobaric environment due to reduced oxygen saturation (SaO_2) (22;23).

The prevalence of EIA and BHR has increased over the last two decades, especially among elite endurance athletes (24-26). This is particular so for skiers (27), possibly due to the cold and dry air exposure during heavy exercise (28). In addition to the type of sports, one major risk factor is atopic disposition (26;29). The International Olympic Committee Medical Commission (IOC-MC) requires at least one positive test of bronchial hyperresponsiveness, as methacholine bronchial provocation, exercise challenge or eucapnic voluntary hyperventilation (EVH) challenge or reversibility to inhaled $ß_2$-agonists, to allow the athletes to inhale $ß_2$-agonists in relation to sports and competitions. Whereas BHR to methacholine has been regarded as a direct measure of non-specific BHR, exercise induced bronchoconstriction (EIB) and eucapnic hyperventilation test are looked upon as indirect measures of non-specific bronchial responsiveness (30).

It has also been dicussed whether a sport specific exercise test, EIB-test in the laboratory, EVH-test or methacholine provocation is the best challenge to diagnose astma and/or BHR in elite athletes. Rundell et al (31) concluded that without relevant

provoking agents, such as a sport specific exercise-field test, one might risk several false negative results on screening for EIB or BHR among athletes. This is in agreement with Ogston and Butcher (32) who reported that by using a sport-specific protocol a larger number of athletes can objectively be screened for EIB. On the other hand Dickinson et al (33) reported that an EVH-test is a more sensitive challenge in asymtomatic athletes than a sport specific and laboratory based challenge.

The present thesis includes five studies performed to evaluate two test protocols for assessing $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$, examine the influence of different climatic conditions upon EIB and exercise capacity (measured by $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$) in subjects with diagnosed EIB (fall in $FEV_1 \geq 10\%$ from before to after exercise) and to evaluate two different provocation tests for diagnosing BHR and asthma in elite cross-country skiers.

2.Aims of the study

1. *To investigate the influence of different environmental climatic conditions upon exercise-induced bronchoconstriction and exercise capacity in subjects with diagnosed EIB.*
    A. *The influence of a hypobaric environment*
    B. *The influence of a humid environment*
    C. *The influence of a cold environment*

2. *To examine if a standard test protocol for assessing EIB also can be used for determination of peak oxygen uptake and peak minute ventilation.*

3. *To compare a methacholine bronchial provocation test with a sport specific exercise- field test in the assessment of bronchial hyperresponsiveness in elite cross- country skiers.*

3. Review of the litterature

3.1 Assessment of exercise capacity

As asthma is a limiting factor for participation in physical activity and sport, assessment of physical fitness may give important information about physical functioning. Physical fitness is an umbrella concept covering a series of qualities related to the performance of physical activity (34). Maximum oxygen uptake ($\dot{V}O_{2\ max}$) or $\dot{V}O_{2\ peak}$ is widely recognized as one of the most important measures of aerobic capacity and physical fitness (34;35). $\dot{V}O_{2\ max}$ provides an accurate measure of aerobic power, and it is highly related to the cardiac output. Åstrand et al (34) define $\dot{V}O_{2\ max}$ as the achievement of a plateau or "levelling off" in $\dot{V}O_2$ despite continued increase in workload (less than 2 $mL \cdot kg^{-1} \cdot min^{-1}$ rise in $\dot{V}O_2$), to be the most important criterion to estimate $\dot{V}O_{2\ max}$. Other factors indicating achievement of $\dot{V}O_{2\ max}$ are respiratory exchange ratio (RER) $\geq$ 1.05 and HR $\geq$ 95% of HR_{max} (based on 220 beats $\cdot$ min^{-1} – age). In young subjects this "levelling off" or the achievement of a plateau often does not occur, and $\dot{V}O_{2\ max}$ may be determined as $\dot{V}O_{2\ peak}$, the highest recorded $\dot{V}O_2$ during the exercise test (36-39).

The choice of exercise ergometer and test protocols may influence the $\dot{V}O_{2\ peak}$ values both in children and in adults, and treadmill running or cycling are the type of exercise most commonly used (34). Åstrand et al (34) conclude that treadmill running is the best exercise ergometer for testing healthy people, especially children, because walking or running is the natural way to move and demands dynamic use of large groups of muscles. Previous studies have compared different test protocols for assessing $\dot{V}O_{2\ peak}$ both in children and in adults. In spite of a general consencus that an incremental test

protocol lasting between 8 and 12 minutes will elicit the highest $\dot{V}O_{2\ peak}$ with the lowest perception of difficulty and discomfort, previous studies did not find any difference in $\dot{V}O_{2\ peak}$ between different test protocols (40;41). Day et al (42) concluded that $\dot{V}O_{2\ peak}$ was not different with a constant workload protocol lasting between 4 and 10 minutes as compared to an incremental ramp protocol. Rossiter et al (43) suggested that $\dot{V}O_{2\ peak}$ can be reached at a constant workload corresponding to105% as well as 95% of maximum workload. This is in agreement with Cooper (44) who maintained that short bouts of high-intensity exercise is the physiologic way of studying children, rather than repeated stepwise exercise testing. Different test protocols may be needed to assess physical fitness in children and adolecents with different diseases. Whereas a gradual increase in speed and inclination of the treadmill may be beneficial for subjects with cardiac diseases, a more rapidly increasing protocol may be better suited for asthmatic children and adolescents as suggested by Cooper (44).

When performing an EIB-test or a test for assessment of $\dot{V}O_{2\ peak}$ at a fixed inclination of the treadmill, it is common to use an inclination of 3^{o} or 5.3% for children, untrained subjects and athletes (7;39). As regards athletes within endurance sports, with exception of runners, both EIB-tests and $\dot{V}O_{2\ max}$-tests are usually performed at 6^{o} or 10.5% inclination to minimize the effects of seasonal changes in $\dot{V}O_{2\ max}$ caused by changes in running technique and running economy as a consequence of seasonal shifts in the type of training, e.g. running and skiing (45). On the other hand a steep inclination can cause local fatigue in the thigh and leg muscles, thus limiting the achievement of $\dot{V}O_{2\ peak}$, especially in children and untrained subjects (46).

The evaluation of the test results should be based on total oxygen uptake ($L \cdot min^{-1}$) because this is correlated with cardiac output, myocardial oxygen consumption and blood flow. The oxygen uptake presented as millilitre per kilogram per minute ($mL \cdot kg^{-1} \cdot min^{-1}$) is a good predictor of the subjects potential to move and lift the body, but it may not reflect the cardiac performance according to Åstrand et al (34).

3.2 EIB - mechanisms

Two main hypotheses have been proposed to explain the relationship between exercise and EIB. The inspired air is conditioned to 37°C and 99.5% relative humidity on the way down the respiratory tract resulting in a net heat and water loss from the respiratory tract. The heat and water loss during exercise or hyperventilation can induce bronchoconstriction and an attack of asthma (12;47)

Gilbert and McFadden (48) suggested that airway cooling due to respiratory heat loss with resulting rewarming by secondary hyperemia and pulmonary vasodilatation is the probable cause of EIB. Airway cooling may also stimulate airways receptors, causing bronchoconstriction through a reflex pathway.

Anderson (49) suggested that the respiratory water loss due to increased ventilation is the main stimulus to provoke EIB. The water loss increase the osmolarity in the extra-cellular fluid in the respiratory mucous membrane resulting in mediator release from mast cells and other inflammatory cells in the airways, thus causing bronchoconstriction (49). This hypothesis is supported by the fact that osmotic agens like mannitol can induce bronchoconstriction (50-52), and that inhaled furosemide, which influence the ion transport in the cell membrane, can protect against EIB (53)

3.3 EIB and different climatic conditions

The two hypotheses referred to above (12;47) are based upon several studies showing that variation in environmental conditions as temperature and humidity, influences bronchoconstriction after exercise. Inspiring cold, dry air during exercise is reported to increase EIB in asthmatic subjects as compared to exercise in a regular, indoor environment and a humid environment (11-14;47). In 1977 Strauss et al studied (11) eight asthmatic subjects inhaling cold air during exercise and found increased bronchoconstriction after exercise as compared to inhaling normal tempered air. The effect of cold air inhalation at rest was small and not significant. Carlsen et al (14) reported markedly increased EIB by cold and dry air inhalation in 32 astmatic children as compared to exercise under regular laboratory conditions. However, most of the previous reports concern the effect of inspiring cold air through a mouthpiece while the subjects are exposed to regular indoor temperature.

Only few studies have been carried out with whole body exposure to cold air. (15-17;54-56). Zeitoun et al (54) concluded that facial cooling combined with either cold or warm air inhalation cause greater bronchoconstriction as compared to isolated inhalation of cold air. They suggested that vagal mechanisms activated by changes in osmolarity play a major role in exercise and cold-induced bronchoconstriction (54). Koskela et al (55) reported that for certain stable asthmatic subjects even a moderate level of exercise can cause bronchoconstriction in climatic conditions similar to a Scandinavian winter. Furthermore, they found that even sitting quiet in a temperature of -20^{o}C caused greater bronchconstriction than moderate exercise in room temperature. They suggested that this could not be explained by hyperventilation-induced airway drying alone, but that a reflex mechanism may be more important than previously thought (55). On the other hand, neither Evans et al (56) nor Sandsund et al (17) found

any additive effect of cold air inhalation upon EIB. The effective temperature of the inhaled air in their studies was actually -1°C and 2°C, respectively, and probably not sufficiently cold for the investigators to be able to discover any difference. Evans et al (56) mentioned that lack of exposure to ambient cold air during inhalation may explain the disappearance of an additive effect.

Humid air reduces EIB (12;13;15;16;57). Bar-Or et al (13) suggested that EIB is more likely to occur in dry air (25% relative humidity and about 25-26°C) than in humid air (90% relative humidity and 25-26°C), possibly due to heat loss caused by evaporation from the airway mucosa. Kallings et al (15) concluded that cold, dry air provoked greater bronchoconstriction than room-tempered humid air (60% relative humidity). They used only PEF measurements as assessment, and their exercise test consisted of only 3 minutes cycling at a intensity of 40% of maximal capacity followed by 6 minutes cycling at 80-85 % of maximal capacity (16).

Boulet and Turcotte (57) reported that EIB was influenced by changes in water content of the air during and after exercise. Bronchoconstriction following exercise was minimal if exercise was performed in humid air (100% relative humidity) and the recovery was in dry air (0% relative humidity), and maximal if the exercise was performed in dry air and the recovery was in humid air.

On the other hand, Zainudin et al (58) found no significant relationship between different humidity levels (41%-90% relative humidity) and EIB in Malaysian schoolchildren, 7-12 years of age. However, in their study the humidity levels were naturally occurring and not standardised, and the study was performed as a cross sectional study with a main objective of determining the prevalence of EIB in a

population of schoolchildren living in a humid, tropical climate in the inner city of Kuala Lumpur (58).

Hypoxic gas inhalation has been reported to enhance airway responsiveness and cause bronchoconstriction in some animal models (18;59) as well as in humans with asthma (19). However, data from humans have so far been conflicting. Saito et al and Alberts et al (20;21) did not find any effect of mild hypoxia upon bronchial responsiveness to methacholine in asthmatic subjects. Only two research-groups (60;61) have as far as we know, investigated the effect of reduced barometric pressure upon EIB. Neither found any additional decrease in bronchoconstriction after exercise in hypobaric conditions. However, in the studies by Louie and Pare (61) and Matsuda et al (60), neither the standardisation of the test protocol nor the inclusions of subjects with a minimum reduction in FEV_1 post exercise of 10% were satisfactory. Louie and Pare (61) investigated the effect of various altitudes on EIB during a 2-week trek through the Himalayas (altitudes between 1500 and 3500 masl) in 10 non asthmatic and 4 asthmatic subjects. EIB was not an inclusion criterion for the asthmatic subjects. Matsuda et al (60) tested 20 children with asthma, but not diagnosed EIB, on a bicycle ergometer in a low-pressure chamber with an incremental test protocol. The highest and last workload corresponded to 75% of HF_{max}. Exercise was performed in 103.1 and 84.5 kPa. The authors did not find any difference in maximum reduction in FEV_1 after exercise or in $\dot{V}O_2$ between the altitudes.

Exercise in medium or high altitudes are normally associated with increased ventilation because of hypoxic conditions (34;62) and this could be expected to result in increased bronchoconstriction. Furthermore, other climatic conditions must be taken into consideration in a mountainous area. The air temperature is lower (a reduction of 6.5°C

per 1000 meters altitude (29), and the water content of the air at these temperatures is substantially lower than the 47 $mg \cdot L^{-1}$ present in fully saturated air at body temperature (30). More knowledge is needed to sort out if the altitude on its own causes increased EIB in addition to the cold and dry air.

3.4 Exercise capacity and different climatic conditions

The influence of environmental conditions upon exercise capacity in subjects suffering from EIB has so far not been properly investigated. The few studies which have been carried out to investigate the influence of cold and humid air upon exercise capacity in asthmatic subjects have given conflicting results (15-17). Only in one study the authors reported on $\dot{V}O_{2\,max}$ (17). The inclusion criteria, test protocols and choice of ergometer are different in the different studies, and the results are thus difficult to compare. Most studies also include few subjects. Kallings et al (15) did not find any differences in $\dot{V}O_2$ or in other physiological variables in asthmatic subjects during exercise in room tempered conditions when inhaling cold, dry air as compared to inhaling warm, humid air. Sandsund et al (17) found no differences in $\dot{V}O_2$ at submaximal workloads, in $\dot{V}O_{2\,max}$ or in lung function in seven mildly asthmatic subjects between inhaling cold air and warm air during exercise in a cold environment. Eschenbacher et al (16) found that the workload in watts performed per $L \cdot min^{-1}$ of oxygen consumed was significantly greater with cold and dry conditions as compared to hot and humid conditions in eight male asthmatic subjects.

The effect of cold air upon physiological variables such as $\dot{V}O_2$, $\dot{V}E$ and HR in healthy subjects is reported to vary depending on factors such as type, intensity and duration of exercise, amount of fatty tissue, wind, ambient temperature, clothing, fluctuations in

body temperature and energy reserves (63). In eight healthy males Quirion et al (63) found significantly reduced $\dot{V}O_{2\ max}$, maximum workload and time to exhaustion whereas $\dot{V}E$ did not change during a short exhaustive exercise at -20°C and 0°C as compared to 20°C. Sandsund et al (64) reported increased $\dot{V}E$ and $\dot{V}O_2$ at submaximal workloads in an environment of -15°C as compared to 23°C, whereas no difference was found for $\dot{V}O_{2\ max}$. They suggested that the exercise stress increased in a cold environment, probably as a response to increased metabolic demand. Their findings in healthy subjects are supported by Claremont et al (65).

Regarding exercise capacity in a hypobaric environment there are to our knowledge, no other studies of $\dot{V}O_{2\ peak}$ or $\dot{V}O_{2\ max}$ in subjects with EIB. However, several authors have investigated how a reduced barometric pressure affects $\dot{V}O_{2\ max}$ in healthy trained and untrained subjects (22;23;62). "Untrained" healthy subjects ($\dot{V}O_{2\ max} \leq 60$ mL·kg^{-1}·min^{-1}) are reported to have a 3.6% reduction in $\dot{V}O_{2\ peak}$ per 1000 meters of increased altitude, while "trained" healthy subjects ($\dot{V}O_{2\ max} \geq 60$ mL·kg^{-1}·min^{-1}) are reported to have a 6.5% reduction (22;23). The larger drop in $\dot{V}O_{2\ max}$, in hypobaric environment in athletes, being more fit, could be explained by diffusion limitation at these high work rates (66).

3.5 Prevalence and diagnosis of asthma and BHR in elite athletes

Asthma represents an increasing problem for athletes competing within endurance sports. The prevalence of EIA has increased over the last two decades, especially among elite endurance athletes (25;27;67). This is particular the case for skiers (27), possibly due to the cold and dry air exposure during heavy exercise.

Heir et al (25) showed that in competitive cross-country skiers the prevalence of doctor diagnosed asthma increased up to 24% in the age group above 28 years, in contrast to 7% in normally physically active control subjects. Larsson (27) demonstrated an unusually high prevalence of asthma and BHR in Swedish top level cross-country skiers. Weiler et al (68) reported a prevalence of EIA of 11% among American athletes participating in the 1984 summer Olympic Games (69), increasing to >20% among the American participants in the 1996 summer Olympic Games in Atlanta (68). Helenius et al (26) found a prevalence of 17% of doctor diagnosed asthma in long distance runners, 8% in speed and power athletes and 3% in the controls, respectively.

The use of inhaled $ß_2$-agonists in asthmatic athletes at the Olympic games increased from 1984 and to later Olympic games (70). Reports and observations of a high frequency in the use of inhaled $ß_2$-agonists among top athletes led IOC Medical Commission to introduce restrictions regarding the use of inhaled $ß_2$-agonists from 1993. From the Salt Lake City Olympic Games in 2002, specific requirements as assessment of bronchial responsiveness, EIB or reversibility to inhaled $ß_2$-agonists were needed before allowing the athletes to use inhaled $ß_2$-agonists. These rules have later been modified. According to the rules from the last Olympic Games in Turin 2006, the athletes must provide evidence of one of the following to be allowed to use inhaled $ß_2$-agonists (IBAs):

1) A 12% or greater increase of the predicted, or baseline, value of FEV_1 after administration of permitted IBAs.

2) A reduction in $FEV_1 \geq 10\%$ from before to after challenge with physical stimuli, such as exercise-field test, treadmill test in the laboratory or eucapnic voluntary hyperventilation (EVH).

3) Obtaining by methacholine bronchial provocation test a dose of inhaled methacholine causing a reduction in FEV_1 of 20% ($PD_{20methacholine}$) less than 2 μmol (400 μg) or a PC_{20} (concentration of methacholine) less than 4 $mg \cdot ml^{-1}$; for those on topical steroids the

$PD_{20methacholine}$ has to be less than 13.2 μmol or the PC_{20} less than 13.2 $mg \cdot ml^{-1}$.

It has been dicussed whether a sport specific exercise test, EIB-test in the laboratory, eucapnic voluntary hyperventilation (EVH-test) or methacholine provocation is the best challenge for elite athletes to diagnose astma and BHR. Whereas bronchial responsiveness to methacholine has been regarded as a direct measure of non-specific bronchial responsiveness, EIB and EVH-tests are looked upon as indirect measures of non-specific bronchial responsiveness (30). Rundell et al (31) reported that by using real life competitive events as the provoking agent among American participants in Winter Olympic Games, 98% of the athletes reporting EIB had a positive test while 48% of the athletes not reporting EIB had a positive test. They concluded that without relevant provoking agents, such as a sport specific exercise-field test, one might risk several false negative results on screening for EIB or BHR among athletes (31). Ogston and Butcher (32) agreed with Rundell et al (31) and concluded that by using a sport-specific protocol a large number of athletes can objectively be screened for EIB. On the other hand Dickinson et al (33) reported that an EVH-test is a more sensitive challenge in asymtomatic athletes than a sport specific and laboratory based challenge.

4. Subjects and methods

4.1 Design and subjects

Study 1, 2, 3 and 4 had an open randomized cross over design and study 5 had an open, non-randomized design. Totally 84 subjects were included in the studies.

Paper 1

In study 1 two test protocols were compared for assessing $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$. An EIB-test protocol was used on one occasion, and a common stepwise protocol was used on the other occasion. The subjects were randomized consecutively to one of the two test protocols first according to random order, generated by a computer programme. The tests were conducted with 3 hours apart in the same laboratory using the same equipment and conducted by the same test leader.

Forty healthy subjects, (♀/♂: 17/23), were included and tested with the two different test protocols for assessing $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$. Twenty subjects (♀/♂: 7/13) performed the two tests at 10.5% inclination of the treadmill and the remaining 20 subjects, (♀ /♂: 10/10), at 5.3%. Ten subjects at each inclination were randomely tested with the EIB protocol first and 10 subjects with the stepwise protocol first. Thirtyone subjects participated in competetive sports and 9 were regular physically active, 3-5 days per week.

Paper 2, 3 and 4

Study 2, 3 and 4 comprised five study days in total aiming to assess the effect of three different climatic conditions (hypobaric, humid and cold conditions) upon EIB and exercise capacity. On day one a skin prick test (SPT) was performed and all subjects

underwent an EIB-test under regular, indoor conditions to assess if they satisfied the inclusion criterion, a reduction in $FEV_1 \geq 10\%$ from before to after exercise. If satisfying the inclusion criterion the subjects were randomized consecutively to one of the four climate blocks in random order generated by a computer programme. The exercise tests were performed in a regular indoor environment, (20°C, 40% relative humidity and an ambient pressure corresponding to 200 meters above sea level (masl)), in a standardised hypobaric environment (20°C, 40 % relative humidity and ambient pressure corresponding to 2500 masl), in a humid environment (20°C and 95% relative humidity) and in a cold environment (-18^{o}C and 40 % relative humidity) on 4 different days. An interval of at least 48 hours was required between each test and not more than 7 days.

Twenty subjects between 10 and 45 years of age with diagnosed EIB were included in the studies. EIB was defined as a reduction in FEV_1 of 10% or more from before to after a standardised EIB-test performed under standard, regular conditions. Exclusion criteria consisted of any other diseases or use of any regular medication which might influence test results and any respiratory tract infection during the last 3 weeks before inclusion in the study. The subjects were also excluded if the baseline FEV_1 measurement varied more than 5% between the test days. Anti asthmatic medication were withheld according to ERS guidelines (7) (described in chapter 4.2.5; exercise measurements) and the lung function measurements were performed according to the procedure described in chapter 4.2.1; lung function measurement.

Seventeen of the 20 subjects were atopic as defined by positive SPT. Seven subjects used regular inhaled steroids; 10 subjects used regular daily long-acting inhaled β_2-agonists. Seventeen subjects used short-acting β_2-agonists on demand, one subject used

oral theophylline, and two subjects used daily the leukotriene antagonist, montelukast. Four subjects used antihistamines, whereas nine subjects were without any regular asthma medication. Five subjects participated in competetive sports, 14 participated in regular physical activity in school or leisure time, and one subject rarely or never participated in physical activity.

Paper 5

Study 5 aimed to compare bronchial responsiveness obtained by an exercise-field test consisted of a 7 kilometers (♀) and 10 kilometers (♂) skiing competition, respectively) and by a bronchial provocation test with methacholine measuring $PD_{20methacholine}$. The study comprised two test days.

Day one: All subjects underwent a methacholine provocation test.

Day two: All subjects competed in a cross-country skiing competition.

Prior to the provocation challenges all athletes refrained from taking any medication that might have confounded the pulmonary function results. Anti asthmatic medication were withheld according to ERS guidelines (7) (described in chapter 4.2.5; exercise measurements) and the lung function measurements were performed according to the procedure described under lung function measurement (chapter 4.2.1).

Twentyfour elite cross-country skiers (♀ /♂:8/16, all members of the Norwegian national teams, five in the men's elite all-round team, eight in the women's elite team, six in the men's elite sprint team and five male skiers in the recruit team were included in the study. Exclusion criteria were any acute or chronic illnesses interfering with the

possibility to perform the study, in addition to upper respiratory tract infections (URTI) during the 4 weeks prior to testing.

4.2 Methods and test procedures

4.2.1 Lung function measurement (paper 1, 2, 3, 4 and 5)

Lung function was measured by maximum forced expiratory flow volume loops (Masterlab, Erich Jaeger®,Germany), forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and forced expiratory flow at 50% of FVC (FEF_{50}).
In study 1 lung function was measured before exercise and 3, 6, 10 and 15 minutes after exercise to ensure that the subjects did not suffer from EIB. In study 2, 3 and 4 lung function was measured before exercise and 1, 3, 6, 10 and 15 minutes after exercise and 15 minutes after inhaled salbutamol (5 $mg \cdot mL^{-1}$; 0.05 $mg \cdot kg^{-1}$). In the hypobaric environment (study 2) the first post exercise measurement was after 3 minutes, as this time was required to balance the barometric pressure in the chamber.

The lung function measurements were performed in a regular, indoor environment outside the climate chamber. All manoeuvres complied with the general acceptability criteria of the European Respiratory Society (ERS) (71). Predicted lung function values, when used, were according to Zapletal et al (72) for subjects ≤18 years and Quanjer et al (71) for subjects > 18 years.

4.2.2 EIB-test protocol (paper 1, 2, 3 and 4)

The EIB-test consisted of 8 minutes treadmill run at a sub-maximal work load (7). In study 1 the inclination of the treadmill was 5.3% and 10.5%, respectively and in study 2, 3 and 4 5.3%. The running speed was adjusted during the first 4 minutes to achieve a work load corresponding to the maximum speed the subjects were able to maintain the

last 4 minutes, about 95% of estimated HR_{max} (220 beats · min^{-1}- age). If the subjects indicated that higher speed was necessary to achieve exhaustion after 8 minutes, the running speed was also adjusted after 5 and 6 minutes. The estimated HR_{max} is elaborated from epidemiological studies and is a circumstantial estimation for individual subjects. The standard deviation for maximum heart rate during exercise is ± 10 beats·min^{-1} (34). Therefore the exercise work load was standardised by a combination of 95% of estimated HR_{max} and the test leader`s evaluation of exhaustion after 8 minutes.

4.2.3 Stepwise protocol (paper 1)

$\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ measurements performed with the stepwise protocol in study 1 was according to the procedure described by Hermansen (73) and Åstrand et al (34). After warming up for 20 minutes at a workload corresponding to 50-60% of estimated $\dot{V}O_{2\ peak}$, the running speed was increased to 80% of estimated $\dot{V}O_{2\ peak}$. The running speed was further increased with 1 km·$hour^{-1}$ at 5.3% inclination and 0.7 km·$hour^{-1}$ at 10.5% inclination, respectively, every minute to a level that brought the subjects close to exhaustion after 4-6 minutes. The increase in running speed corresponds to an increase in $\dot{V}O_2$ of 3-5 mL·kg^{-1}·min^{-1} at each step. Oxygen uptake, $\dot{V}E$, RER and HR were measured during the test. Three criteria to identify $\dot{V}O_{2\ peak}$ were used, achievement of a plateau defined as less than 2 mL·kg^{-1}·min^{-1} rise in $\dot{V}O_2$ despite continued increase in workload, RER≥1.05 and $HR_{peak} \geq 95\%$ of estimated HR_{max}.

4.2.4 Exercise measurements (paper 1)

The exercise tests in study 1 were performed on a motor-driven treadmill (Woodway, USA). $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ were assessed by use of Champion, Jaeger, Germany. The

subjects wore a nose clip and breathed through a low restistance Hans Rudolph mouthpiece (2700 Series; Hans Rudolph Inc, USA). Gas samples of oxygen and carbon dioxide were collected from a mixing chamber with average values obtained over 30 seconds periods and used for analysis. The heart rate was recorded electronically and registered every minute (Polar Sports tester PE 3000®, Polar Electro OY, Kempele, Finland). The highest recorded values of $\dot{V}O_2$, $\dot{V}E$, respiratory exchange ratio (RER), HR and running speed were used in the statistical analysis and expressed as peak values.

4.2.5 Exercise measurements (paper 2, 3 and 4)

The EIB-tests in study 2, 3 and 4 were performed on a motor-driven treadmill ("Bodyguard" 2313, Sweden) with an inclination of 5.3%. Douglas-bags were used for collecting gas samples of the expired air 5, 6 and 7 minutes after the start of exercise (74). The variations reported for the Douglas bag method used with cycle ergometry are 2.3 -2.5% for daily variations and 3.3-5.1% for between days variations (75). The Douglas bag system was chosen because measurements using the automatic, electronic equipment gave unstable and not reproducible results in the humid and cold environments. The subjects wore a nose clip and breathed through a Hans Rudolph mouthpiece (2700 Series; Hans Rudolph Inc, USA). Expiratory gas samples were taken for at least 30 seconds and analysed for the oxygen and carbon dioxide content (Oxygen analyzer model S-3A/1 and Carbon dioxide analyzer model CD-3A; Ametek Inc, USA). The volume, temperature and pressure of the expired gas were measured at the time the air was analyzed ("Ventilation measuring system", model S-430, KL-Enginering, Northridge, California, USA). Oxygen uptake, $\dot{V}E$, breathing frequency (BF) and RER were measured and recorded. The heart rate was recorded electronically and registered every minute (Polar Sports tester PE 3000®, Polar Electro OY, Kempele,

Finland). The highest recorded values of $\dot{V}O_2$, $\dot{V}E$, RER, HR and running speed were used in the statistical analysis and expressed as peak values.

In the hypobaric environment (study 2), the arterial oxygen saturation was recorded during the exercise tests with a self calibrating pulse oximeter (Nellcor N-395, Nelcor Puritan Bennet Inc, Pleasanton, USA) with an RS-10 forehead sensor (RS-10, Nelcor Puritan Bennet Inc, Pleasanton, USA). The sensor was taped to the skin above arcus superciliaris to minimize poor signal detection (76). The SpO_2 measurements were only performed in the hypobaric and normobaric environment because the pulse oximeter did not function properly under the extreme humid and cold conditions (study 3 and 4).

Antiasthmatic medication was withheld according to the ERS guidelines. Inhaled short-acting β_2-agonists and sodium cromoglycate were withheld for eight hours prior to testing; inhaled long-acting β_2-agonists, theophylline and leukotriene antagonists for the last 72 hours, anti-histaminic for the last 7 days and orally administered glucocorticosteroids for the last month (7).

4.2.6 Skin prick test (paper 2, 3 and 4)

The skin prick test was performed on the first study day according to the Nordic guidelines (77) with the following prevalent ambient allergens: moulds (Cladosporium herbarum), house dust mites (Dermatohagoideus pteronyssimus), dog dander, cat dander, birch pollen, grass pollen (timothy), mug worth pollen, cow's milk, shrimp and hen's white egg (Soluprick, ALK, Copenhagen, Denmark). To be considered allergic to an allergen, a positive skin prick test of at least ++ (1/2 of the reaction to histamine 10 $mg \cdot mL^{-1}$) was required. The size was recorded by measuring (maximum + minimum diameter (mm)) $\cdot 2^{-1}$.

4.2.7 Sport specific exercise challenge (paper 5)

The exercise-field test consisted of a cross-country skiing competition. The men performed a 10 kilometers cross-country skiing competition and the women a 7 kilometers competition. Lung function (maximal expiratory flow volume loops) was measured before the start of the competition, immediately after finishing, and 5, 10, and 20 minutes after. The competitions took place at an altitude of 1100 and 1250 masl for the women and men, respectively, and the ambient temperature and the relative humidity during the competition were measured and recorded (specified under ambient conditions; chapter 4.3).

4.2.8 Methacholine bronchial provocation (paper 5)

Non-specific bronchial hyper responsiveness was measured by bronchial provocation to methacholine. Methacholine was delivered by the inspiration triggered nebuliser Aerosol Provocation System Jäger® (Würzburg, Germany), and inhaled in doubling doses until FEV_1 decreased 20 percent from baseline, as measured after inhaled nebulised isotonic saline. The dose causing 20 % reduction in FEV_1 (PD_{20}) was determined by linear interpolation on the semi logarithmic dose response curve. All tests were performed according to current guidelines from American Thoracic Society (78). After bronchial provocation testing subjects were given salbutamol inhalations to reverse bronchial constriction.

4.3 Ambient conditions

Study 2, 3 and 4 were conducted in a conditioned low pressure climate chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway), shown at the picture on the next page with a description of the chamber's possible environmental variations.

Altitude: 0-8000 masl

Relative humidity: 15-100%

Temperature: -20°C to 45°C

Wind: 0 -15 m·sec^{-1}

The subjects performed exercise testing according to identical test procedures but under four different environmental conditions. In study 2, a hypobaric environment, 75.5 kPa (± 0.2), corresponding to about 2500 masl with a relative humidity of 40.2% (± 3.1) and a temperature of 20.1°C (±1.2) was compared to a normobaric environment, 98.7 kPa (± 1.1). In study 3, a humid environment, 19.9 °C (±1.0) and 95.0% (± 1.7) relative humidity was compared to a regular indoor environment. A cold environment, -18.0°C (±1.4) and a relative humidity of 39.2 % (±3.8) was compared to a regular indoor environment in study 4. The regular indoor environment had a relative humidity of

40.0% (± 3.3) and a temperature of 20.2°C (±1.1). The barometric pressure during the exercise tests were 98.7 (± 1.1) kPa except for the hypobaric environment.

The environmental conditions during the skiing competitions (study 5) were a temperature of -2 ^{o}C and -4 ^{o}C and a relative humidity of 36 % and 34% for the women and men's competitions, respectively. The competitions took place at an altitude of 1100 and 1250 masl for the women and men, respectively.

4.4 Ethical considerations

The present thesis was performed according to the principles stated in the Declaration of Helsinki. All subjects signed a written consent form and in addition for subjects below18 years of age, one of their parents gave the written consent. The Regional Medical Ethics Committee approved the study.

4.5 Statistical analysis

Demographic results are given as mean values with standard deviation (SD) and results as means with 95% confidence intervals (CI). When satisfying normal distribution, differences between two measurements were analysed by Student's paired t-tests, otherwise a non parametric test for paired observations, Wilcoxon signed ranks test, was used. All tests were two-tailed with a significance level of 5%. In study 1 the Bland-Altman approach was used to calculate the limits of agreement for $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ between the two test protocols (79). Correlation was calculated by Pearson's correlation coefficient. Differences in categorical data were assessed by the Fischer exact test.

The bronchoconstrictor response following exercise was measured as the maximum per cent fall in FEV_1 and FEF_{50} after exercise, and the area under the curve (AUC) as per cent fall of the preexercise value in $FEV_1 \cdot time^{-1}$, up to 15 minutes postexercise, using the trapezoid rule. Identical analysis were made for FEF_{50}. If FEV_1 or FEF_{50} increased from baseline after exercise, the corresponding area was subtracted from the AUC measurements.

Assuming that the inhaled air during exercise is fully saturated with vapor and reaches the temperature of 37°C, the respiratory water loss during the last 3 minutes of exercise was calculated by using a web-based on-line calculator designed by the Department of Physics and Astronomy, Georgia State University Atlanta, based on empirical fit for density data,

(http;//hyperphysics.phyastr.gsu.edu/hbase/kinetic/relhum.html 2004).

Hofstra et al (80) found that a sample size of 12 subjects was sufficient to obtain differences in EIB. Based upon FEV_1 and $\dot{V}O_{2\ peak}$ as main variables, with pre-existing knowledge of the variation of these variables and assuming a power of 80%, a sample size of 20 subjects was calculated as necessary to obtain a significance level of 5% (80). Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 11.0 and MedCalc version 8.1.1.0.

5. Results

Paper I

To compare a common stepwise protocol with a standardised EIB-test protocol for assessing $\dot{V}O_{2\,peak}$ *and* $\dot{V}E_{peak}$

The results from study 1 showed no significant difference in $\dot{V}O_{2\,peak}$ or $\dot{V}E_{peak}$ between the EIB protocol and the stepwise protocol, neither at 10.5% inclination of the treadmill, $\dot{V}O_{2\,peak}$: 63.9 mL·kg^{-1}·min^{-1}, (60.0, 68.0) [mean (95% confidence intervals)] vs 63.3 mL·kg^{-1}·min^{-1} (59.3, 67.4) respectively, $\dot{V}E_{peak}$: 158 L·min^{-1} (144, 173) vs 161 L·min^{-1} (145, 176) respectively, nor at 5.3% inclination, $\dot{V}O_{2\,peak}$: 56.1 mL·kg^{-1}·min^{-1} (51.9, 60.2) vs 56.0 mL·kg^{-1}·min-^{1} (52.1, 60.0) respectively, $\dot{V}E_{peak}$: 123 L·min^{-1} (114, 132) vs 127 L·min^{-1} (116, 138), respectively.

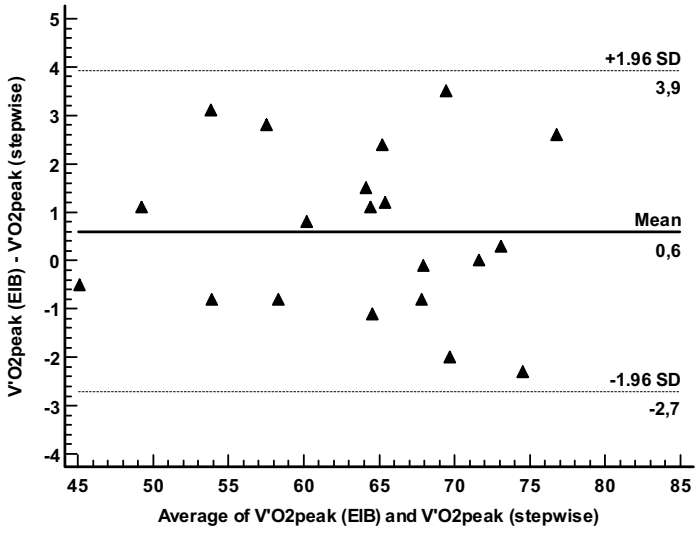

Figure 1. Bland-Altman plot (95% limits of agreement) for peak oxygen uptake ($\dot{V}O_{2\,peak}$) assessed with the EIB protocol and the stepwise protocol at 10.5% inclination of the treadmill.

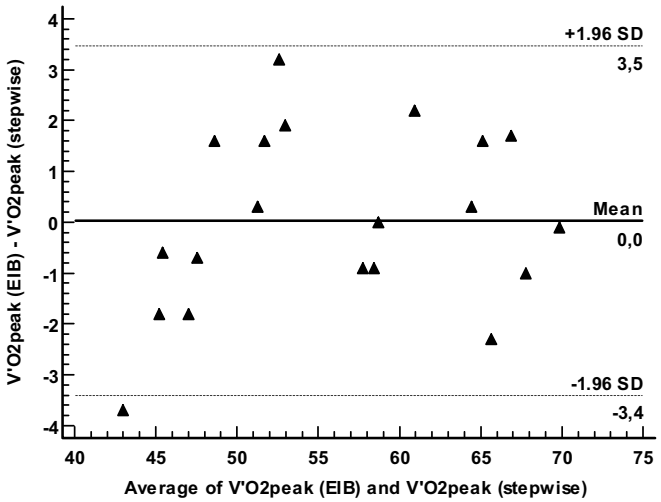

Figure 2. Bland-Altman plot (95% limits of agreement) for peak oxygen uptake ($\dot{V}O_{2\ peak}$) (▲) assessed with the EIB protocol and the stepwise protocol at 5.3% inclination of the treadmill.

Compared to the stepwise protocol, HR_{peak} and peak running speed were significantly lower with the EIB-test protocol at both inclinations ($p < 0.001$). Also RER_{peak} was significantly reduced with the EIB protocol at 10.5% inclination and 5.3% inclination ($p=0.001$ and $p=0.01$, respectively).

Paper 2

To examine the influence of a hypobaric environment upon EIB, and secondarily to examine the effect of reduced barometric pressure upon exercise capacity in subjects with EIB.

Neither the maximum reduction in FEV_1, FEF_{50} and PEF (% of baseline) nor AUC were significantly different after running in hypobaric as compared to normobaric

environment. No significant correlation was found between the decrease in S_PO_2 during running in the hypobaric environment and maximal fall in $FEV_{1.}$

There was a significant decrease in $\dot{V}O_{2\ peak}$ in the hypobaric environment. The reduction was 10.1% (7.2, 13.0) ($p<0.001$), or 4.4% (3.1, 5.7) per 1000 masl The S_PO_2 at $\dot{V}O_{2\ peak}$ was 94.4% (92.2, 96.6) in the normobaric environment and 85.6% (82.8, 88.4) in the hypobaric environment. The mean percentage reduction in S_PO_2 at $\dot{V}O_{2\ peak}$ was 9.3% (7.0, 11.6) ($p<0.001$), or 4.1% (3.0, 5.1) per 1000 masl, respectively.

Paper 3

To examine the effect of changing the humidity of the environmental air upon exercise capacity and secondarily to assess the influence of changing environmental humidity upon EIB in subjects with diagnosed EIB.

Peak oxygen uptake and peak running speed, increased significantly, 4.5% and 5.9% respectively, during exercise in the humid environment as compared to exercise in the regular indoor environment. $\dot{V}O_{2\ peak}$ increased from 46.5 (43.9, 49.9) to 48.6 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ (45.5, 52.5), respectively; and peak running speed from 10.2 (9.3, 10.7) to 10.8 $km{\cdot}hour^{-1}$ (10.0, 11.3), respectively ($p=0.001$). HR_{peak} was significantly higher under humid conditions ($p=0.003$), whereas BF_{peak} was significantly reduced ($p<0.001$). There were no significant differences in $\dot{V}E_{\ peak}$ and RER_{peak} during exercise between the two climatic conditions.

Maximum reduction in FEV_1 as per cent of baseline lung function after exercise in the humid environment was half of the reduction in FEV_1 after exercise under regular indoor conditions, 12% (7, 17) vs 24% (19,29), respectively ($p=0.0007$).

Maximum reduction in FEF_{50} as per cent of baseline lung function was also almost reduced to the half after exercise in humid environment, 20% (12, 29) compared to exercise under regular conditions, 38% (30, 46) (p=0.0004). AUC for FEV_1 decreased significantly after exercise in the humid environment, 103.3 (163.9, 42.8) vs exercise under regular conditions, 249.5 (316.9, 182.2), respectively (p=0.001).

No correlation was found between maximum reduction in lung function after exercise or water loss during exercise and the increase in $\dot{V}O_{2\ peak}$ in the humid compared to the regular indoor environment.

Paper 4

To examine the influence of changing environmental temperature upon exercise capacity, and secondarily to assess the influence of changing the environmental temperature upon EIB in subjects with diagnosed EIB.

$\dot{V}O_{2\ peak}$ decreased significantly, 6.5%, from 47.9 (45.0, 51.8) to 44.8 $mL \cdot kg^{-1} \cdot min^{-1}$ (41.2, 48.4), respectively (p=0.004) during exercise under regular indoor conditions as compared to exercise in the cold environment. Peak running speed was also significantly lower in the cold environment; 10.2 (9.5, 11.0) vs 9.7 $km \cdot hours^{-1}$ (8.9, 10.5), respectively (p= 0.02). There were no differences in $\dot{V}E_{peak}$, RER_{peak}, HR_{peak} or BF_{peak} during exercise between the two climatic conditions.

Mean values of maximum reduction in FEV_1 and AUC for FEV_1 increased significantly after exercise in the cold environment. Maximum reduction in FEV_1 post exercise in the cold environment was 31% (24, 38) vs 24% (19, 29), respectively after exercise

under regular conditions (p=0.04). AUC for FEV_1 was increased after exercise in the cold air, 358 (261, 455) vs exercise under regular conditions, 250 (182, 317), respectively (p=0.01). Increased maximum reduction in FEF_{50} after exercise in the cold environment was also found; 47% (38, 55) vs 38% (30, 46), but on the border of significance (p=0.06). Maximum reduction in FVC as per cent of baseline lung function or AUC for FEF_{50} did not differ between the climatic conditions. No correlation was found between maximum reduction in lung function (FEV_1 or FEF_{50}) after exercise or water loss during exercise and the reduced $\dot{V}O_{2\ peak}$ in the cold conditions as compared to the regular indoor conditions.

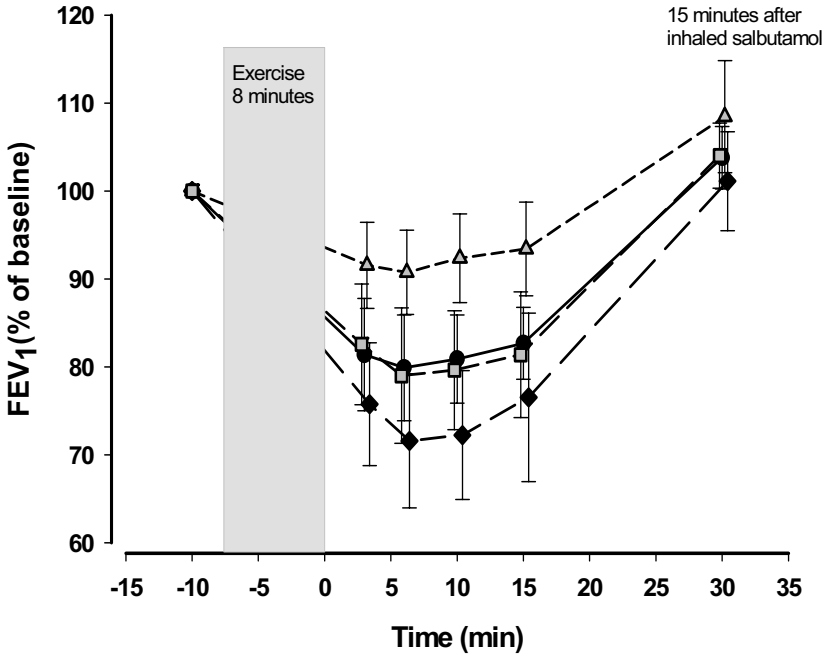

Figure 3. Lung function (FEV_1) before and 3,6,10 and 15 minutes after exercise and 15 minutes after inhaled salbutamol in a standardised regular environment (●) , in a hypobaric environment (■), in a cold environment (♦) and in a humid environment (▲) (n=20). Results are given as mean with 95% confidence intervals.

Table 1. Peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak minute ventilation ($\dot{V}E_{peak}$) peak heart rate (HR_{peak}) peak respiratory exchange ratio (RER_{peak}), peak breathing frequency (BF_{peak}), and peak running speed during exercise in hypobaric conditions (2500 masl), in humid conditions (95% relative humidity) and in cold conditions (-18°C) compared to measurements in regular indoor conditions (200 masl, 20°C and 40% relative humidity) (n=20).

Variables	Regular env.	Hypobaric env.	Regular env.	Humid env.	Regular env.	Cold env,
$\dot{V}O_{2\ peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	47.6 (44.3, 50.8)	42.7 (39.7, 45.8)*	46.5 (43.9, 49.9)	48.6 (45.5, 52.5)*	47.9 (45.0, 51.8)	44.8 (41.2, 48.4)*
$\dot{V}E_{peak}$ ($L \cdot min^{-1}$)	101 (89, 114)	105 (92, 119)	99 (86, 112)	100 (87, 113)	99 (86, 112)	95 (80,110)
HR_{peak} ($beat \cdot min^{-1}$)	187 (182, 192)	185 (180, 190)	186 (181, 192)	189 (186, 194)*	186 (181, 192)	187 (181, 192)
RER_{peak}	1.03 (1.00, 1.07)	1.06 (1.02, 1.10)*	1.03 (1.00, 1.07)	1.00 (0.96, 1.03)	1.02 (0.95, 1.06)	1.03 (1.00,1.07)
BF_{peak} ($breath \cdot min^{-1}$)	46 (41, 51)	47 (43, 51)	46 (41, 51)	43 (39, 48)*	46 (41, 51)	47 (42, 51)
Peak running speed ($km \cdot hour^{-1}$)	10.5 (10.0, 11.0)	9.5 (9.0, 10.5)*	10.2 (9.3, 10.7)	10.8 (10.0, 11.3)*	10.2 (9.5, 11.0)	9.7 (8.9, 10.5)*

Values are given as mean with 95% confidence intervals in parentheses.
* = significant difference between each climate and its respective regular environment

Paper 5

To compare methacholine bronchial provocation with a sport specific exercise- field test in the diagnosis of asthma and/or bronchial hyperresponsiveness among elite cross-country skiers.

Bronchial hyperresponsiveness, defined as $PD_{20methacholine}$ <16 µmol, was found in 13 out of 24 skiers (54.2 %), in three of eight females and in ten of 16 males, whereas nine skiers (37.5 %) had a $PD_{20methacholine}$ <8 µmol. The distribution of bronchial responsiveness to methacholine is shown in figure 1. Two out of 24 subjects (8.3%) experienced a positive exercise-field test and a maximum reduction in $FEV_1 \geq 10\%$. Both had a maximum reduction in FEV_1 20 minutes after exercise. One of the skiers with a positive exercise-field test had a positive methacholine provocation with $PD_{20methacholine}$ of 5.79 µmol, whereas the other had $PD_{20methacholine}$ of 9.55 µmol.

Subjects older than 25 years of age had significantly more often $PD_{20methacholine}$ below 8µmol than subjects 25 years and younger (p=0.036). A significant negative correlation was found between age and log values of $PD_{20methacholine.}$ No significant difference in BHR to methacholine was found related to gender.

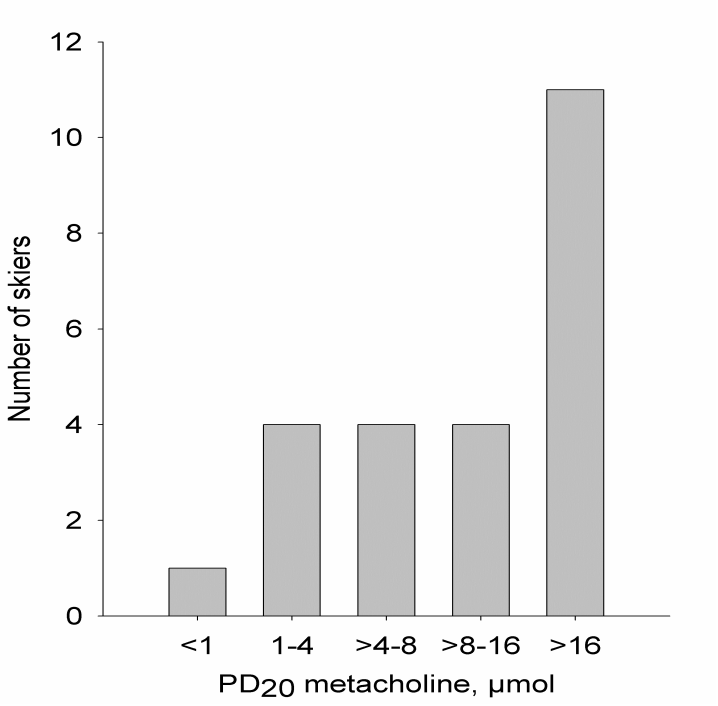

Figure 4. $PD_{20methacholine}$ in 24 elite cross-country skiers. Dose of methacholine causing a 20 % reduction in forced expiratory volume in one second (FEV_1), versus number of skiers.

6. Discussion

The main objective of the present theses was to investigate the influence of different environmental conditions upon exercise capacity and EIB in asthmatic subjects and evaluate different exercise protocols for both determining exercise capacity (study 1) and assessing BHR in elite cross-country skiers (study 5). The same design, subjects and test protocols were employed in study 2, 3 and 4. The ambient conditions were the same except for the barometric pressure in study 2, relative humidity in study 3 and temperature in study 4. The subjects were temporary exposed to the standardised environmental conditions during the exercise tests. Exercising under hypobaric conditions, extreme humid conditions and extreme cold conditions was compared to exercising under regular indoor conditions.

The overall findings of the entire study showed that exercise capacity can be measured with an EIB-test protocol. $\dot{V}O_{2\ peak}$ is reduced in a hypobaric and in a cold environment and increased in a humid environment. $\dot{V}E_{peak}$ did not differ in any of the climates. There was no difference in EIB in a hypobaric environment, EIB was increased in a cold environment and reduced to the half in a humid environment as compared to a regular, indoor environment. Methacholine provocation is more sensitive than a sport specific exercise-field test in order to assess BHR in elite cross-country skiers.

Paper 1

No differences were found for $\dot{V}O_{2\ peak}$ or $\dot{V}E_{peak}$ either at 10.5% inclination or at 5.3% inclination of the treadmill between the two test protocols.

Achievement of a plateau as described in the method section was fulfilled with the stepwise protocol. Although $\dot{V}O_2$ reached a plateau also with the EIB protocol, this

could not be confirmed since the EIB-test protocol has constant speed the last 3-4 minutes. However, criteria were fulfilled for RER and HR. Thus, the criteria for achieved $\dot{V}O_{2\ peak}$ described by Åstrand et al (34) seem to be fullfilled with both protocols.

The criterion of a constant speed during the last 4 minutes of the EIB-test protocol was fulfilled at both inclinations as also the criterion of a HR of 90-95% of estimated HR_{max} during the last 4 minutes. In study 1 all subjects were tested based on estimated HR_{max} and the test leader`s evaluation of exhaustion. Mean estimated HR_{max} for the group running at 10.5% was 196 beats·min^{-1} and mean HR_{peak} measured with the EIB protocol was 186 beats·min^{-1}. For the group running at 5.3% inclination, the mean estimated HR_{max} was 194 beats·min^{-1} and mean HR_{peak} measured with the EIB protocol was 185 beats·min^{-1}. This was 94.8% and 95.3%, respectively of estimated HR_{max}. Thus, both the use of an experienced test leader and the use of estimated HR_{max}, are important during clinical exercise testing.

In the most commonly used stepwise test protocols for assessing $\dot{V}O_{2\ max}$ warming up is included in the test procedure (41;81-83). The final running phase lasted between 4 and 6 minutes in the stepwise protocol after 20 minutes warming up, whereas the EIB-test lasted totally for 8 minutes without warming up. The initial workload was approximately 80% of estimated maximum workload for both protocols. However, the effect of the warming up was reduced to a minimum with the EIB protocol, since the main target for provoking EIB is a fast increase in ventilation. In the present study, warming up in the stepwise protocol was not associated with higher $\dot{V}O_{2\ peak}$ measurements in healthy trained subjects as compared to the EIB protocol without warming up. This is supported by the study of Chwalbinska-Moneta and Hanninen and

Busuttil and Ruhling (84;85). According to Mc Ardle et al (86) the beneficial effect of warming up upon $\dot{V}O_{2\ max}$ and exercise performance lacks justification by laboratory studies. On the other hand Ingjer and Strømme (87) and Inbar and Bar-Or (88) have reported increased $\dot{V}O_{2\ max}$ after warming up.

The two test protocols at both inclinations demonstrate different ways to reach peak values. The main difference is the increased running speed with the stepwise protocol. There is an "overshoot" in relation to the speed required to reach $\dot{V}O_{2\ peak}$ with the stepwise protocol as compared to the EIB protocol. The difference in HR between the protocols accompanies the difference in the running speed, whereas $\dot{V}O_2$ increased with both protocols until reaching a plateau. Neither the running speed nor the HR differed during the last 4 minutes with the EIB protocol. The criterion of a steady state speed during the last 4 minutes of the EIB protocol is fulfilled at both inclinations as also the criterion of a HR of 90-95% of estimated HR_{max} during the last 4 minutes.

Recently De Fuccio et al (89) concluded that rapid-incremental protocols can be as useful as standard high-intensity constant work rate protocols in diagnosing EIB in subjects with suspected EIB. In their study both $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ were significant lower with the constant work rate protocol, presumably because the workload with the constant work rate was too low as compared to the constant workload the last 4 minutes in the present study.

Even though no significant differences in $\dot{V}O_{2\ peak}$ or $\dot{V}E_{peak}$ were found in the present study of healthy subjects, we cannot conclude that the results may apply to all asthmatic subjects. Anti-asthmatic treatment should be withheld before EIB-testing according to

the guidelines for exercise challenge testing from European Respiratory Society and American Thoracic Society (7;78), and this might influence $\dot{V}O_{2\ peak}$ if bronchoconstriction occurs during the test. According to several previous studies on EIB, bronchoconstriction usually occurs immediately or soon after the exercise and not during the exercise (7;8;78;90). $\dot{V}O_2$ measurements during an 8 minutes exercise test may thus not be affected. If the asthmatic patient becomes obstructive during the exercise test a new test for assessing $\dot{V}O_{2\ peak}$ with pre-medication may be performed. A similar study with EIB-positive subjects should optimally be performed.

Paper 2

An additional reduction in FEV_1 or FEF_{50} after running in the hypobaric environment was not found. HR_{peak} and $\dot{V}E_{peak}$ were not higher during exercise in the hypobaric environment. $\dot{V}O_{2\ peak}$ was 10.1% lower in the hypobaric environment. Mean reduction in S_PO_2 at $\dot{V}O_{2\ peak}$ was 9.3% below running in the normobaric environment.

Some authors have speculated whether inhalation of hypoxic gas could enhance airway responsiveness (18) and result in bronchoconstriction (91). Our study did not at all confirm this. Exercise in medium or high altitudes is usually associated with increased ventilation due to the hypoxic conditions (92), which may explain a possible increased reduction in lung function. In addition, the temperature and the water content of the air at these altitudes are substantially lower than at sea level. The present study was however conducted in a low-pressure chamber enabling control of air temperature and relative humidity. Except for the barometric pressure, the climatic conditions were equal.

Reduced $\dot{V}O_{2\ peak}$ and running speed during exercise in the hypobaric environment could partly explain why the subjects did not increase their $\dot{V}E_{peak}$. The reduction in $\dot{V}O_{2\ peak}$ is most probably due to the lower SpO_2. Since the temperature and water content of the inspired air together with $\dot{V}E_{peak}$ were identical for both conditions, airway cooling and water loss from the airways should be the same. Our findings are in agreement with results from other studies dealing with exercise challenges in a hypobaric environment (60;61). Louie and Pare (61) and Matsuda et al (60) did not find any additional increase in bronchoconstriction in a hypobaric environment. However, the number of subjects with EIB was far too low to reach any conclusion, and the exercise protocol was not sufficiently standardised (78). Numerous studies have used running on a treadmill for 6-8 minutes near maximum load to assess EIB both in clinical setting and in epidemiological studies
(8;93-95).

Paper 3

In study 3 it was demonstrated that exercise capacity measured by $\dot{V}O_{2\ peak}$ and peak running speed increased significantly during exercise under extreme humid conditions compared to regular indoor conditions. HR_{peak} increased and BF_{peak} was reduced in the humid climate, whereas $\dot{V}E_{peak}$ and RER_{peak} were unchanged.
The reduction in FEV_1 after exercise in the humid environment was reduced to the half compared to after exercise under regular conditions. Similar findings were made for the reduction in FEF_{50}. However, even under humid climatic conditions there was still a significant EIB compared to baseline lung function.

Neither Kallings et al (15) nor Eschenbacher et al (16) found any difference in $\dot{V}O_2$ or in other physiological variables at submaximal workloads in asthmatic subjects. However, only six and eight subjects, respectively were included in their studies(15;16), and their results can only be used for generation of hypotheses for further investigations. The workload, ventilation and the oxygen demand in their studies were probably too low to discover any difference in $\dot{V}O_2$. In the present study, the differences in $\dot{V}O_2$, running speed, HR and BF occurred when the subjects were close to their maximal aerobic capacity. In previous reports it has been concluded that bronchoconstriction occurs after exercise(8;9;96;97), and therefore it should not be expected that $\dot{V}O_{2\ peak}$ is influenced by bronchoconstriction during exercise. For detection of EIB during exercise, recording of tidal breathing loops may be a help.

The breathing pattern seems to be different during exercise in the humid as compared to the regular indoor environment. Peak tidal volume (V_{tpeak}) increased significantly in the humid environment. Consequently the subjects had a slower and deeper breathing pattern in the humid environment. All except two subjects reported spontaneously that breathing during exercise in the humid environment was much easier than it was under regular indoor conditions. This is in agreement with the observation that the subjects ran faster with increased HR_{peak} and $\dot{V}O_{2\ peak}$ and with less effort (no change in RER_{peak} and $\dot{V}E_{peak}$) in the humid environment. The mechanism of increased $\dot{V}O_{2\ peak}$ in the humid environment is unknown, but in the present study a different breathing pattern was seen when the subjects were close to maximum aerobic capacity and the increased $\dot{V}O_{2\ peak}$ might be explained by less dead volume in the respiratory tract and increased inspiratory capacity.

Humid environmental conditions seem to have a protective effect on EIB. The respiratory water loss was significantly decreased in the humid environment compared to regular, indoor conditions, but there was still a signficant loss of water from the airways. Boulet and Turcotte (57) reported that EIB was influenced by the changes in water content during and after exercise. The recovery period in the present study took place in standard environmental conditions, which is, according to Boulet and Turcotte (57), the best recovery environment to protect against EIB.

Zainudin et al (58) reported no significant relationship between different humidity levels, (41-90% relative humidity) and EIB (defined as reduction in FEV_1 ≥15%) among Malaysian school children. Their humidity levels were naturally occurring and not standardised. Their investigation was performed as a cross sectional study with a main objective to determine the prevalence of EIB in a population of school children living in a humid, tropical climate in the inner city of Kuala Lumpur.

Paper 4

In study 4 it was demonstrated that the exercise capacity judged by $\dot{V}O_{2\ peak}$ and peak running speed decreased significantly during exercise in a cold environment as compared to regular indoor conditions, whereas $\dot{V}E_{\ peak}$, RER_{peak} and BF_{peak} did not differ. Maximum reduction in FEV_1 after exercise and AUC for FEV_1 increased significantly in the cold environment, in contrast to the maximum reduction in FEF_{50} which did not reach statistically significant difference between the two climatic conditions. The increased reduction in FEF_{50} reached statistical significance only at 1 and 6 minutes after exercise in the cold environment, whereas AUC for FEF_{50} did not change.

According to the present study, the differences in $\dot{V}O_2$ and running speed occured when the subjects were close to their maximal aerobic capacity, the last 3 minutes of the EIB-test. No correlation was found between maximum reduction in lung function (FEV_1 or FEF_{50}) after exercise or water loss during exercise and the reduced $\dot{V}O_{2\ peak}$ in the cold conditions compared to the regular indoor conditions. The lack of correlation is possibly due to too weak statistical power because of the relatively low number of subjects included. All except three subjects reported spontaneously that breathing during exercise in the cold environment was much more difficult than breathing under room tempered conditions. These statements support that the subjects ran slower the last 4 minutes of the test with decreased $\dot{V}O_{2\ peak}$ in the cold environment.

The explanation of reduced $\dot{V}O_{2\ peak}$ and peak running speed in the cold environment might be an increased strain level, especially for asthmatics, when they start the exercise at a high intensity level in a cold environment without a properly warming-up period. The subjects were only exposed to the cold environment for 10 minutes and no freezeing or shivering were observed or reported. Since they wore warm clothes suited for the cold environment, the decrease in $\dot{V}O_{2\ peak}$ was probably caused by reduced running speed. These findings are supported by the study of Quirion et al (63) on healthy subjects. They demonstrated that $\dot{V}O_{2\ max}$ significantly decreased and $\dot{V}E_{peak}$ did not change in a temperature of -20^{o}C and 0^{o}C as compared to a temperature of 20^{o}C. Their subjects reported that submaximal exercise intensities were more tiring in a cold environment as compared to a warm environment. They suggested that the net efficiency of exercise at low temperatures is lower than under normal conditions. On the other hand Sandsund et al (64) reported increased $\dot{V}O_2$ in eight healthy male athletes at submaximal exercise intensities in a cold environment compared to regular

indoor conditions, but no difference in $\dot{V}O_{2\ max}$. Time to exhaustion was shorter in the cold environment. They suggested that exercise stress is higher at submaximal exercise in a cold environment. This is in agreement with the reduced running speed during exercise in the present study. Claremont et al (65) explained the same observation by a catecholamine calorigenic effect.

In the studies of asthmatics of Kallings et al (15), Sandsund et al (17) and Eschenbacher et al (16) only six, seven and eight subjects, respectively were included. Thus, their results may merely serve as pilot studies indicating the need for further investigations. The workload differed markedly between these studies and also to the present study. The workload, ventilation and the demand for oxygen is too low in the study from Kallings et al (15) and Eschenbacher et al (16) in order to be able to discover any difference in $\dot{V}O_{2\ peak}$ as compared to the exercise load at which the difference occured in the present study.

The results of the present study confirms previous reports showing that inhalation of cold air increase EIB in asthmatic subjects (14;47). On the other hand neither Evans et al (56) nor Sandsund et al (17) could find any additive effect of cold air inhalation upon EIB. The temperature of the inhaled air in their studies was actually -1^{o}C and 2^{o}C, respectively, and possibly not cold enough to disclose any difference. Evans et al (56) suggested that lack of exposure to ambient cold air during inhalation might explain the lack of an additive effect.

Cold environmental conditions seem to aggravate the effect on EIB, and the respiratory water loss significantly increased in the cold environment as compared to the regular, indoor conditions. These findings support earlier reports on EIB and cold environment

and indicate that the worsening effect on EIB in asthmatics is due partly to increased water loss and partly to heat loss (12-15;57). Our findings are supported by Zeitoun et al (54), Koskela et al (55) and Boulet and Turcotte (57). The recovery period in the present study was in regular, indoor environmental conditions which is, according to Boulet and Turcotte (57), the best recovery environment to protect EIB.

Paper 5

The results from study 5 suggest that $PD_{20methacholine}$ is more sensitive than exercise-field testing using the competitive sport in order to assess BHR and asthma in elite cross-country skiers. Also Dickinson et al (33) concluded that a sport specific exercise test is not the optimal challenge for diagnosing EIA. They proposed that an EVH-test provides a more sensitive diagnosis of EIA in elite winter athletes. On the other hand, our observations do not agree with the findings of Rundell et al and Ogston and Butcher (31;32) who maintained that a sport specific exercise-field test is the method of choice in the diagnosis of EIB among top athletes. In connection with the Olympic Games in Salt Lake City in 2002 an exercise-field test was recommended because it is considered to be an effective and more sensitive test to identify EIB in cold weather than an exercise test performed under laboratory conditions with respect to temperature and humidity (98;99). It can be assumed that by performing their usual exercise in their usual environment, the athletes would be in the best position to unveil their respiratory problems. However, in the present study, the sport specific exercise-field test did not reveal any subjects not already recognized by the methacholine provocation test ($PD_{20methacholine}$), and 11 out of 13 subjects with some degree of BHR to methacholine were not detected by the exercise-field test (31).

Crimi et al (100) claimed that direct stimuli, like methacholine, can allow the identification of asthmatic subjects, who do not exhibit EIB because of low degree of

airway inflammation at the time of study, but may eventually become ill if exposed to sensitizing allergens or after virus infections. Langdeau et al (101) investigated the Canadian Olympic Team and found that nearly 50% were positive to methacholine compared with 18% of healthy controls. This is in concurrence to the findings in the present study.

A significantly higher percentage of BHR was found among subjects older than 25 years of age ($p=0.036$) This is in accordance with the findings of Heir et al (25). One possible explanation may be that the continued stress on the airways over many years caused by high ventilations rates during exercise, often in dry cold environments, increases BHR over time by increasing airways inflammation as also found by Sue-Chu et al and Karjalainen et al in their bronchial biopsy studies of young athletes from a skiing high school (102;103).

This corresponds to the findings in a study of exercising mice with increasing epithelial damage with continued exercise (104). Others have suggested that immune suppression may come into play in the development of BHR, at least in endurance athletes. Thus, Heir et al (105) found that physical training with an upper respiratory tract infection (URTI) induced a long-lasting (≥6 weeks) increased BHR to histamine, whereas this did not occur in subjects who were not training actively during the infection.

No significant gender difference was found in the present study with six out of 16 men and three out of eight women displayed BHR to methacholine with a PD_{20} below 8µmol The results of previous studies have implied a slightly higher prevalence in female athletes (27;98), and females have been shown to have a slightly higher prevalence of BHR to methacholine (106;107). The number of athletes participating in

the present study was possibly to low to reveal significant gender differences.

The present study fully demonstrates the discrepancy between direct and indirect tests of BHR. Furthermore it should be emphasized that asthma is a clinical diagnosis, based upon reports of recurring epidsodes of bronchial obstruction. Both direct and indirect BHR changes over time due to changing exposure to allergens and other environmental agents, as well as being dependent on anti-inflammatory treatment. The diagnosis of asthma in athletes should be based upon a combination of clinical history and clinical signs with the use of supplementary objective tests as used in the present study.

6.1 Strengths and limitations

6.1.1 Design

The studies included in the present thesis have a randomized cross over design (paper 1, 2, 3, and 4) and an open, non-randomized design (paper 5). The subjects are their own controls and the random variance is reduced. Thus, fewer subjects are needed to detect significant differences as compared to other designs. The number of subjects included were strictly calculated based upon previous studies and existing knowledge of the variation of the main variables, $\dot{V}O_{2\ peak}$ and FEV_1 and are based upon assessing a power of 80% and a significance level of 5%.

The cross over design is a suitable method to detect differences in $\dot{V}O_{2\ peak}$ between two test protocols (paper 1) and also for detecting the influence of different environmental conditions upon exercise capacity and EIB (paper 2, 3 and 4). All the subjects in study 2, 3 and 4 had a chronic and stable asthma diagnosis and the wash out period was at least 48 hours. The subjects in study 1 were healthy and physical active and the wash out period between the exercise tests was at least 3 hours. In study 5 all

the cross-country skiers of the Norwegian national teams were included and the wash out period was at least 24 hours between the methacholine provocation and the skiing competition.

6.1.2 Strength of the study

A major advantage of the present study is that all subjects included in study 2, 3 and 4 have a diagnosed EIB according to the definition by ERS and ATS (7;78). Most of the previous studies within this field have only included asthmatic subjects and not subjects with an objective EIB diagnosis. If differences in EIB is one of the main outcome a verified diagnosis of EIB should be an important inclusion criterion.

Another advantage of the present study is that all ambient conditions were kept equal except for variation in the assessed climatic conditions; barometric pressure (study 2), relative humidity (study 3) and temperature (study 4). Furthermore, all lung function tests, exercise tests and SPT were performed by one experienced investigator, and the clinical examinations carried out by one paediatrician working within allergology and respiratory medicine.

6.1.3 Limitations of the study

There are limitations in the present studies that need to be addressed. Firstly, even though no significant differences in $\dot{V}O_{2\ peak}$ or $\dot{V}E_{peak}$ were found in healthy subjects in paper 1, the results may not be transferrable to all asthmatic subjects. Anti-asthmatic treatment has to be withheld in asthmatics according to ERS Task Force before EIB-testing and this might influence $\dot{V}O_{2\ peak}$ if bronchoconstriction occurs during the test. Although authors of previous studies on EIB have concluded that bronchoconstriction usually occurs immediately or soon after the test, it is also known from clinical practise

that asthmatic patients can respond different upon exercise. Most probably the recording of tidal breathing loops during exercise would help to assess if EIB occured during exercise.

Secondly, one can argue against the relatively wide range in age of the subjects, from youth to adulthood. A more homogeneous population would have been preferable, but the duration of the study was the limiting factor. It turned out that it was difficult to include subjects with EIB. More than 200 subjects with respiratory symptoms during or after exercise were pre-tested to include the 20 subjects who fulfilled the inclusion criterion.

7. Conclusions

1. Different climatic environmental conditions influences both EIB and exercise capacity in different ways.

   A. A barometric pressure corresponding to an altitude of 2500 metres does not influence EIB in subjects with asthma. $\dot{V}O_{2peak}$ and S_pO_2 are significantly reduced in the hypobaric environment. This result suggests that subjects with EIB, like their healthy nonasthmatic peers, can participate in sports activities such as altitude climbing, skiing and tracking in medium high altitudes. However, the low temperature and the subsequent reduced water content of the air in the mountainous area can influence both heat loss and dehydration of the airways.

   B. Exercising in a humid environment improves exercise capacity and protects against EIB in subjects suffering from EIB. This result suggests that a warm, humid climate is the best climate to practice physical activity for asthmatic subjects.

   C. Exercising in a cold environment reduces exercise capacity and increase EIB in subjects suffering from EIB. These findings have important implications for medical treatment and training procedures in a cold environment for patients and athletes with EIB.

2. $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ are comparable using the two test protocols both at 10.5%and at 5.3% inclinations of the treadmill. EIB-test may thus be employed for both provoking EIB and assessing $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$,

thus giving information about both EIB and physical fitness at the same time. In clinical practice this may save one test day, and simplify diagnostic and monitoring procedures of the patients.

3. Methacholine provocation ($PD_{20methacholine}$) is more sensitive than exercise-field testing in confirming the diagnosis of BHR in elite cross-country skiers.

8. **Future research**

Based upon the results of the present thesis the following research question should be addressed in the future:

Our results of the effect of environmental climatic conditions upon exercise capacity in asthmatic subjects should be confirmed in a new study with a group of EIB-positive subjects and a control group consisting of healthy subjects.

Furthermore it is possible that bronchoconstriction may occur during an 8 minutes exercise test in subjects with EIB. This may be resolved by performing a study in subjects with verified EIB and analysing tidal breathing loops every 30 seconds or every minute during the exercise test in addition to measure lung function after exercise as commonly performed.

Further studies are needed to investigate the possible variation in sensitivity, specificity, positive and negative predictive values of the different provocation tests of BHR and asthma between different asthma phenotypes.

9. Reference list

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ERRATA

Paper 1 and 5

Since submission to the doctorial committee, paper 1 has been accepted for publication in a revised form. Corrections have been made both in the introduction, methods and discussion chapter.

Paper 5 was published in October in its original form.

Misprints are corrected in the abbreviations:

RER: **respiratory** exchange ratio

RER_{peak}: peak **respiratory** exchange ratio

Misprints are corrected in the introduction:

Page 9, #2: An **individual's** pattern of physical activity and future activity level **is** often founded during childhood and adolescence and **is**…

Page 10, #1: a comparison between the EIB-test protocol and a common stepwise protocol for determination of $\dot{V}O_{2\ peak}$ and peak minute ventilation ($\dot{V}E_{peak}$) **is** required.

#2: sub **arctic** climate , …than **at** sea level.

Page 11, #3: a **larger** number

Page 14, #1: Other factors indicating achievement of $\dot{V}O_{2\ max}$ are respiratory exchange ratio (RER) ≥ 1.05 and HR ≥ 95% of $\mathbf{HR}_{max}$

Can one single test protocol for provoking exercise-induced bronchoconstriction also be used for assessing aerobic capacity?

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**The study is performed within the ORAACLE (the Oslo Research group for Asthma and Allergy in Childhood; the Lung and Environment), which is member of the Ga[2]len, European Network of Centers of Excellence.*

Title for running head: $\dot{V}O_{2\ peak}$ – a comparison of two test protocols

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Abstract

Introduction: Several different exercise protocols are used to assess exercise-induced bronchoconstriction (EIB) and to measure peak oxygen uptake ($\dot{V}O_{2\ peak}$) and peak minute ventilation ($\dot{V}E_{peak}$).

Objective: To evaluate if one single test protocol for assessing EIB also can be used to determine aerobic capacity measured by ($\dot{V}O_{2\ peak}$) and ($\dot{V}E_{peak}$).

Methods: In a randomized cross over design, 40 healthy subjects (♀/♂=17/23), aged 14-40 years performed two exercise tests on a treadmill. Twenty subjects, (♀/♂=7/13), performed the two exercise tests at a treadmill inclination of 10.5%; the remaining 20 subjects, (♀/♂=10/10), at an inclination of 5.3%. A common stepwise protocol with 20 minutes warming up was compared to an EIB-test protocol of eight minutes treadmill run without warming up with a workload corresponding to 95% of maximum heart rate the last four minutes.

Results: $\dot{V}O_{2\ peak}$ did not differ significantly between the two test protocols at 10.5% inclination; 63.3 $mL \cdot kg^{-1} \cdot min^{-1}$ (59.3, 67.4) [mean (95% confidence intervals)] and 63.9 $mL \cdot kg^{-1} \cdot min^{-1}$ (60.0, 68.0), respectively or at 5.3% inclination; 56.0 $mL \cdot kg^{-1} \cdot min^{-1}$ (52.1, 60.0) and 56.1 $mL \cdot kg^{-1} \cdot min^{-1}$ (51.9, 60.2), respectively. Also $\dot{V}E_{peak}$ did not differ between the protocols, 158 (144, 173) vs.161 $L \cdot min^{-1}$ (145, 176) at 10.5% inclination, and 123 (114, 132) vs. 127 $L \cdot min^{-1}$ (116, 138) at 5.3% inclination, with the EIB protocol and the stepwise protocol, respectively.

Conclusion: $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ did not differ between the two test protocols, and one single standardised EIB-test may thus be used both for both provoking EIB and assessing $\dot{V}O_{2\ peak}$.

Key words: peak oxygen uptake; peak minute ventilation; exercise-induced bronchoconstriction, EIB protocol, stepwise exercise test

Introduction

Different test protocols are used to diagnose exercise-induced bronchoconstriction (EIB) and to assess aerobic capacity measured by peak oxygen uptake ($\dot{V}O_{2\ peak}$). In the assessment of EIB, exercise is used as a provocation factor, commonly by running on a treadmill for 6-8 minutes at a submaximal work load (1;2). It has recently been suggested that an exercise load corresponding to 95% of estimated maximum heart rate (HR_{max}) is preferable to increase the sensitivity of the test (3).

Maximum oxygen uptake ($\dot{V}O_{2\ max}$) or $\dot{V}O_{2\ peak}$ is widely recognized as one of the most important indices of aerobic capacity and physical fitness (4;5), usually assessed with treadmill running or cycling as ergometer (4). Previous studies comparing different test protocols for assessing $\dot{V}O_{2\ peak}$ in children and adults failed to detect differences in $\dot{V}O_{2\ peak}$ (6;7), but it is nevertheless a general consensus that a test protocol lasting between 8 and 12 minutes will elicit the highest $\dot{V}O_{2\ peak}$ with the lowest perception of difficulty and discomfort. Previously Day et al (8) showed that $\dot{V}O_{2\ peak}$ was similar with a constant load protocol lasting between 4 and 10 minutes and an incremental ramp protocol. Recently Rossiter et al (9) found that $\dot{V}O_{2\ peak}$ can be reached at a constant workload corresponding to 105% as well as 95% of maximum workload. Cooper (10) maintained that in children, testing with short bouts of high-intensity exercise is more physiologic than repeated stepwise exercise testing. However, it is important to allow for the impact of different diseases when choosing a test protocol. Asthmatic subjects may find the slow increase of speed and inclination boring, thus a more rapid protocol, suggested by Cooper (10) may be preferable.

Assessment of physical fitness may give important information about physical functioning in health and illness, and there is increasing interest for testing aerobic capacity in patients with chronic lung disorders, including asthma.

To obtain information about both EIB and $\dot{V}O_{2\ peak}$ with one single exercise test is useful both in clinical practice and in research. Up to now, the EIB-test and the $\dot{V}O_{2\ peak}$ measurement have been performed with two different test protocols on separate days. The possibility of combining assessment of EIB and $\dot{V}O_{2\ peak}$ into one exercise test reduces the burden for the patient and the costs for the health system. The objective of the present study was to assess if one single standard test protocol for assessing EIB also can be used to determine aerobic capacity measured by $\dot{V}O_{2\ peak}$ and peak minute ventilation ($\dot{V}E_{peak}$).

Material and methods

Design

The present study was an open randomized, cross-over design, comparing an EIB-test protocol and a common stepwise protocol for assessing $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$. Subjects were allocated consecutively to start with one or the other test protocol, according to a computer generated random order. The tests were performed three hours apart. All tests were undertaken in one laboratory by one test leader using the same equipment.

The study was performed according to the principles stated in the Declaration of Helsinki. All subjects signed a written consent form and in addition for subjects < 18 years, one of their parents gave the written consent. The Regional Medical Ethics committee approved the study.

Subjects

Forty healthy, non-smoking, physically active subjects (but not elite athletes) aged 14-40 years (23 males and 17 females) were included in the study. Twenty subjects were tested with the two test protocols at 10.5% (13 males and 7 females) and the remaining (10 males and 10 females) at 5.3% inclination of the treadmill. Ten subjects at each inclination were tested with the EIB protocol first and 10 subjects with the stepwise protocol first. All subjects were familiar with exercise testing on treadmill. The subjects were not allowed to exercise, drink alcohol or smoke cigarettes 24 hours prior to the tests, and no food or drink was allowed within 90 minutes of the tests. Exclusion criterion was EIB; a reduction in forced expiratory volume in one second (FEV_1) ≥10% from before to after exercise.

Exercise testing

Both tests were conducted in one laboratory at a temperature of 21.0^{o}C (±2 ^{o}C) and a relative humidity of 38.0% (± 4%), running on a manually controlled, motor driven treadmill (Woodway, USA). $\dot{V}O_{2\,peak}$ was measured with a Champion (Jaeger, Germany) analyser. The subjects wore a nose clip and breathed through a low resistance Hans Rudolph mouthpiece (2700 Series; Hans Rudolph Inc, USA). Gas samples of oxygen and carbon dioxide were collected from a mixing chamber with average values obtained over 30 seconds periods used for analysis. Heart rate (HR) was recorded electronically and registered every minute (Polar Sports tester PE 3000®, Polar Electro OY, Kempele, Finland).

The highest measured value of $\dot{V}O_2$, $\dot{V}E$, respiratory exchange ratio (RER) and HR from both protocols were used in the statistical analysis and expressed as peak values.

EIB-test protocol

Measurement of $\dot{V}O_{2\,peak}$ with the EIB-test protocol was performed by running for eight minutes. The inclination of the treadmill in the two groups was 10.5% and 5.3%, respectively. The starting running speed was approximately 70-80% of estimated HR_{peak} based on (220 beats·min^{-1} – age). The speed was subsequently adjusted during the first four minutes to achieve a work load corresponding to the maximum speed the subjects were able to sustain the last four minutes, about 95% of estimated HR_{max} (1-3). The work load of the EIB-test protocol has been validated by Carlsen et al (3). $\dot{V}O_2$, $\dot{V}E$, RER and HR were measured during the exercise test. Lung function was measured by maximal forced expiratory flow volume loops (Masterlab, Erich Jaeger®, Germany). FEV_1, forced vital capacity (FVC), and forced expiratory flow at 50% of FVC (FEF_{50}) were measured before and 1, 3, 6, 10 and 15 minutes after the treadmill run with the EIB protocol to ensure that the subjects did not suffer from EIB. All manoeuvres complied

with the general acceptability criteria of the ERS (11). Predicted lung function values, when used, were according to Quanjer et al (11) for subjects > 18 years and Zaplethal et al (12) for subjects ≤18 years.

Stepwise protocol

$\dot{V}O_{2\ peak}$ was measured with a stepwise protocol according to the procedure described and validated by Hermansen (13) and Åstrand et al (4). After warming up for 20 minutes at a workload corresponding to approximately 50-60% of $\dot{V}O_{2\ peak}$, the running speed was increased to a workload corresponding to approximately 80% of predicted $\dot{V}O_{2\ peak}$ (based on a Norwegian normal population (14). The running speed was further increased every minute with one km·hour^{-1} for the group running at 5.3% inclination and 0.7 km·hour^{-1} for the group running at 10.5% inclination, to a level bringing the subjects close to exhaustion after 4-6 minutes. The increase in running speed corresponds to an increase in $\dot{V}O_2$ of approximately 3-5 ml·kg^{-1}·min^{-1} at each step. $\dot{V}O_2$, $\dot{V}E$, RER and HR were measured during the test. Three criteria to identify $\dot{V}O_{2\ peak}$ were used, achievement of a plateau defined as less than 2 ml·kg^{-1}·min^{-1} rise in $\dot{V}O_2$ despite continued increase in workload, RER ≥ 1.05 and HR_{peak} ≥ 95% of estimated HR_{max} (220 beats·min^{-1} – age). Lung function was measured before, 6 and 10 minutes after exercise.

Statistical analysis

Results are given as mean values with 95% confidence intervals (CI) unless otherwise stated. Demographic data are given as mean values ± standard deviation (SD). The data was normally distributed, and differences between the two test protocols were analysed by standard t-tests for paired samples. Association between test

protocols was assessed by Pearson's correlation coefficient (r) with 95% CI. Limits of agreement for $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$ between the two test protocols were calculated according to Bland and Altman (15).

Based upon $\dot{V}O_{2\,peak}$ as main variable, with pre-existing knowledge of the variation of this variable and assuming a power of 80%, a sample size of 20 subjects at each inclination of the treadmill was calculated to obtain a significance level of 5%. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 11.0 and MedCalc version 8.1.1.0.

Results

Demographic data of the included subjects are given in table 1, stratified for exercising at an inclination of 5.3% and 10.5% respectively. None of the subjects had a significant reduction in lung function (FEV_1, FEF_{50} and FVC) after the EIB-test or the stepwise protocol either at 10.5% inclination or at 5.3% inclination of the treadmill.

10.5% inclination of the treadmill

No significant difference in mean $\dot{V}O_{2\ peak}$ was found between the EIB-test protocol and the stepwise protocol, 63.9 $ml \cdot kg^{-1} \cdot min^{-1}$ (60.0, 68.0) [mean (95% confidence intervals)] vs. 63.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (59.3, 67.4), respectively, or in mean $\dot{V}E_{peak}$, 158 $L \cdot min^{-1}$ (144, 173) vs.161 $L \cdot min^{-1}$ (145, 176), respectively (table 2). Both $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ measurements correlated highly between the protocols; r=0.98 (0.95, 0.99) and r=0.97 (0.94, 0.99), respectively (both p-values<0.0001). The Bland-Altman plots show the 95% limits of agreement for $\dot{V}O_{2\ peak}$ and for $\dot{V}E_{peak}$ between the two test protocols, 0.6 ±1.69 (arithmetic mean ± SD) and -2.53 ±6.84, respectively (fig.1).

Compared to the stepwise protocol, HR_{peak}, 186 $beats \cdot min^{-1}$ (183,189) vs. 190 $beats \cdot min^{-1}$ (188, 193), respectively (p<0.001) and peak running speed, 12.6 $km \cdot hour^{-1}$ (12.0, 13.2) vs. 13.4 $km \cdot hour^{-1}$ (12.7, 14.0), respectively (p<0.001), were significantly lower with the EIB protocol. Also RER_{peak} was significantly lower with the EIB protocol (p=0.001) (table 2).

As demonstrated in figure 3, $\dot{V}O_2$ did not differ significantly between the test protocols during the last two minutes. The running speed and HR were significantly higher with the stepwise compared to the EIB protocol during the last three minutes.

5.3% inclination of the treadmill

No significant difference was found between the EIB-test protocol and the stepwise protocol for mean $\dot{V}O_{2\ peak}$, 56.1 ml·kg^{-1}·min^{-1} (51.9, 60.2) vs. 56.0 ml·kg^{-1}·min^{-1} (52.1, 60.0), respectively or for mean $\dot{V}E_{peak}$, 123 L·min^{-1} (114, 132) vs. 127 L·min^{-1} (116, 138), respectively (table 2). Both $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ measurements correlated significantly between the test protocols; r=0.98 (0.95, 0.99) and r=0.94 (0.86, 0.98), respectively (both p-values<0.0001). The Bland-Altman plots show the 95% limits of agreement for $\dot{V}O_{2\ peak}$ and for $\dot{V}E_{peak}$ between the two test protocols, 0.03 ±1.75 (arithmetic mean ± SD) and -3.72±7.99, respectively (fig.2). Peak heart rate and peak running speed were significantly lower with the EIB protocol, 185 beats·min^{-1} (181, 190) vs. 189 beats·min^{-1} (185, 193), respectively and 12.6 km·hour^{-1} (11.9, 13.3) vs. 14.0 km·hour^{-1}(13.3, 14.8), respectively (both p-values <0.001) (table 2). Also RER_{peak} was significantly lower with the EIB protocol (p=0.01) (table 2).

As demonstrated in figure 3, $\dot{V}O_2$ was significantly increased with the EIB protocol after -3 and -2 minutes, but with no significant differences between the test protocols during the last two minutes. The running speed was significantly higher with the stepwise protocol during the last three minutes, and the HR was significantly higher with the stepwise protocol during the last two minutes.

Discussion

The present study demonstrated that $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$ were similar with the two test protocols, with no significant differences according to protocols either at 10.5% or at 5.3% inclination of the treadmill. Neither Day et al (8) found any difference in $\dot{V}O_{2\,peak}$ between an incremental cycle ergometer test and a constant speed test. Thus, the EIB protocol may be employed for both provoking EIB and assessing $\dot{V}O_{2\,peak}$ and $\dot{V}E_{peak}$ at the same time, thus giving information about both EIB and physical fitness.

Achievement of a plateau as described in the method section was fulfilled with the stepwise protocol. Although $\dot{V}O_2$ reached a plateau also with the EIB protocol, this could not be confirmed since the EIB-test protocol has constant speed the last 3-4 minutes. This is supported by the study of Rossiter et al (9). However, criteria were fulfilled for RER_{peak} and HR_{peak}.

In the present study aiming at comparing two methods (test protocols) we preferred to include healthy physical active subjects to exclude factors which might influence the results, such as bronchoconstriction occuring during exercise. The relatively heterogeneous group reflect the period of life extending from youth to adulthood, when human beings are physically active and spend much time on physical activity. We chose to compare the two different test protocols at two different fixed inclinations of the treadmill, 5.3% and 10.5% respectively, since these are commonly used for exercise testing among adolescents, untrained subjects and athletes (1;16).

Even though no significant differences in $\dot{V}O_{2\,peak}$ or $\dot{V}E_{peak}$ were found in the present study of healthy subjects, we cannot conclude that the results may apply all asthmatic

subjects. Anti-asthmatic treatment should be withheld before EIB-testing according to the guidelines for exercise challenge testing from European Respiratory Society and American Thoracic Society (1;2), and this might influence $\dot{V}O_{2\ peak}$ if bronchoconstriction occurs during the test. According to several previous studies on EIB, bronchoconstriction usually occurs immediately or soon after the exercise and not during the exercise (1-3;17;). $\dot{V}O_2$ measurements during an eight minutes exercise test may thus not be affected. If bronchoconstriction occurs during the exercise test, this might affect the test results as regards $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ and a new test for assessing $\dot{V}O_{2\ peak}$ with pre-medication may be performed. A similar study with subjects with EIB should optimally be performed to confirm the present results and recording of tidal breathing during the exercise test, may be a help in assessing possible occurrence of EIB during testing.

The two test protocols at both inclinations demonstrate different ways to reach $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$. The main difference is the increased running speed with the stepwise protocol, inducing an "overshoot" in relation to the speed required to reach $\dot{V}O_{2\ peak}$ compared to the EIB protocol, as shown in fig. 3.

Whereas $\dot{V}O_2$ increased to similar levels until reaching a plateau with both protocols, both HR_{peak}, RER_{peak} and peak running speed was significantly higher with the stepwise protocol indicating a higher level of exhaustion and a higher level of anaerobic energy consumption. As demonstrated in fig.3, $\dot{V}O_2$ was significantly higher with the EIB protocol until the last two minutes of the tests. This is not surprising as the stepwise protocol lasted totally 4-6 minutes after warming up, and there is a delay in the $\dot{V}O_2$ measurements in relation to the minute-by minute increase in running speed. The EIB-test has a constant exercise load the last four minutes, and thus $\dot{V}O_2$ measurements had

almost reached a plateau by the last 3-4 minutes of the test. Although the present study compared $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ with two different test protocols, neither of the protocols could be used for assessment of anaerobic threshold or sub-maximal exercise responses.

The final running phase lasted between four and six minutes in the stepwise protocol after 20 minutes warming up, whereas the EIB-test lasted totally for eight minutes without warming up. The initial workload was approximately 80% of estimated maximum workload for both protocols. However, the effect of the warming up was reduced to a minimum with the EIB protocol since the main target for provoking EIB is a fast increase in ventilation. In the present study, warming up in the stepwise protocol was not associated with higher $\dot{V}O_{2\ peak}$ measurements in healthy trained subjects as compared to the EIB protocol without warming up. This is supported by the study of Chwalbinska-Moneta and Hanninen and Busuttil and Ruhling (18;19). According to Mc Ardle et al (20) the beneficial effect of warming up upon $\dot{V}O_{2\ max}$ and exercise performance lacks justification by laboratory studies. On the other hand Ingjer and Strømme (21) and Inbar and Bar-Or (22) have described increased $\dot{V}O_{2\ max}$ after warming up.

Neither the running speed nor the HR differed during the last four minutes in the EIB protocol (fig. 3). The criterion of a constant speed during the last four minutes of the EIB-test protocol was fulfilled at both inclinations as also the criterion of a HR of 90-95% of estimated HR_{max} during the last four minutes (fig.3). In the present study all subjects were tested based on estimated HR_{max} (220 beats·min^{-1}-age) and the test leader`s evaluation of exhaustion. Mean estimated HR_{max} for the group running at 10.5% was 196 beats·min^{-1} and mean HR_{peak} measured with the EIB protocol was 186 beats·min^{-1}. For the group running at 5.3% inclination, the mean estimated HR_{max} was

194 beats·min^{-1} and mean HR_{peak} measured with the EIB protocol was 185 beats · min^{-1}. This was 94.8% and 95.3%, respectively of estimated HR_{max}. Thus, both the use of an experienced test leader and the use of estimated HR_{max}, are important during clinical exercise testing.

In conclusion $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ were comparable using the two test protocols both at 10.5% and at 5.3% inclinations of the treadmill. EIB-test may thus be employed for both provoking EIB and assessing $\dot{V}O_{2\ peak}$ and $\dot{V}E_{peak}$ at the same time, thus giving information about both EIB and physical fitness. In clinical practice this may save one test day and simplify diagnostic and monitoring procedures of the patients.

Acknowledgement

The authors thank Petter Mowinckel for good support and eminent statistical advice.

Table 1. Demographic data and baseline lung function (FEV_1 and FEF_{50}, % of predicted) of the 40 subjects included in the study before exercise tests at 5.3% and 10.5% inclination of the treadmill. Data are given as mean ± standard deviation with range in parenthesis.

Variables	5.3% inclination Mean± SD	(Range)	10.5% inclination Mean± SD	(Range)
Age (years)	26±11	(14-40)	24 ± 6	(17-40)
Male/female	10/10		13/7	
Bodyweight (kg)	65.2 ± 10.1	(48-85)	71.1±9.4	(53-93)
Height (cm)	173± 8.5	(159-192)	178±7.3	(164-188)
Baseline FEV_1 (% pred.)	112±13.9	(86-153)	110±12.8	(81-129)
Baseline FEF_{50} (% pred.)	97±26.9	(52-151)	99±23.6	(68-152)

Table 2. Peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak ventilation ($\dot{V}E_{peak}$), peak respiratory exchange ratio (RER_{peak}), peak heart rate (HR_{peak}) and peak running speed assessed with the EIB protocol and the stepwise (SW) protocol at 10.5% and 5.3% inclination of the treadmill.

	EIB protocol 10.5% Mean (95%CI)	SW- protocol 10.5% Mean (95%CI)	p	EIB protocol 5.3% Mean (95%CI)	SW- protocol 5.3% Mean (95%CI)	p
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	63.9 (60.0,68.0)	63.3 (59.3,67.4)	*ns*	56.1 (51.9,60.2)	56.0 (52.1,60.0)	*ns*
VE_{peak} ($L \cdot min^{-1}$)	158 (144,173)	161 (145,176)	*ns*	123 (114,132)	127 (116,138)	*ns*
RER_{peak}	1.13 (1.12,1.14)	1.17 (1.15,1.19)	*0.001*	1.09 (1.05,1.12)	1.13 (1.09,1.17)	*0.01*
HR_{peak} ($beats \cdot min^{-1}$)	186 (183,189)	190 (188,193)	*<0.001*	185 (181,190)	189 (185,193)	*<0.001*
Running speed ($km \cdot hour^{-1}$)	12.6 (12.0,13.2)	13.4 (12.7,14.0)	*<0.001*	12.6 (11.9,13.3)	14.0 (13.3,14.8)	*<0.001*

ns= not significant

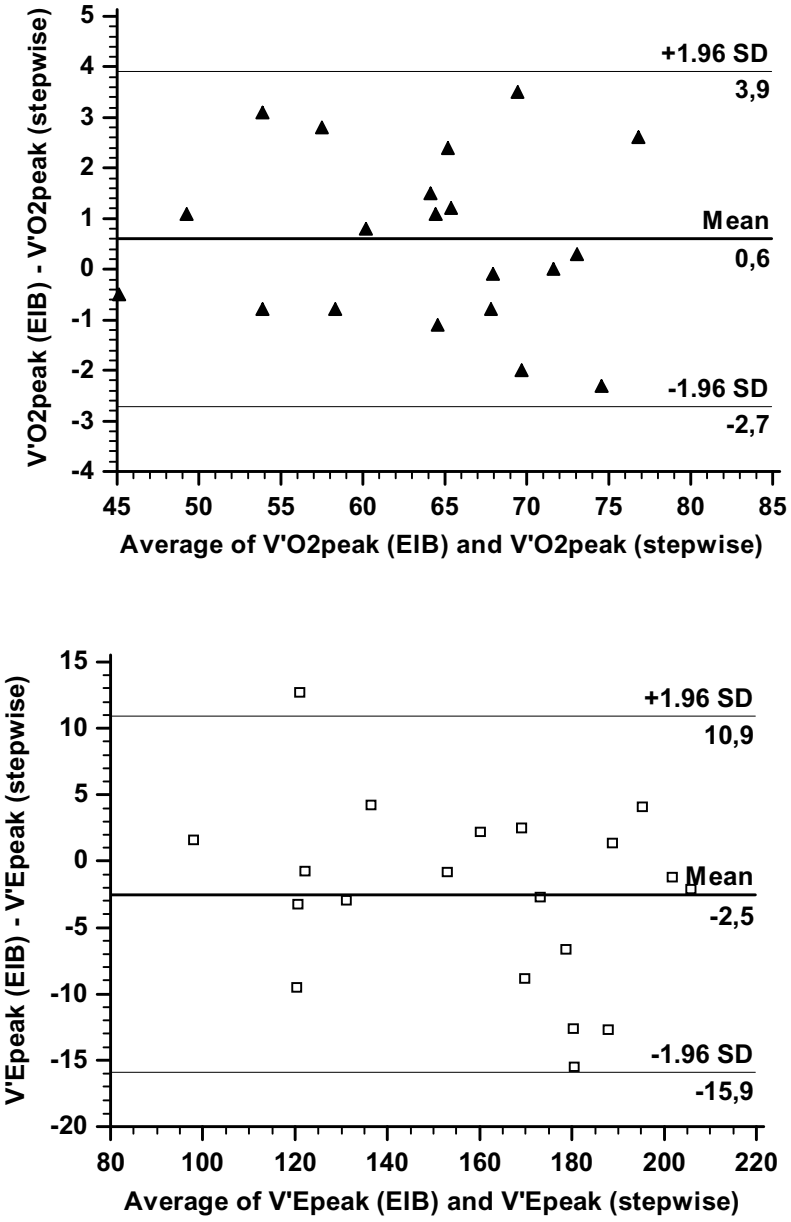

Figure1. Bland-Altman plot (95% limits of agreement) for peak oxygen uptake ($\dot{V}O_{2\ peak}$) (▲) and peak minute ventilation ($\dot{V}E_{peak}$) (□) assessed with the EIB protocol and the stepwise protocol at 10.5% inclination of the treadmill.

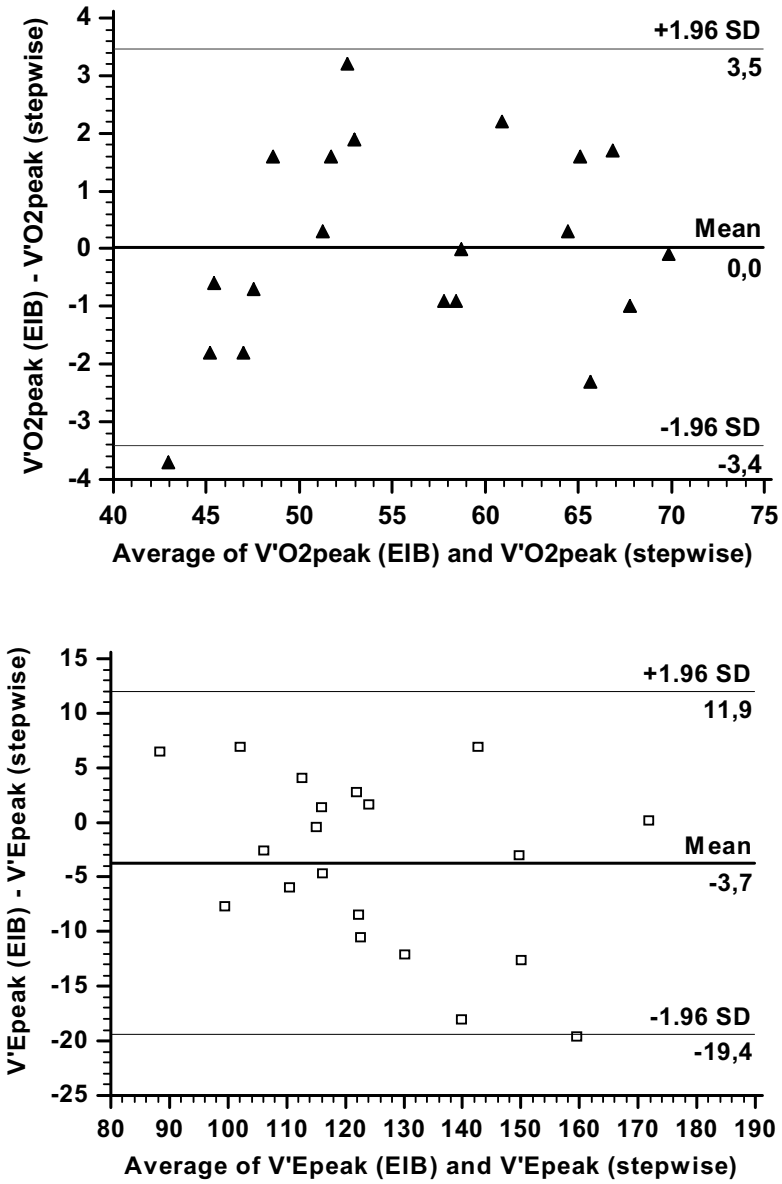

Figure 2. Bland-Altman plot (95% limits of agreement) for peak oxygen uptake ($\dot{V}O_{2\ peak}$) (▲) and peak minute ventilation ($\dot{V}E_{peak}$) (□) assessed with the EIB protocol and the stepwise protocol at 5.3% inclination of the treadmill.

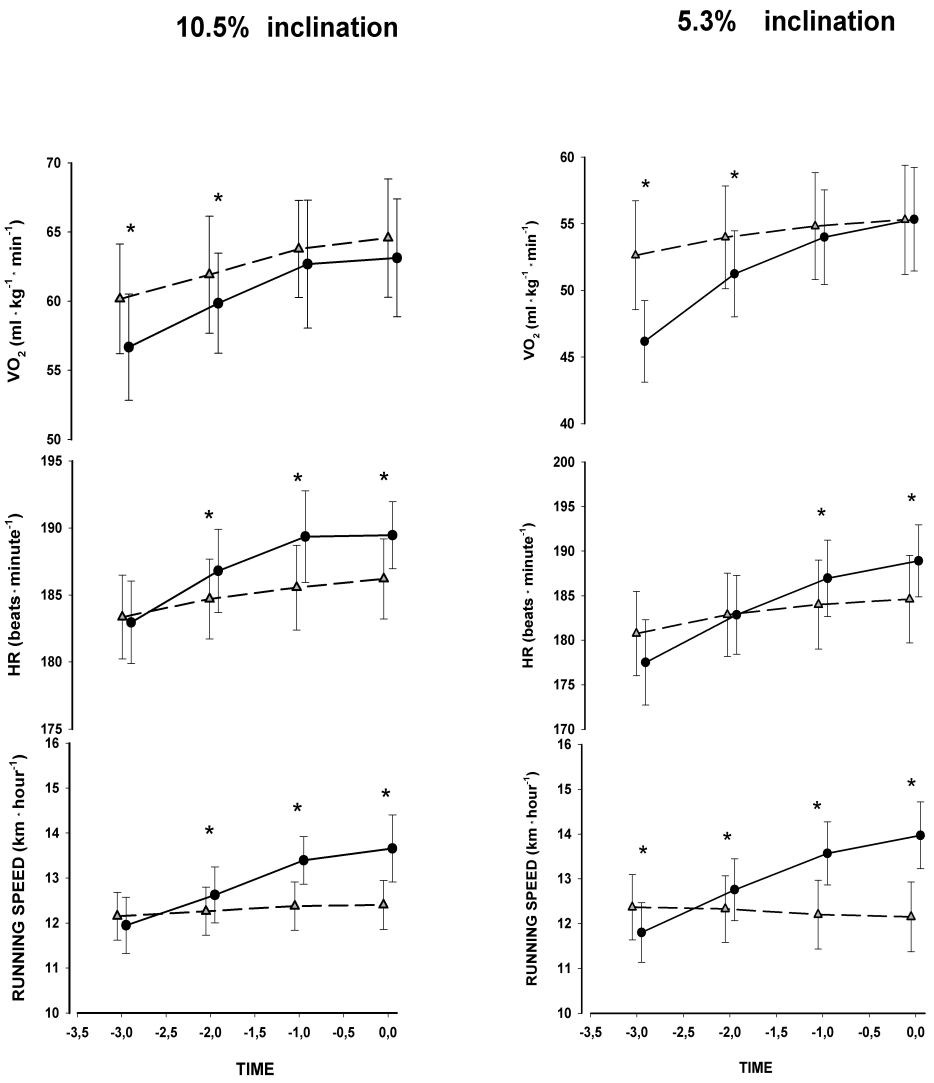

Figure 3. Oxygen uptake ($\dot{V}O_2$), heart rate (HR) and running speed assessed with the EIB protocol (▲) and the stepwise protocol (●) during the last four minutes of the exercise tests at 10.5% and 5.3% inclination of the treadmill. Results are given as mean with 95% confidence intervals. (* = significant difference).

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Original article

Asthma in medium altitude – exercise-induced bronchoconstriction in hypobaric environment in subjects with asthma

Background: Hypoxic gas inhalation has been reported to enhance airway responsiveness and results in bronchoconstriction in animal models and in humans with asthma. However, the data have so far been conflicting. The aim of the present study was to examine the effect of reduced barometric pressure on exercise-induced bronchoconstriction (EIB) in subjects with asthma.
Methods: Twenty subjects (10–45 years old, ♂/♀ = 13/7) with asthma (at least 10% reduction in forced expiratory volume in 1-second postexercise) participated in exercise testing in barometric pressure corresponding to altitudes of 200 (normobaric) and 2500 (hypobaric) m above sea level in random order on separate days. Lung function was measured before and after exercise, as well as after inhalation of salbutamol. Heart rate, oxygen uptake ($\dot{V}O_2$), arterial oxygen saturation (S_pO_2), respiratory gas exchange ratio (RER) and minute ventilation ($\dot{V}_E$) were measured during exercise.
Results: There was no difference in lung function after exercise. The $\dot{V}_{Epeak}$ and HR_{peak} during exercise did not differ. The RER_{peak} was higher ($P = 0.04$) in hypobaric environment. The $\dot{V}O_{2peak}$ decreased 10.1% (7.2–13.0) [mean (95% confidence intervals)] ($P < 0.001$) from normobaric to hypobaric environment. At the same time, S_pO_2 at $\dot{V}O_{2peak}$ decreased from 94.4 (92.2–96.6) to 85.6% (82.8–88.4) ($P < 0.001$).
Conclusions: A barometric pressure corresponding to altitude of 2500 m did not increase EIB in subjects with asthma. The reduction in $\dot{V}O_{2peak}$ is most probably due to the lower S_pO_2 in hypobaric environment.

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Key words: asthma; exercise-induced bronchoconstriction; hypobaric environment; lung function; oxygen saturation; oxygen uptake.

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Exercise-induced bronchoconstriction (EIB) has been reported to occur in 70–90% of asthmatics who have not received anti-inflammatory treatment (1, 2). The prevalence of EIB among children and adolescents in the general population has been reported to be 16–20% (3, 4). The EIB may limit participation in sports, modern lifestyle activities like skiing and activities of daily living.

Hypoxic gas inhalation enhanced airway responsiveness and resulted in bronchoconstriction in animal models (5, 6) and asthmatic subjects (7). However, the data on humans have so far been conflicting, and others have found a decrease (8) or no change (9, 10) in methacholine bronchial responsiveness from normal to medium or higher altitudes.

Standardized exercise tests are used to diagnose EIB in asthmatics with a history of shortness of breath during or after exertion. The EIB cannot be diagnosed by methacholine bronchial challenge, and not excluded by negative methacholine challenge (11). To our knowledge, only two groups (12, 13) have studied the effect of reduced barometric pressure on EIB. They found no additional increase in EIB. However, the exercise tests were not satisfactorily standardized (11, 14–17), and not all subjects had a reduction of ≥10% in forced expiratory volume in 1 s (FEV_1) after exercise. The main objective of the present study was to examine whether running in a hypobaric environment increases EIB in subjects with asthma, and secondarily to examine the effect of reduced barometric pressure on oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}_E$), heart rate (HR) and arterial oxygen saturation (S_pO_2) during running.

Materials and methods

Study design

The study had a randomized, crossover design to identify EIB in normobaric (ambient pressure corresponding to 200 m above sea level) and hypobaric environments (ambient pressure corresponding to 2500 m above sea level). On day one, all subjects underwent a standardized EIB test in normobaric environment to see if they satisfied the inclusion criteria (fall in $FEV_1 \geq 10\%$ postexercise). The subjects then participated in exercise testing in normobaric and hypobaric environments in random order on separate days. The study was performed in accordance with the Declaration of Helsinki (18).

Subjects

Twenty subjects with asthma, who where 10–45 years of age, were included in the study. Seventeen subjects were atopic. Exclusion criteria were any other disease, which might influence the results and respiratory tract infection during the last 3 weeks before inclusion in the study, and use of medication, which interfered with the results, according to standard criteria (19). Seven subjects used daily-inhaled steroids, 17 short-acting inhaled β_2-agonists on demand, 10 long-acting inhaled β_2-agonists, 1 theophylline, 4 regular antihistamines and 2 subjects used leukotriene antagonists. Nine subjects were without regular asthma medication.

Methods

Lung function was measured outside the pressure chamber by maximum forced expiratory flow–volume curves (Masterlab, Erich Jaeger® GmbHrzburg, Germany) (20) with predicted values (21, 22). Lung function [(FEV_1); forced vital capacity (FVC); and forced expiratory flow at 50% of FVC (FEF_{50})] was measured before, immediately after, 3, 6, 10 and 15 min after exercise, and 15 min after inhalation of salbutamol (5 mg/ml; 0.05 mg/kg).

The skin prick test was performed according to Nordic guidelines (23) with the following prevalent allergens: *Cladosporium herbarum*, *Dermatohagoideus pteronyssimus*, dog dander, cat dander, birch, timothy and mug worth pollen, cow's milk, shrimp and egg (Soluprick, ALK, Copenhagen, Denmark).

The EIB was determined by running on a treadmill ('Bodyguard' 2313, Cardionics AB, Sweden) for 6–8 min with sub-maximal workload. The inclination of the treadmill was 5.3% and the speed adjusted during running to achieve a workload of 90–95% of maximum (11). Douglas-bags were used for collecting gas samples of the expired gas every minute during the running (24). The subjects, wearing nose clips, breathed through a mouthpiece (2700 Series; Hans Rudolph Inc., Kansas City, KS), with expiratory gas samples of 30 s. The collected samples were analysed for oxygen and carbon dioxide content (Oxygen analyzer, model S-3A/1 and Carbon dioxide analyzer, model CD-3A; Ametek Inc., Pittsburgh, PA). The volume, temperature and pressure of the expired gas were measured at the time the air was analysed ('Ventilation measuring system', model S-430, KL-Engineering, Northridge, CA). The S_PO_2 was recorded during running with a pulse oximeter (Nellcor N-395, Nellcor Puritan Bennet Inc., Pleasanton, CA) with an RS-10 forehead sensor (Nellcor Puritan Bennet Inc.) taped to the skin above arcus superciliaris (25). The heart rate was recorded electronically and registered every minute (Polar Sports tester PE 3000®, Polar Electro OY, Kempele, Finland).

Running was performed in a conditioned pressure chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway) with temperatures between 19 and 23°C and relative humidity of approximately 40%. The barometric pressures during running were 98.7 (±1.1) and 75.5 (±0.2) kPa.

Statistical analysis

Demographics are given as mean values and standard deviation (SD) and results as means with 95% confidence intervals (CI). Differences between the two tests were assessed by Student's paired *t*-test. The bronchoconstrictor response following exercise was measured as the maximum per cent fall in FEV_1 after exercise and the area under the curve (AUC) per cent fall of the preexercise value in FEV_1/time, up to 15-min postexercise, using the trapezoid rule. Identical analysis was made for FEF_{50}. If FEV_1 or FEF_{50} was greater than baseline values, area was subtracted from the AUC measurements. Baseline FEV_1, FVC and FEF_{50} were measured on two consecutive days to calculate the coefficient of repeatability (26). The significance level was set to 5% using two-sided tests. Using preexisting knowledge of the main variable in previous studies monitoring EIB (27, 28), we found that 20 subjects were needed to obtain a power of at least 80% with a significance level of 5%.

Results

Demographics and baseline lung function are given in Table 1. Neither maximum reduction in FEV_1 and FEF_{50} (per cent of baseline) nor AUC was significantly different after running in hypobaric compared to normobaric environments (Fig. 1). No significant correlation was found between decrease in S_PO_2 during exercise in hypobaric environment and maximum drop in FEV_1. The response, 15 min after inhalation of salbutamol, was not significantly different between tests (Fig. 1), but FEV_1 and FEF_{50} increased significantly ($P < 0.001$) after inhalation of salbutamol compared to baseline values. Baseline FEV_1, FEF_{50} and FVC did not differ significantly on the two test days.

The $\dot{V}O_{2peak}$, S_PO_2 at $\dot{V}O_{2peak}$, HR_{peak}, highest recorded oxygen pulse, RER_{peak}, $\dot{V}_{Epeak}$ and highest recorded running speed during treadmill run in normobaric and hypobaric environments are shown in Table 2. The reduction in $\dot{V}O_{2peak}$ between normobaric and hypobaric conditions was 10.1% (7.2–13.0) ($P < 0.001$). Mean reduction in S_PO_2 at $\dot{V}O_{2peak}$ was 9.3% (7.0–11.6) ($P < 0.001$). The highest recorded oxygen pulse decreased 8.6% (5.9–11.3) ($P < 0.001$) in hypobaric environment (Table 2). On the other hand, the AUC was not different.

Discussion

The present study did not demonstrate an additional reduction in FEV_1 or FEF_{50} after running in hypobaric environment. The HR_{peak} and $\dot{V}_{Epeak}$ were not higher during running in hypobaric environment. The $\dot{V}O_{2peak}$ was 10.1% lower in hypobaric environment. Mean reduction in S_PO_2 at $\dot{V}O_{2peak}$ was 9.3% below running in the normobaric environment.

Table 1. Demographic data of the 20 subjects (♂/♀ = 7/13) included in the study

	Mean	SD	Range
Age (years)	24	10.5	10–45
Weight (kg)	67.0	11.1	37–112
Height (cm)	171.5	18.2	147–197
Body mass index (kg/m^2)	22.6	4.7	17.1–37.2
Baseline FEV_1 (l) (% predicted)	3.7 (101)	0.91 (14.9)	1.9–5.7 (79–126)
Baseline FEF_{50} (l) (% predicted)	3.7 (77)	1.40 (22.2)	1.6–6.8 (45–127)
Baseline FVC (l) (% predicted)	4.5 (106)	1.11 (11.7)	2.6–7.2 (84–134)

Data are given as mean, SD and range.

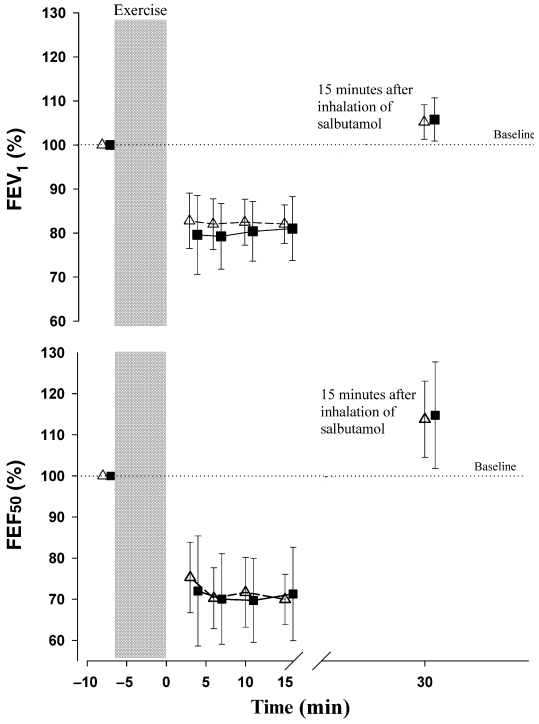

Figure 1. Lung function (FEV_1 and FEF_{50}% of baseline) before and after exercise in normobaric (Δ) and hypobaric (■) environments ($n = 20$). Results are shown as mean values with 95% confidence intervals.

Table 2. Peak oxygen uptake ($\dot{V}O_{2peak}$), S_PO_2 at $\dot{V}O_{2peak}$, peak heart rate (HR_{peak}), oxygen pulse at $\dot{V}O_{2peak}$ ($\dot{V}O_2/HR_{peak}$), peak respiratory exchange ratio (RER_{peak}), peak minute ventilation ($\dot{V}O_{Epeak}$) and peak running speed (V_{peak}) during treadmill run in normobaric and hypobaric environment ($n = 20$)

	Normobaric	Hypobaric	Significance
$\dot{V}O_{2peak}$ (ml/kg/min)	47.6 (44.3–50.8)	42.7 (39.7–45.8)	$P < 0.001$
S_PO_2 at $\dot{V}O_{2peak}$ (%)	94.4 (92.2–96.6)	85.6 (82.8–88.4)	$P < 0.001$
HR_{peak} (beats/min)	187 (182–192)	185 (180–190)	ns
$\dot{V}O_2/HR_{peak}$ (ml)	17.0 (14.9–19.2)	15.4 (13.6–17.3)	$P < 0.001$
RER_{peak}	1.03 (1.00–1.07)	1.06 (1.02–1.10)	$P = 0.04$
$\dot{V}_{Epeak}$ (l/min)	101 (89–114)	105 (92–119)	ns
V_{peak} (km/h)	10.5 (10–11)	9.5 (9.0–10.5)	$P < 0.001$

Values are given as mean with 95% confidence intervals in parentheses.
ns, not significant.

The major factors that determine severity of EIB are the pulmonary ventilation reached and sustained during exercise and water content and temperature of the inspired air. The stimulus by which exercise causes the airways to narrow is respiratory water and heat loss (29, 30). This is thought to stimulate the release of inflammatory mediators such as histamine and leukotrienes. The EIB in hypobaric environment may be due to the same mechanisms, even though some have speculated whether inhalation of hypoxic gas could enhance airway responsiveness (6) and result in bronchoconstriction (31). Exercise in medium or high altitudes is usually associated with increased ventilation due to hypoxic conditions (32). In addition, the temperature and water content of the air at these altitudes are substantially lower than at sea level. The present study was, however, conducted in a low-pressure chamber enabling control of air temperature and relative humidity. Except for the barometric pressure, the climatic conditions were equal. Reduced $\dot{V}O_{2peak}$ and running speed during exercise in hypobaric environment could partly explain why the subjects did not increase their $\dot{V}O_{Epeak}$. The reduction in $\dot{V}O_{2peak}$ is most probably due to the lower S_PO_2. Since the temperature and water content of the inspired air together with $\dot{V}_{Epeak}$ were identical for both conditions, airway cooling and water loss in the airways should be the same. Our findings are in agreement with results from other studies dealing with exercise challenges in hypobaric environment (12, 13). Louie and Paré (12) investigated the effect of various altitudes on EIB during a 2-week trek through the Himalayas (altitudes between 1500 and 3500 m above sea level) in 10 nonasthmatic and 4 asthmatic subjects. The EIB was not an inclusion criterion for the asthmatic subjects. Matsuda et al. (13) tested 20 children with asthma on a bicycle ergometer in a low-pressure chamber. Exercise was performed in 103.1 and 84.5 kPa. The authors in the op. cit. did not find any additional increase in bronchoconstriction in hypobaric environment. However, the number of subjects with EIB was far too low to reach any conclusion, and the exercise protocol was not acceptably standardized (11). Numerous studies have used running on a treadmill for 6–8 min near maximum load to assess EIB both in a clinical setting and in epidemiological studies (14–17).

In conclusion, running in a barometric pressure corresponding to an altitude of 2500 m did not increase EIB in subjects with asthma. The reduction in $\dot{V}O_{2peak}$ is most probably due to the lower S_PO_2 in the hypobaric environment. However, the low temperature and the subsequent reduced water content of the air in the mountainous area can influence both heat loss and dehydration of the airways. The present study suggests that subjects with EIB, like their healthy nonasthmatic peers, can participate in sport activities such as altitude climbing, skiing and tracking in medium high altitudes. The results should, however, be confirmed in a normal altitude setting outside the laboratory.

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Humidity influences exercise capacity in subjects with exercise-induced bronchoconstriction (EIB) ☆

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KEYWORDS
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Summary
Rationale: Exercise-induced bronchoconstriction (EIB) increases in cold and dry air and decreases in humid air in subjects with asthma. Few reports have reported on the effect of humid environment upon exercise capacity in subjects with EIB.
Objective: The primary aim of the present study was to examine the effect of changing the humidity of the environmental air upon exercise capacity measured by peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak ventilation ($\dot{V}E_{peak}$) and peak running speed ($\dot{V}_{peak}$) and secondarily to assess the influence of environmental humidity upon EIB in subjects suffering from EIB.
Methods: Twenty subjects (10–45 years old, male/female:13/7) with diagnosed EIB performed exercise testing under standardised, regular environmental conditions, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)], and under standardised humid environmental conditions; 19.9 °C (±1.0) and 95% (±1.7) relative humidity in random order on separate days. Lung function was measured before and 1, 3, 6, 10 and 15 min after exercise. Heart rate (HR), oxygen uptake ($\dot{V}O_2$), respiratory gas exchange ratio (RER), breathing frequency (BF) and minute ventilation ($\dot{V}E$) were measured during exercise.
Results: $\dot{V}O_{2\ peak}$ and $\dot{V}_{peak}$ increased significantly from 40% to 95% relative humidity of the environmental air, 4.5% and 5.9%, respectively ($P = 0.001$). HR_{peak} increased significantly in the humid environment, while BF_{peak} decreased significantly. RER_{peak} and $\dot{V}E_{peak}$ did not change significantly. Post-exercise reduction in FEV_1 (ΔFEV_1) and FEF_{50} (forced expiratory flow at 50% of FVC) (ΔFEF_{50}) significantly decreased after exercise in a humid environment as compared to regular conditions, ΔFEV_1: 12% (7,17) vs. 24% (19,29) [mean (95% confidence intervals)], respectively, ΔFEF_{50}: 20% (12,29) vs. 38% (30,46), respectively ($P<0.001$).

☆The study is performed within the ORAACLE (the Oslo Research group for Asthma and Allergy in Childhood; the Lung and Environment), which is member of the Ga²len, Network of Centers of Excellence.

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Conclusion: Exercise capacity ($\dot{V}O_{2\ peak}$ and $\dot{V}_{peak}$) markedly improved during exercise in humid air in subjects with EIB, whereas EIB was reduced to the half.

Introduction

Exercise-induced bronchoconstriction (EIB) is common in asthmatic children and adolescents and has been stated to occur in as much as 70–90% of untreated asthmatics.[1,2] As EIB influences daily life activities and sports activities in children and adolescents, an accurate assessment of EIB is important to enable optimal choice of treatment. EIB is best assessed by a standardised exercise test, commonly used is running on a treadmill for 6–8 min at a submaximal workload.[3–5] Lately it has been maintained that an exercise load corresponding to 95% of estimated maximum heart rate (HR_{max}) (220 beats min^{-1} - age) is preferable to obtain a high sensitivity of the test.[6–8]

EIB consists of bronchoconstriction occurring immediately or soon after physical exercise and is mainly thought to be caused by the increased ventilation during exercise. Two main hypotheses have been proposed to explain the relationship between exercise and EIB. Gilbert and McFadden[9] suggested that airway cooling due to respiratory heat loss with resulting rewarming by secondary hyperemia and pulmonary vasodilatation is the probable cause of EIB. Airway cooling also stimulates airways receptors, causing bronchoconstriction through a reflex pathway.

Anderson[10] suggested that respiratory water loss due to increased ventilation is the main stimulus to provoke EIB. The water loss causes increased osmolarity in the extracellular fluid in the respiratory mucous membrane, with a secondary influx of extracellular ions (Cl^- and Ca^{2+}) into the cells. Activation of adenylcyclase and phospholipase with new formation of mediators as well as release of preformed mediators from mast cells and other inflammatory cells in the airways are thought to cause bronchoconstriction.[10] Variation in environmental conditions as temperature and humidity of the inspired air influences the degree of bronchoconstriction after exercise. Inspiring cold and dry air during exercise leads to increased bronchoconstriction.[11–13] Warm, humid air has been reported to reduce EIB,[14–17] but, on the other hand, Zainudin et al.[18] found no significant relationship between different humidity levels (41–90% relative humidity) and EIB in Malaysian school children, 7–12 years of age.

The influence of a humid environment upon exercise capacity in asthmatic subjects has so far not been properly investigated. Kallings et al.[16] did not find any difference in $\dot{V}O_2$ or $\dot{V}E$ during exercise in six asthmatic subjects in humid climate as compared to dry, cold climate. Eschenbacher et al.[19] found that the workload in watts performed per L min^{-1} oxygen consumed was significantly higher under cold and dry conditions compared to hot and humid conditions in eight male asthmatic subjects.

However, it is not known if increased humidity of the environmental air, known to reduce EIB occurring mainly after exercise, also may influence $\dot{V}O_2$ or $\dot{V}E$ during running, or if there is a relationship between the magnitude of EIB and $\dot{V}O_2$ and $\dot{V}E$ during exercise.

Such knowledge is needed for giving optimal advice and treatment to asthmatic children and adolescents competing in different sports, especially endurance sports, and also as related to regular physical training of asthmatic children and adolescents.

The null hypothesis of the present study was that there is no difference in exercise capacity in subjects suffering from EIB exercising under regular, indoor conditions (20 °C and 40% relative humidity) as compared to exercising under humid conditions (20 °C and 95% relative humidity).

The primary aim of the present study was to investigate if conditioned, humid air, 20 °C and 95% relative humidity as compared to regular, indoor environmental conditions, 20 °C and 40% relative humidity influence exercise capacity measured by peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak ventilation ($\dot{V}E_{peak}$) and peak running speed ($\dot{V}_{peak}$) during exercise in subjects with EIB.

The secondary aim was to assess the influence of humidity upon EIB, and if there was any relationship between the changes in EIB to changes in exercise capacity.

Material and methods

Design

The present study was randomised, cross-over with one test for exercise-induced bronchoconstriction (EIB-test) in a standardised, regular indoor environment, 20.2 °C (±1.1) and 40% (±3.3) relative

humidity [mean (±SD)], and another test in a standardised humid environment 19.9 °C (±1.0) and 95% (±1.7) relative humidity on two different test days. Intervals of at least 48 h were required between each of the two tests. There were three study days in total. On day one, all subjects underwent an EIB-test to assess if they satisfied the inclusion criterion, a reduction in forced expiratory volume in 1 s (FEV_1) ≥ 10% from before to after exercise. If satisfying the inclusion criterion, the subjects were randomised consecutively to one of the two climate blocks according to random order generated by a computer programme. Eleven subjects were tested under regular indoor conditions first and nine subjects under humid conditions the first test day. The study could not be blinded because the subjects could immediately feel which climate they went into. The present study is part of a larger study also investigating the effect of changes in barometric pressure[20] and temperature.

The study was performed according to the principles stated in the Declaration of Helsinki. The Regional Medical Ethics committee approved the study.

Ambient conditions

On study days 2 and 3 the subjects performed exercise testing according to identical test protocols. The exercise tests were performed in a conditioned pressure chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway) with temperature 20.2 (±1.1) and relative humidity of 40% (±3.3) on one of the study days, and temperature 19.9 (±1.0) and relative humidity of 95% (±1.7) on the other study day. The barometric pressure during the exercise tests was 98.7 kPa (±1.1) or 740 mmHg (±8).

Subjects

Twenty subjects, 10–45 years of age, with documented EIB (≥10% decrease in FEV_1 after a standardised EIB-test) were included into the study. The EIB-test on the screening day was performed under standardised, regular indoor conditions. Exclusion criteria consisted of any other diseases or use of any regular medication which might influence test results and any respiratory tract infection during the last 3 weeks before study inclusion. Another exclusion criterion was if the FEV_1 baseline measurement varied more than 5% between the two test days.

Seventeen of the 20 subjects were atopic as defined by positive skin prick test (SPT). Seven subjects used regular inhaled steroids, and 10 subjects used regular daily long-acting inhaled β_2-agonists. Seventeen subjects used short-acting β_2-agonists on demand, one subject used oral theophylline, and two subjects used daily leukotriene antagonist. Four subjects used antihistamines, whereas nine subjects were without any regular asthma medication.

Five subjects participated in competitive sports, 14 participated in regular physical activity in school or leisure time, and one subject rarely or never participated in physical activity.

Methods

Lung function

Lung function was measured by maximally forced expiratory flow volume loops (Masterlab, Erich Jaeger®, Germany). FEV_1, forced vital capacity (FVC), and forced expiratory flow at 50% of FVC (FEF_{50}) were measured before exercise, 1, 3, 6, 10, 15 min after exercise and 15 min after inhaled salbutamol (5 mg mL^{-1}; 0.05 mg kg^{-1}). All lung function measurements were performed in a regular, indoor environment outside the climatic chamber.

All manoeuvres complied with the general acceptability criteria of The European Respiratory Society.[21] Predicted lung function values, when used, were according to Zapletal et al.[22]

EIB-test

EIB was determined by running on a motor-driven treadmill ("Bodyguard" 2313, Sweden) for 8 min at a submaximal workload (6,7). The inclination of the treadmill was 5.3%. The speed of the treadmill ($\dot{V}$) was adjusted during the first 4 min to achieve a workload corresponding to the maximum speed the subjects were able to keep the last 4 min, at about 95% of estimated maximum heart rate (220 beats min^{-1} - age). If the subjects indicated that higher speed was necessary to achieve exhaustion after 8 min, the running speed was adjusted also after 5 and 6 min. The estimated maximum heart rate is elaborated from epidemiological studies and it is a circumstantial estimation for individual subjects. The standard deviation for maximum heart rate during exercise has been reported to be ±10 beats min^{-1}.[23] Therefore, the exercise workload was standardised by a combination of 95% of estimated maximum heart rate and the test leader's evaluation of exhaustion after 8 min. Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), breathing frequency (BF) and respiratory exchange ratio (RER) were measured 5, 6 and

7 min after starting exercise test. The EIB-test protocol used in our study is different from a standard, incremental protocol to determine $\dot{V}O_{2\ peak}$. However, a previous study showed no difference in $\dot{V}O_{2\ peak}$ between the two test protocols.[24] Douglas bags were used for collecting gas samples of the expired gas.[25] The variations reported for the Douglas-bag method used with cycle ergometry are 2.3–2.5% for daily variations and 3.3–5.1% for between days variations.[26] The Douglas-bag system was chosen because of technical problems with the automatic equipment for measuring $\dot{V}O_2$ in the humid environment in the chamber. The automatic measurements were unstable and not reproducible.

The subjects, wearing a nose clip, breathed through a Hans Rudolph mouthpiece (2700 Series; Hans Rudolph Inc., USA). Expiratory gas samples were taken for at least 30 s and analysed for oxygen and carbon dioxide content (Oxygen analyzer model S-3A/1 and Carbon dioxide analyzer model CD-3A; Ametek Inc., USA). The volume, temperature and pressure of the expired gas were measured at the time the air was analysed ("Ventilation measuring system", model S-430, KL-Enginering, Northridge, California, USA). The heart rate was recorded electronically and registered every minute (Polar Sports tester PE 3000™, Polar Electro OY, Kempele, Finland).

Anti-asthmatic medication were withheld before the exercise tests. Inhaled short-acting β_2-agonists and sodium cromoglycate were withheld for 8 h prior to testing; inhaled long-acting β_2-agonists, theophylline and leukotriene antagonists for the last 72 h, anti-histamines for the last 7 days and orally administered glucocorticosteroids for the last month.[27]

Maximum percentage reduction in FEV_1 after exercise test was calculated by: (pre-exercise FEV_1 − minimum post-exercise FEV_1)/(pre-exercise FEV_1) × 100%. Minimum post-exercise FEV_1 was the lowest recorded value at 1, 3, 6, 10 or 15 min after exercise test. Similar calculations were performed for FEF_{50} and FVC. The highest recorded HR, $\dot{V}O_2$, $\dot{V}E$, BF and RER values during exercise test were determined as HR_{peak}, $\dot{V}O_{2\ peak}$, $\dot{V}E_{peak}$, BF_{peak} and RER_{peak}. Peak tidal volume ($V_{t\ peak}$) during exercise was calculated by $\dot{V}E_{peak}\,BF_{peak}^{-1}$.

Assuming that the inhaled air during exercise is fully saturated with vapour and reaches the temperature of 37 °C, the respiratory water loss during the last 3 min of exercise was calculated by using a web-based on-line calculator designed by the Department of Physics and Astronomy, Georgia State University, Atlanta, based on empirical fit for density data.[28]

Skin prick test

The SPT was performed according to the Nordic guidelines[29] with the following prevalent ambient allergens: moulds (Cladosporium herbarum), house dust mites (Dermatohagoideus pteronyssimus), dog dander, cat dander, birch pollen, grass pollen (timothy), mug worth pollen, milk, shrimp and hen's egg white (Soluprick, ALK, Copenhagen, Denmark). To be considered allergic to an allergen, a positive SPT of at least ++ (1/2 of the reaction to histamine 10 mg mL^{-1}) was required. The size was recorded by measuring (maximum+minimum diameter (mm)) × 2^{-1}.

Statistical analysis

Demographics are given as mean values and standard deviation (SD) and results as means with 95% confidence intervals (CI). Differences between the two tests were analysed by standard *t*-tests for paired samples when satisfying normal distribution. Correlation was calculated by Pearson's correlation coefficient. The bronchoconstrictor response following exercise was measured as the maximum per cent fall in FEV_1 and FEF_{50} from before to after exercise and the area under the curve (AUC) as per cent fall of the pre-exercise value in FEV_1 time^{-1}, up to 15-min post-exercise, using the trapezoid rule. Identical analysis was made for FEF_{50}. If FEV_1 or FEF_{50} increased from baseline after exercise, the corresponding area was subtracted from the AUC measurements. All tests were two-tailed with a significance level of 5%.

Based upon $\dot{V}O_{2\ peak}$ and FEV_1 as main variables, with pre-existing knowledge of the variation of these variables and assuming a power of 80%, a sample size of 20 subjects was calculated to obtain a significance level of 5%.[30]

Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 11.0.

Results

Demographic data and baseline lung function are given in Table 1. Baseline lung function (FEV_1, FEF_{50}, and FVC) did not differ significantly on the two test days.

Exercise capacity, $\dot{V}O_{2\ peak}$ and $\dot{V}_{peak}$, increased significantly, 4.5% and 5.9%, respectively, during exercise in humid air. $\dot{V}O_{2\ peak}$ from 46.5 ml kg^{-1} min^{-1} (43.9, 49.9) [mean (95% CI)] to 48.6 ml kg^{-1} min^{-1} (45.5, 52.5), respectively, and $\dot{V}_{peak}$ from 10.2 km h^{-1} (9.3, 10.7) to 10.8 km h^{-1} (10.0, 11.3), respectively ($P = 0.001$) (Table 2). HR_{peak} also significantly increased under humid

Table 1 Demographic data and baseline lung function (% of predicted) before exercise in standard, regular environment, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)] and in a standard humid environment, 19.9 °C (±1.0) and 95% (±1.7) relative humidity of the 20 subjects included in the study.

Variables	Mean±SD	(Range)
Age (years)	24±10.3	(10–45)
Gender ♀/♂	7/13	
Bodyweight (kg)	66.2±19.1	(34–111)
Height (cm)	171.1±11.0	(149–197)
Baseline FEV_1 (% predicted), 40% rel.hum.	100±13.6	(79–122)
Baseline FEV_1 (% predicted), 95% rel.hum.	100±15.7	(77–127)
Baseline FEF_{50} (% predicted), 40% rel.hum.	74±20.0	(45–111)
Baseline FEF_{50} (% predicted), 95% rel.hum.	77±22.4	(44–115)
Baseline FVC (% predicted), 40% rel.hum.	106±12.5	(84–137)
Baseline FVC (% predicted), 95% rel.hum.	105±14.2	(80–135)

Data are given as mean±standard deviation with range in parentheses.

Table 2 Peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak heart rate (HR_{peak}), peak respiratory exchange ratio (RER_{peak}), peak breathing frequency (BF_{peak}), peak minute ventilation ($\dot{V}E_{peak}$) and peak running speed ($\dot{V}_{peak}$) during exercise test under standardised, regular conditions, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)] and under standardised humid conditions, 19.9 °C (±1.0) and 95% (±1.7) relative humidity ($n = 20$).

Variables	40% relative humidity	95% relative humidity	Mean difference (95% CI)	Significance (P)
$\dot{V}O_{2\ peak}$ ($ml\,kg^{-1}\,min^{-1}$)	46.5	48.6	−2.13 (−3.30, −0.96)	0.001
HR_{peak} ($beats\,min^{-1}$)	186	189	−3.20 (−5.17, −1.23)	0.003
RER_{peak}	1.03	1.00	0.03 (−0.01, 0.07)	ns
BF_{peak}($breath\,min^{-1}$)	46	43	2.22 (1.11, 3.33)	<0.001
$\dot{V}E_{peak}$ ($L\,min^{-1}$)	99	100	−1.00 (−5.11, 3.11)	ns
$V_{t\ peak}$ ($L\,breath^{-1}$)	2.24	2.34	−0.10 (−0.18, −0.031)	0.008
$\dot{V}_{peak}$ ($km\,h^{-1}$)	10.2	10.8	−0.66 (−1.01, −0.31)	0.001

Values are given as mean and mean difference between the groups with 95% confidence intervals in parentheses.
ns = not significant.

conditions ($P = 0.003$), while BF_{peak} significantly decreased ($P<0.001$) (Table 2). There were no significant differences in mean $\dot{V}E_{peak}$ and RER_{peak} during exercise between the two climatic conditions (Table 2).

The increase in $\dot{V}O_2$ from 5 to 7 min differed significantly between the two test climates, 2.8 $ml\,kg^{-1}\,min^{-1}$ (1.9, 3.6) under regular conditions vs. 4.4 (3.5, 5.3) under humid conditions, respectively ($P = 0.001$). Also the increase in running speed differed significantly from 5 to 7 min between regular and humid conditions ($P<0.001$). No significant differences were found in the increase of $\dot{V}E$, HR or BF from 5 to 7 min between the two climates.

Maximum reduction in FEV_1, FEF_{50}, FVC and AUC changed significantly after exercise in the humid environment as compared to regular, indoor conditions ($P \leqslant 0.002$). Maximum reduction in FEV_1 as per cent of baseline lung function after exercise in humid environment was half of the reduction in FEV_1 after exercise under regular conditions, 12% (7,17) vs. 24% (19,29), respectively ($P = 0.0007$) (Table 3).

Maximum reduction in FEF_{50} as per cent of baseline lung function was also almost reduced to the half after exercise in humid environment, 20% (12,29) compared to exercise under regular conditions, 38% (30,46) ($P = 0.0004$) (Table 3). AUC for FEV_1 decreased after exercise in humid environment, 103.3 (163.9, 42.8) vs. exercise under regular conditions, 249.5 (316.9, 182.2), respectively ($P = 0.001$).

Calculated respiratory water loss during the last 3 min of exercise under regular indoor conditions was 10.4 g (9.3, 11.5) vs. 7.8 g (6.8, 8.8) in humid environment, respectively ($P<0.001$).

No significant correlation was found between reduction in lung function after exercise and water loss during the last 3 min of exercise. Neither was

Table 3 Difference (Δ) in maximum reduction in FEV_1, FEF_{50} and FVC (% of baseline) after exercise test under standardised, regular conditions, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)] and under standardised humid conditions, 19.9 °C (±1.0) and 95% (±1.7) relative humidity ($n = 20$).

Variables	40% rel. humidity	95% rel. humidity	Significance (P)
ΔFEV_1 (%)	24 (19,29)	12 (7,17)	0.0007
ΔFEF_{50} (%)	38 (30,46)	20 (12,29)	0.0004
ΔFVC (%)	15 (11,19)	9 (5,12)	0.002

Values are given as mean with 95% confidence intervals in parentheses.

there any significant correlation between maximum reduction in lung function after exercise (measured by FEV_1, FEF_{50}) or AUC or water loss during exercise and increased $\dot{V}O_{2\ peak}$ in the humid environment.

Discussion

The present study demonstrated that exercise capacity measured by $\dot{V}O_{2\ peak}$, $\dot{V}_{peak}$ and HR_{peak} increased significantly during exercise under humid environmental conditions compared to regular indoor conditions. BF_{peak} was decreased in humid climate, whereas $\dot{V}E_{peak}$ and RER_{peak} did not differ (Table 2).

The reduction in FEV_1 after exercise in humid environment was reduced to the half compared to after exercise under standard, regular conditions. Similar findings were made for reduction in FEF_{50}. However, even under humid climatic conditions there was still a significant EIB compared to the baseline lung function. Mean FEF_{50} at baseline was only 74% and 77% of predicted (Table 1), and this demonstrates the presence of peripheral airway obstruction in this group of asthmatics. Only seven out of 20 subjects used anti-inflammatory treatment (inhaled steroids).[31] The relatively large age range of the subjects in the present study reflects the period of life extending from school-age to adulthood, where human beings are physically active and spending time to physical activity.

The standardisation of the exercise load was based upon the screening test of the individual subjects aiming a submaximal to maximal exercise load as assessed by HR. The speed of the treadmill thus becomes a measure of performance during the two different climatic conditions.

Kallings et al.[16] reported on $\dot{V}O_2$ measurement in subjects with asthma during exercise in a humid environment compared to a dry, cold environment. They did not find any differences in HR, $\dot{V}O_2$, $\dot{V}E$, RER, CO_2 elimination or subjective ratings of perceived exertion and breathlessness between the two climates. Also Eschenbacher et al.[19] investigated the effect of changing temperature and humidity in an environmental chamber upon lung function and work capacity in eight healthy and eight asthmatic subjects. The workload in their study was adjusted for the subsequent environmental exposure in order to keep $\dot{V}E$ similar for each subject on the different test days. They did not find any difference in $\dot{V}O_2$ or HR at submaximal workloads. However, only six and eight subjects, respectively, were included in their studies, and their results can only be used for generation of hypotheses for further investigations. The workload, ventilation and the oxygen demand were probably too low to discover any difference in $\dot{V}O_2$. In the present study, the differences in $\dot{V}O_2$, $\dot{V}$, HR and BF first occur when the subjects were close to their maximal aerobic capacity (Table 2 and Fig. 1).

$\dot{V}O_2$ did not differ significantly between the two climatic conditions after 5 min exercise, but $\dot{V}O_2$ increased significantly more from 5 to 7 min in the humid environment compared to regular environment (Fig. 1). A similar pattern is shown for the running speed. These findings support that there is no significant difference in $\dot{V}O_2$ at submaximal workloads, but that the humid environment improves $\dot{V}O_2$ especially during maximum aerobic performance.

No correlation was found between maximum reduction in lung function after exercise or water loss during exercise and the increase in $\dot{V}O_{2\ peak}$ in the humid compared to the standard, indoor environment. Although a significant reduction in FEV_1 from baseline to 1 min after exercise was found in the regular environment but not in the humid environment, no correlation was found to the increased $\dot{V}O_{2\ peak}$ in the humid environment. FEF_{50} did not change from baseline to 1 min after exercise in any of the environmental conditions (Fig. 2). Many previous reports have concluded that bronchoconstriction occurs after exercise,[3,6,32,33] and thus it should not be expected that $\dot{V}O_{2\ peak}$ is influenced by bronchoconstriction during exercise. Nevertheless, the understanding of the present

study might have been improved if tidal breathing loops had been recorded during exercise.

The breathing pattern seems to be different during exercise in the humid as compared to the regular indoor environment. BF_{peak} was reduced during exercise in humid environment with no difference in $\dot{V}E_{peak}$. Peak tidal volume ($V_{t\ peak}$) increased significantly in the humid environment (Table 2). Consequently the subjects had a slower and deeper breathing pattern in the humid environment. All except two subjects reported spontaneously that breathing during exercise in the humid environment was much easier as compared to the regular indoor conditions. This is in agreement with the fact that the subjects ran faster with increased

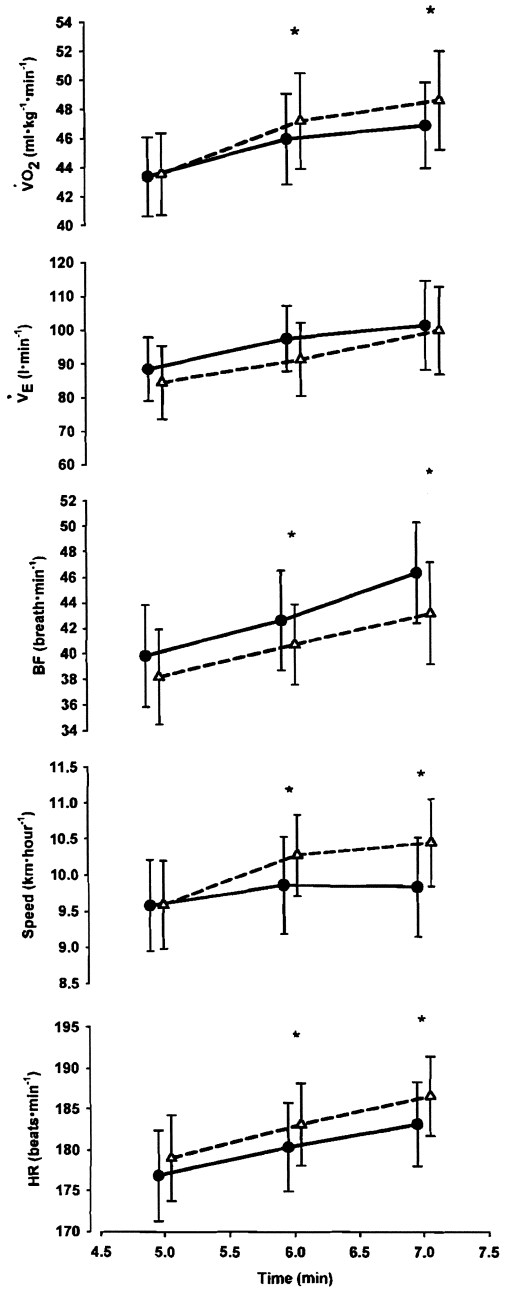

Figure 1 Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), breathing frequency (BF), running speed ($\dot{V}$) and heart rate (HR) after 5,6 and 7 min exercise test under standard, regular conditions, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)] (●) and under standard humid conditions, 19.9 °C (±1.0) and 95% (±1.7) relative humidity (Δ) ($n = 19$). Results are given as mean with 95% confidence intervals (*statistical significance).

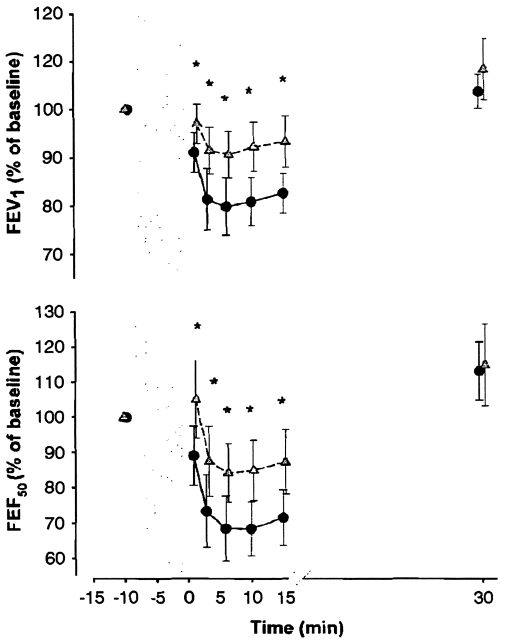

Figure 2 Lung function (FEV_1 and FEF_{50}) before, 1, 3, 6, 10 and 15 min after exercise and 15 min after inhaled salbutamol under standard, regular conditions, 20.2 °C (±1.1) and 40% (±3.3) relative humidity [mean (±SD)] (●) and under standard humid conditions (19.9 °C (±1.0) and 95% (±1.7) relative humidity) (Δ) ($n = 20$). Results are given as mean with 95% confidence intervals (*statistical significance).

$\dot{V}_{peak}$, HR_{peak} and $\dot{V}O_{2\ peak}$ with less effort (no change in RER_{peak} and $\dot{V}E_{peak}$) in the humid environment. The mechanism of increased $\dot{V}O_{2\ peak}$ in the humid environment is unknown, but we observed in the present study a different breathing pattern when the subjects were close to maximum aerobic capacity.

Humid environmental conditions thus seem to have a protective effect on EIB. The respiratory water loss was significantly decreased in humid environment compared to regular, indoor conditions, but there was still a significant loss of water from the airways. Air of 37 °C fully saturated with vapour contains 44 g H_2O/m^3. Air of temperature 20 °C with 40% relative humidity contains 6.9 g H_2O/m^3 and air of 20 °C and 95% relative humidity contains16.4 g H_2O/m^3.[28] With increasing ventilation rates during exercise, the water loss increases. The reduced loss of water from the airways is probably the main reason of the protective effect on EIB in a humid environment.[14–17]

Bar-Or et al.[15] suggested that EIB is more likely in dry air (25% relative humidity and about 25–26 °C) than in humid air (90% relative humidity and 25–26 °C), possibly due to heat loss at the airway mucosa caused by evaporation. Also Boulet and Turcotte[17] reported that EIB was influenced by the changes in water content during and after exercise. In their study, 12 mild asthmatics performed a 6 min steady state exercise at 80% of maximum workload in four different environmental conditions. They repeated that bronchoconstriction following exercise was minimal if exercise was performed in humid air with the recovery periode in dry air, and maximal if the exercise was performed in dry air with recovery in humid air.[17] The recovery period in our study took place in standard environmental conditions and according to Boulet and Turcotte[17] the best recovery environment to protect against EIB. Kallings et al.[16] concluded that cold, dry air provoked more bronchoconstriction than room-tempered humid air (60% relative humidity). Their study also supports our findings, although they used PEF measurements only as lung function variable, and their exercise test differed and consisted of only 3 min cycling at an intensity of 40% of maximal capacity followed by 6 min cycling at 80–85% of maximal capacity.[16]

On the other hand, Zainudin et al.[18] reported no significant relationship between different humidity levels, (41–90% relative humidity) and EIB (defined as reduction in $FEV_1 \geqslant 15\%$) among Malaysian school children. Their humidity levels were naturally occurring and not standardised. Their study was performed as a cross-sectional study with a main objective to determine the prevalence of EIB in a population of school children living in a humid, tropical climate in the inner city of Kuala Lumpur.

The test procedure, the use of drugs before testing and ambient conditions were precisely standardised in the present study. The ambient conditions were similar during the two test days except for the relative humidity. Several of the earlier reports included fewer subjects, and neither the exercise workload nor the ambient conditions were standardised.

In conclusion, exercising in a humid environment improves exercise capacity as measured by $\dot{V}O_{2\ peak}$ and $\dot{V}_{peak}$, and protects against EIB in subjects suffering from EIB.

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Exercise capacity and exercise-induced bronchoconstriction (EIB) in a cold environment ☆

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KEYWORDS
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Summary
Introduction: Exercise in a cold environment has been reported to increase exercise-induced bronchoconstriction (EIB). However, the effect of a cold environment upon exercise capacity in subjects with EIB has, to our knowledge, not been previously reported.
Purpose: Primary: To examine the influence of changing environmental temperature upon exercise capacity measured by peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak ventilation ($\dot{V}E_{peak}$) and peak running speed in subjects with diagnosed EIB.
Secondary: To assess the influence of changing environmental temperature upon EIB.
Methods: Twenty subjects (10–45 years old, male/female: 13/7) with EIB underwent exercise testing by running on a treadmill in a climate chamber under standardised, regular conditions, 20.2 °C (±1.1) and 40.0% (±3.3) relative humidity [mean(±SD)], and in a standardised cold environment, −18.0 °C (±1.4) and 39.2% (±3.8) relative humidity in random order on separate days. Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER), heart rate (HR) and running speed were measured during exercise.
Lung function (flow volume loops) was measured before and 1, 3, 6, 10 and 15 min after exercise and 15 min after inhalation of salbutamol.
Results: $\dot{V}O_{2\ peak}$ decreased 6.5%, from 47.9 (45.0, 50.8) to 44.8 ml kg^{-1} min^{-1} (41.2, 48.4) [mean (95% confidence intervals)] ($p = 0.004$) in the cold environment. Also running speed was significantly lower in the cold environment ($p = 0.02$). No differences were found for $\dot{V}E_{peak}$, RER_{peak} or $HR_{peak.}$ The post-exercise reduction in forced expiratory volume in 1 s (FEV_1) (ΔFEV_1) increased significantly from 24% (19,29) to 31% (24,38), respectively ($p = 0.04$) after exercise in the cold environment. No correlation was found between reduction in $\dot{V}O_{2\ peak}$ and the increased maximum fall in FEV_1 in the cold environment.

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Conclusion: Exercise capacity ($\dot{V}O_{2\ peak}$ and peak running speed) was markedly reduced during exercise in a cold environment whereas EIB increased in subjects suffering from EIB.

Introduction

Inspiring cold, dry air during exercise is reported to increase exercise-induced bronchoconstriction (EIB) in asthmatic subjects compared with regular, indoor environment and humid environment.[1–3]

Most of the previous reports concern the effect of inspiring cold air through a mouthpiece, while the subjects are exposed to regular, laboratory environmental temperature. Only very few studies have investigated the effect of the whole body exposure to cold air upon exercise capacity and/or lung function in asthmatic subjects.[4–8]

As far as we know, only three studies have investigated the influence of cold air upon oxygen uptake ($\dot{V}O_2$) in asthmatic subjects[6–8] and only one of them has reported on maximum oxygen uptake ($\dot{V}O_{2\ max}$).[6] Kallings et al.[7] did not find any differences in $\dot{V}O_2$ or other physiological parameters in asthmatic subjects during exercise under room tempered conditions when inhaling cold, dry air as compared with inhaling warm, humid air. Also Sandsund et al.[6] concluded with no differences in $\dot{V}O_2$ at submaximal workloads, in $\dot{V}O_{2\ max}$ or in lung function in seven mild asthmatic subjects between inhaling cold air and warm air in a cold environment during exercise. Eschenbacher et al.[8] found that the workload in watts performed per $L\,min^{-1}$ of oxygen consumed was significantly greater during the cold and dry conditions than during hot and humid conditions in eight male asthmatic subjects.

The effect of cold air on physiological parameters in healthy subjects is reported to vary depending on different factors such as type, intensity and duration of exercise, amount of fatty tissue, wind, ambient temperature, clothing, fluctuations in body temperature and energy reserves.[9] Quirion et al.[9] found significantly decreased $\dot{V}O_{2\ max}$, maximum workload and time to exhaustion, whereas minute ventilation ($\dot{V}E$) did not change during a short exhaustive exercise at −20 and 0 °C as compared with 20 °C in eight healthy males. Sandsund et al.[10] reported increased $\dot{V}E$ and $\dot{V}O_2$ at submaximal workloads in an environment of −15 °C as compared with 23 °C whereas no difference was found for $\dot{V}O_{2\ max}$. They suggested that exercise stress increased in a cold environment, probably as a response to increased metabolic demand. Their findings in healthy subjects are supported by Claremont et al.[11]

As EIB influences daily life activities and sports activities in children and adolescents, an accurate assessment of EIB is important to enable optimal choice of treatment. EIB is best assessed by a standardised exercise test, commonly used is running on a treadmill for 6–8 min at a submaximal work load.[12,13] Lately it has been maintained that an exercise load corresponding to 95% of maximum heart rate (HR_{max}) is preferable to obtain a high sensitivity.[14] EIB consists of bronchoconstriction occurring immediately or soon after physical exercise triggered by increased ventilation during exercise.[12,14–16] Two main hypotheses have been proposed to explain the relationship between exercise and EIB. Gilbert and McFadden[17] suggested that airway cooling is probably the cause of EIB. Anderson[18] suggested that respiratory water loss due to increased ventilation is the main stimulus to provoke EIB.

Although it has been generally accepted that cold air inhalation increases EIB, this has recently been challenged by Evans et al.[19] They concluded that cold air inhalation had no additive effect upon the severity of EIB after exercise or decrease in lung function after eucapnic voluntary hyperventilation.

However, it is not known if cold environment may influence exercise capacity or if there is a relationship between the magnitude of EIB and exercise capacity in subjects with EIB. Such knowledge is needed for giving optimal advice and treatment to asthmatic children and adolescents competing in different sports, especially endurance winter sports. It is also needed in relationship to regular physical training of asthmatic children and adolescents especially in the Scandinavian countries and in other countries with temperature to subartic climate where the winter season can be quite cold.

The aims of the present study were primarily to assess any possible change in exercise capacity measured by peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak ventilation ($\dot{V}E_{peak}$) and peak running speed during exercise in a cold environment as compared to regular indoor environmental conditions and secondarily to assess the influence of cold environment upon EIB in subjects with diagnosed EIB.

Material and methods

Design

The present study has an open randomised, cross-over design with one exercise test performed under standard, regular indoor conditions, temperature of 20 °C and 40.0% relative humidity, and another test in a standardised cold environment, −18 °C and 40% relative humidity on two different days. An interval of at least 48 h was required between the two tests. There were three study days in total. On day one, all subjects underwent an EIB-test to assess if they satisfied the inclusion criterion, reduction in forced expiratory volume in 1 s (FEV_1) $\geqslant$ 10% from before to after exercise. If satisfying inclusion criterion the subjects were randomised consecutively to one of the two climate blocks in random order generated by a computer programme. The study could not be blinded because the subjects could immediately feel which climate they went into. The present study was part of a larger study aiming to assess the effect of different environments, altitude[20] and humidity[21] upon exercise capacity and upon EIB in subjects suffering from EIB.

The study was performed according to the principles stated in the Declaration of Helsinki. The Regional Medical Ethics committee approved the study and all subjects signed an informed written consent before inclusion.

Ambient conditions

On study days 2 and 3, the subjects performed exercise testing according to identical test procedures. The exercise tests were performed in a conditioned climate chamber (Norwegian Sub diving Techniques A/S, Haugesund, Norway) with relative humidity of 40.0% ($\pm$3.3) and temperature 20.2 °C ($\pm$1.1) [mean($\pm$SD)] on one of the study days and −18 °C ($\pm$1.4) and relative humidity of 39.2% ($\pm$3.8) on the other study day. The barometric pressure during the exercise tests were 98.7 kPa ($\pm$1.1) or 740 mmHg ($\pm$8).

Subjects

Twenty subjects between 10 and 45 years of age with diagnosed EIB were included in the study. EIB was defined by a reduction in FEV_1 of 10% or more from before to after a standardised EIB-test performed under standard, regular conditions. Exclusion criteria consisted of any other diseases or use of any regular medication that might influence test results and any respiratory tract infection during the last 3 weeks before study inclusion. The subjects were also excluded if the baseline FEV_1 measurement varied more than 5% between the two test days.

Antiasthmatic medication was withheld according to ERS guidelines. Inhaled short-acting β_2-agonists and sodium cromoglycate were withheld for 8 h prior to testing, inhaled long-acting β_2-agonists, theophylline and leukotriene antagonists for the last 72 h, anti-histaminic for the last 7 days and orally administered glucocorticosteroids for the last month.[12]

Seventeen of the 20 subjects were atopic as defined by positive skin prick test (SPT). Seven subjects used regular inhaled steroids and ten subjects used regular daily long-acting inhaled β_2-agonists. Seventeen subjects used short-acting β_2-agonists on demand, one subject used oral theophylline and two subjects used a leukotriene antagonist daily. Four subjects used antihistamines, whereas nine subjects were without any regular asthma medication. Five subjects participated in competitive sports, 14 participated in regular physical activity in school or leisure time, and one subject rarely or never participated in physical activity.

Lung function

Lung function was measured by maximally forced expiratory flow volume loops (Masterlab, Erich Jaeger®, Germany). FEV_1, forced vital capacity (FVC) and forced expiratory flow at 50% of FVC (FEF_{50}) were measured before exercise and 1, 3, 6,10 and 15 min after exercise and 15 min after inhaled salbutamol (5 mg mL^{-1}; 0.05 mg kg^{-1}). All lung function measurements were performed in a regular, indoor environment outside the climate chamber. All manoeuvres complied with the general acceptability criteria of The European Respiratory Society (ERS).[22] Predicted lung function values, when used, were according to Zapletal et al.[23] and Quanjer et al.[22]

Exercise test

EIB was determined by running on a motor-driven treadmill ("Bodyguard" 2313, Sweden) for 8 min at a submaximal work load.[12] The inclination of the treadmill was 5.3%. The running speed was adjusted during the first 4 min to achieve a work load corresponding to the maximum speed the subjects were able to maintain the last 4 min, about 95% of estimated HR_{max} (220 beats min^{-1}-age). If the subjects indicated that higher speed was necessary to achieve exhaustion after 8 min the running speed was also adjusted after 5 and 6 min. The estimated HR_{max} is elaborated from epidemiological studies, and it is a circumstantial estimation for individual subjects. The standard deviation for maximum heart rate during exercise is $\pm$10 beats min^{-1}. Therefore, the exercise workload was standardised by a combination of 95% of estimated HR_{max} and the test leader's evaluation of exhaustion after 8 min. $\dot{V}O_2$, $\dot{V}E$, breathing frequency (BF) and respiratory exchange ratio (RER) were measured 5, 6 and 7 min after starting exercise test. The EIB protocol used in our study is different from a standard, incremental protocol for assessing $\dot{V}O_{2\ peak}$, but has been evaluated in a previous study. A comparison of the EIB protocol and a stepwise protocol showed no difference in $\dot{V}O_{2\ peak}$ or $\dot{V}E_{peak}$.[24] Douglas bags were used for collecting gas samples of the expired gas.[25] The variations reported for the Douglas bag method used with cycle ergometry are 2.3–2.5% for daily variations and 3.3–5.1% for between days variations.[26] The Douglas bag system was chosen because the measurements with the automatic equipment were unstable and not reproducible in the cold environment.

The subjects, wearing a nose clip, breathed through a Hans Rudolph mouthpiece (2700 Series; Hans Rudolph Inc, USA). Expiratory gas samples were taken for at least 30 s and analysed for the oxygen and carbon dioxide content (Oxygen analyser model S-3A/1 and Carbon dioxide analyzer model CD-3A; Ametek Inc, USA). The volume, temperature and pressure of the expired gas were measured at the time the air was analysed ("Ventilation measuring system", model S-430, KL-Engineering, Northridge, California, USA). The heart rate (HR) was recorded electronically and registered every minute (Polar Sports tester PE 3000®, Polar Electro OY, Kempele, Finland).

Maximum percentage reduction in FEV_1 after exercise test was calculated by (pre-exercise FEV_1—minimum post-exercise FEV_1)/(pre-exercise FEV_1) $\times$ 100%. Minimum post-exercise FEV_1 was the lowest recorded value at 1, 3, 6, 10 or 15 min after exercise test. Similar calculations were performed for FEF_{50} and FVC. The highest recorded HR, $\dot{V}O_2$, $\dot{V}E$, BF, RER and running speed during exercise tests were determined as HR_{peak}, $\dot{V}O_{2\ peak}$, $\dot{V}E_{peak}$, BF_{peak} $RER_{\ peak}$ and peak running speed.

Assuming that the inhaled air during exercise is fully saturated with vapour and reaches the temperature of 37 °C, the respiratory water loss during the last 3 min of exercise was calculated by using a web-based online calculator designed by the Department of Physics and Astronomy Georgia State University Atlanta, based on

empirical fit for density data (http://hyperphysics.phyastr.gsu.edu/hbase/kinetic/relhum.html 2004).

Skin prick test

The skin prick test was performed according to the Nordic guidelines[27] with the following prevalent ambient allergens: moulds (Cladosporium herbarum), house dust mites (Dermatohagoideus pteronyssimus), dog dander, cat dander, birch pollen, grass pollen (timothy), mug worth pollen, milk, shrimp and egg (Soluprick, ALK, Copenhagen, Denmark). To be considered allergic to an allergen, a positive skin prick test of at least ++ (1/2 of the reaction to histamine 10 mg mL^{-1}) was required. The size was recorded by measuring (maximum+minimum diameter (mm)) $\times 2^{-1}$.

Statistical analysis

Demographics are given as mean values and standard deviation (SD) and results as means with 95% confidence intervals (CI). Differences between the two tests were analysed by Student's paired *t*-tests when satisfying normal distribution. Correlation was calculated by Pearson's correlation coefficient. The bronchoconstrictor response following exercise was measured as the maximum per cent fall in FEV_1 and FEF_{50} after exercise and the area under the curve (AUC) per cent fall of the pre-exercise value in $FEV_1 \cdot time^{-1}$, up to 15-min post-exercise, using the trapezoid rule. Identical analysis was made for FEF_{50}. If FEV_1 or FEF_{50} increased from baseline after exercise, the corresponding area was subtracted from the AUC measurements. All tests were two-tailed with a significance level of 5%.

Based upon FEV_1 and $\dot{V}O_{2\ peak}$ as main variables, with pre-existing knowledge of the variation of these variables and assuming a power of 80%, a sample size of 20 subjects was calculated as necessary to obtain a significance level of 5%.[28] Statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 11.0.

Table 1 Demographic data and baseline lungfunction (% of predicted) before exercise in a standardised regular environment, 20.2 °C (±1.1) and 40.0% (±3.3) relative humidity [mean(±SD)] and in a standardised cold environment, −18 °C (±1.4) and 39.2% (±3.8) relative humidity.

Variables	*Mean±SD (range)*
Age (years)	24±10.3 (10–45)
Gender ♀/♂	7/13
Bodyweight (kg)	66.2±19.1 (34–111)
Height (cm)	171.1±11.0 (149–197)
Baseline FEV_1 (% predicted), 20 °C	100±13.6 (79–122)
Baseline FEV_1 (% predicted, −18 °C	99±14.6 (75–122)
Baseline FEF_{50} (% predicted), 20 °C	74±20.0 (45–111)
Baseline FEF_{50} (% predicted), −18 °C	76±20.4 (45–119)
Baseline FVC (% predicted), 20 °C	106±12.5 (84–137)
Baseline FVC (% predicted), −18 °C	104±14.1 (78–133)

Data are given as mean±standard deviation and range in paranthesis ($n = 20$).

Results

Demographic data and baseline lung function are given in Table 1. Baseline lung function (FEV_1, FEF_{50} and FVC) did not differ significantly on the two test days. $\dot{V}O_{2\ peak}$ decreased significantly, 6.5%, from 47.9 ml kg^{-1} min^{-1} (45.0, 51.8) [mean (95% confidence intervals)] to 44.8 ml kg^{-1} min^{-1} (41.2, 48.4), respectively ($p = 0.004$) during exercise under regular conditions as compared with exercise in the cold environment (Table 2). Four subjects reduced $\dot{V}O_{2\ peak}$ more than 10%, nine subjects had a reduction between 5 and 10% and six subjects reduced $\dot{V}O_{2\ peak}$ less than 5% in the cold environment. One subject increased $\dot{V}O_{2\ peak}$ 5% in the cold environment. Peak running speed was also significantly lower in the cold environment: 10.2 km h^{-1} (9.5, 11.0) vs. 9.7 km h^{-1} (8.9, 10.5), respectively ($p = 0.02$) (Table 3). There were no differences in $\dot{V}E_{peak}$, RER_{peak}, HR_{peak} or BF_{peak} during exercise between the two climatic conditions (Table 2). $\dot{V}O_2$ was significantly reduced after 5, 6 and 7 min run in the cold environment ($p = 0.01$) (Fig. 1). The running speed was also significantly lower in the cold environment after 5 and 7 min ($p = 0.01$ and $p = 0.03$, respectively) (Fig. 1). No significant differences were found for $\dot{V}E$, RER,

Table 2 Peak oxygen uptake ($\dot{V}O_{2\ peak}$), peak heart rate (HR_{peak}) peak respiratory exchange ratio (RER_{peak}), peak breathing frequency (BF_{peak}), peak minute ventilation ($\dot{V}E_{peak}$) and peak running speed during exercise under standardised, regular conditions, 20.2 °C (±1.1) and 40.0% (±3.3) relative humidity [mean(±SD)] and under standardised cold conditions, −18 °C (±1.4) and 39.2%(±3.8) relative humidity ($n = 20$).

Variables	20 °C	−18 °C	Mean difference (95%CI)	*p*
$\dot{V}O_{2\ peak}$ (ml kg^{-1} min^{-1})	47.9	44.8	3.1 (1.2, 5.1)	0.004
HR_{peak} (beats min^{-1})	186	187	−1.5 (−4.3, 1.3)	ns
RER_{peak}	1.02	1.03	−0.006 (−0.04, 0.03)	ns
BF_{peak} (breath min^{-1})	46	47	−0.24 (−2.36, 1.89)	ns
$\dot{V}E_{peak}$ (L min^{-1})	99	95	3.4 (−8.4, 15.3)	ns
Peak running speed (km h^{-1})	10.2	9.7	0.5 (−0.1, −0.9)	0.02

ns = not significant.
Values are given as mean and mean difference between the groups with 95% confidence intervals in parentheses.

Table 3 Difference (Δ) in maximum reduction in FEV_1, FEF_{50} and FVC (% of baseline) and area under curves (AUC) for FEV_1 and FEF_{50} after exercise test in a standardised regular environment, 20.2 °C (±1.1) and 40.0% (±3.3) relative humidity [mean(±SD)] and in a standardised cold environment, −18 °C (±1.4) and 39.2%(±3.8) relative humidity ($n = 20$).

Variables	20 °C	−18 °C	*p*
ΔFEV_1 (%)	24 (19,29)	31 (24,38)	0.04
ΔFEF_{50} (%)	38 (30,46)	47 (38,55)	ns
ΔFVC (%)	15 (11,19)	20 (14,27)	ns
AUC (FEV_1)	250 (182,317)	358 (261,455)	0.01
AUC (FEF_{50})	386 (276,495)	485 (364,606)	ns

Values are given as mean with 95% confidence intervals in parentheses.
ns = not significant.

BF or HR after 5, 6 and 7 min run between the two climatic conditions.

Maximum reduction in FEV_1 and AUC for FEV_1 increased significantly after exercise in the cold environment as compared with regular, indoor conditions. Maximum reduction in FEV_1 as per cent of baseline lung function after exercise in the cold environment was 31% (24, 38) vs. 24% (19, 29), respectively, after exercise under regular conditions ($p = 0.04$) (Table 3). AUC for FEV_1 was higher after exercise in the cold air, 358 (261, 455) vs. exercise under regular conditions, 250 (182, 317), respectively ($p = 0.01$) (Table 3).

Increased maximum reduction in FEF_{50} after exercise in the cold environment was also found; 47% (38, 55) vs. 38% (30, 46), but on the border of significance ($p = 0.06$). Maximum reduction in FVC as per cent of baseline lung function or AUC for FEF_{50} did not differ significantly between the climatic conditions (Table 3). Reduction in FEF_{50} was significantly higher 1 and 6 min after exercise in the cold environment (Fig. 2).

Calculated respiratory water loss during the last 3 min of exercise in the cold environment was 12.5 g (10.8, 14.3) vs. 10.8 g (9.7, 12.0) under regular indoor conditions ($p = 0.03$).

No significant correlation was found between reduction in lung function after exercise and water loss during the last 3 min of exercise. Nor was there any significant correlation between increased maximum fall in lung function (measured by FEV_1 and FEF_{50}) or increased AUC after exercise and reduced $\dot{V}O_{2\ peak}$ in the cold environment.

Discussion

The present study demonstrated that exercise capacity measured by $\dot{V}O_{2\ peak}$ and peak running speed decreased significantly during exercise in a cold environment as compared with regular environmental conditions, whereas $\dot{V}E_{peak}$, RER_{peak} and BF_{peak} did not differ in subjects suffering from EIB (Table 2).

Maximum reduction in FEV_1 after exercise and AUC for FEV_1 increased significantly in the cold environment as compared with exercise under standard, regular conditions. Maximum reduction in FEF_{50} did not reach statistically significant difference. The increased reduction in FEF_{50} reached statistical significance only at 1 and 6 min after exercise in the cold environment whereas AUC for FEF_{50} did not change (Fig. 2 and Table 3). Mean FEF_{50} at baseline was only 74% and 76% of that predicted (Table 1). This demonstrates the presence of airway obstruction in the peripheral airways in this group of asthmatics. Only seven out of 20 subjects used anti-inflammatory treatment (inhaled steroids).

According to the present study, the differences in $\dot{V}O_2$ and running speed occur when the subjects were close to their maximal aerobic capacity, the last 3 min of the EIB-test (Table 2 and Fig. 1). No correlation was found between maximum reduction in lung function (FEV_1 or FEF_{50}) after exercise or water loss during exercise and the reduced $\dot{V}O_{2\ peak}$ in the cold compared to the regular environment. The lack of correlation is possibly due to the number of subjects included. The power is probably too weak to detect any association. Nor can the reduction in $\dot{V}O_2$ be explained by reduction in $\dot{V}E$. No significant difference was found in $\dot{V}E$ during the last minutes of the tests or in $\dot{V}E_{peak}$ (Fig. 1 and Table 2) between the two climatic conditions.

All except three subjects reported spontaneously that breathing during exercise in the cold environment was much more difficult as compared with that in regular conditions. These statements support that the subjects ran slower during the last 4 min of the test with decreased $\dot{V}O_{2\ peak}$ in the cold environment. Studies aiming to imitate "real climatic conditions", like the present study, cannot be blinded and psycological factors might influence the results. To minimise these effects, objective measurements and well-standardised test procedures are necessary. In the present study the standardisation of the exercise load was based upon the screening test of the individual subjects aiming a submaximal to maximal exercise load as assessed by HR. The speed of the treadmill thus becomes a measure of performance during the two different climatic conditions.

The measurement of $\dot{V}O_2$ in the cold environment was challenging because the instruments used for direct and continuously $\dot{V}O_2$ measurements during exercise did not work in −18 °C. The Douglas Bag System used in the present study is a precise and well-documented instrument, and it is in fact recognised as a "gold standard". The disadvantage using the Douglas Bag system was that the $\dot{V}O_2$ measurements during the entire exercise period and the feasibility to measure tidal breathing flow volume loops during exercise were missed.

The causes of reduced $\dot{V}O_{2\ peak}$ and peak-running speed in the cold environment are unknown. Possibly, an increased

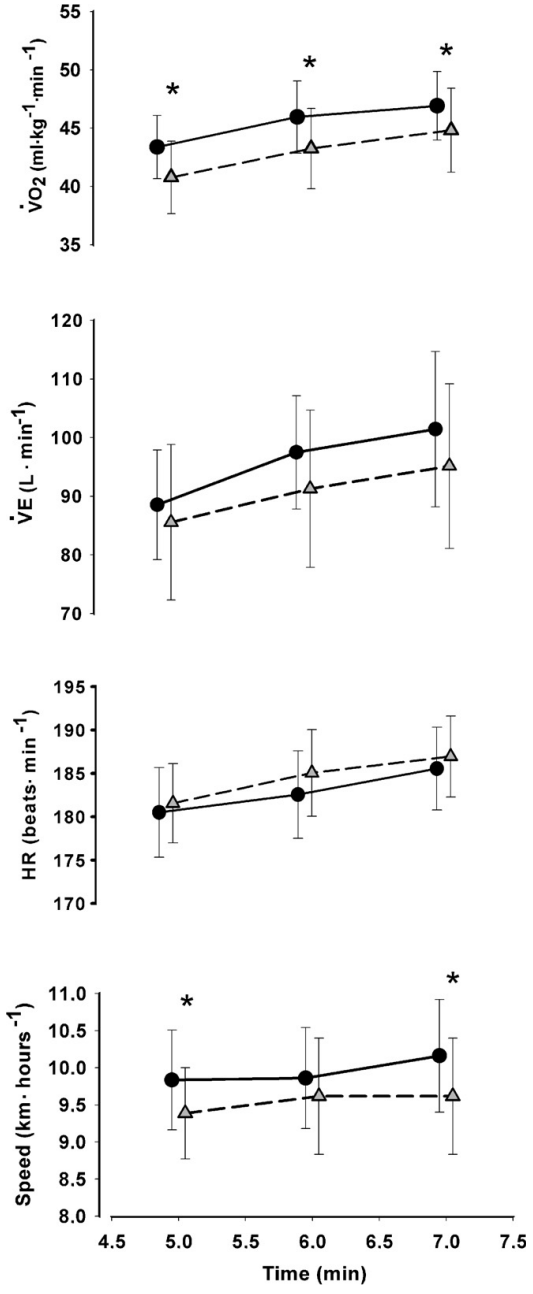

Figure 1 Oxygen uptake ($\dot{V}O_2$), minute ventilation ($\dot{V}E$), heart rate (HR) and running speed after 5,6 and 7 min exercise test under standardised regular conditions (●) and under standardised cold conditions (△) ($n = 20$). Results are given as mean with 95% confidence intervals. (*) = statistical significance.

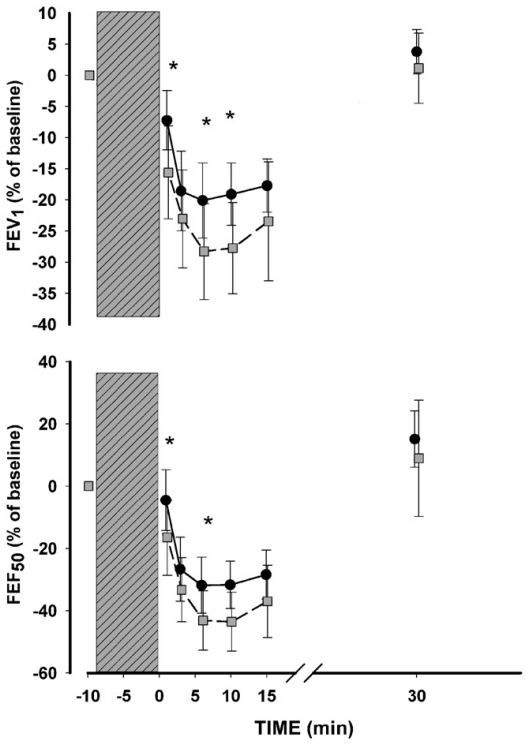

Figure 2 Lung function (FEV_1 and FEF_{50}) before and 1, 3, 6, 10 and 15 min after exercise and 15 min after inhaled salbutamol in a standardised regular environment (●) and in a standardised cold environment (▩) ($n = 20$). Results are given as mean with 95% confidence intervals. (*) = statistical significance.

strain level, especially for asthmatics starting exercise on a high intensity in a cold environment without warming-up, might reduce the performance. Neither HR_{peak} nor RER_{peak} differed during the two tests and indicate that the subjects achieved equal level of exhaustion even though the running speed was reduced in the cold environment. The subjects were only exposed to the cold environment for 10 min and no freezeing or shivering were observed or reported. As they wore warm clothes suited for the cold environment, the decrease in $\dot{V}O_{2\ peak}$ had probably a direct relation to reduced running speed during exercise. An EIB-test with pre-medication of inhaled β_2-agonists in the cold environment or a control group of EIB-negative subjects might explain if the airway calibre is a possible reason. Our findings are supported by the study from Quirion et al.[9] on healthy subjects. They demonstrated that $\dot{V}O_{2\ max}$ significantly decreased and the $\dot{V}E$ did not change in −20 and 0 °C as compared with that in 20 °C, and their subjects reported that submaximal exercise intensities were more tiring in a cold environment as compared with those in a warm environment. They suggested that the net efficiency of exercise at low temperatures is lower than under normal conditions. On the other hand, Sandsund et al.[10] reported

increased $\dot{V}O_2$ in eight healthy male athletes at submaximal exercise intensities in a cold environment compared with those in standard, indoor conditions, but there was no difference in $\dot{V}O_{2\ max}$. Time to exhaustion was shorter in the cold environment. They suggested that exercise stress is higher at submaximal exercise intensities in a cold environment in agreement with the reduced running speed during exercise in the present study. Claremont et al.[11] tried to explain the same observation by a catecholamine calorigenic effect.

In the studies of asthmatics from Kallings et al.,[7] Sandsund et al.[6] and Eschenbacher et al.,[8] only six, seven and eight subjects, respectively, were included, and their results only serve as pilot studies indicating the need for further investigations. The workload differed markedly between these studies and also from the present study. The workload, ventilation and the demand for oxygen is too low in the study from Kallings et al. and Eschenbacher et al. in order to be able to discover any difference in $\dot{V}O_{2\ peak}$ as compared with the exercise load at which the difference occurred in the present study.

Sandsund et al.[6] found no differences in $\dot{V}O_{2\ max}$, $\dot{V}E_{max}$, HR_{peak} or blood lactic acid when inhaling cold or warm air during exercise. However, in their study the temperature of the environmental air was $-15\,^{\circ}C$, the breathing mouthpiece acted as a heat exchanger and increased the inspired cold air to $2\,^{\circ}C$. This is most probably not cold enough to observe any differences in lung function or in the physiological variables. Their exercise protocol was in fact not an exercise test for provoking EIB but a stepwise protocol for measuring anaerobic threshold and $\dot{V}O_{2\ max}$ with a 20 min warming-up period.

The present study confirms previous reports that inhalation of cold air increases EIB in asthmatic subjects.[1,2] On the other hand, neither Evans et al.[19] nor Sandsund et al.[6] could find any additive effect of cold air inhalation upon EIB. The temperature of the inhaled air in their studies was actually -1 and $2\,^{\circ}C$, respectively, and probably not cold enough to discover any difference. Evans et al.[19] mentioned that lack of exposure to ambient cold air during inhalation may explain the lack of an additive effect.

However, cold environmental conditions seem to aggravate the effect on EIB, and the respiratory water loss significantly increased in the cold environment as compared with that in the regular, indoor conditions. Air of $37\,^{\circ}C$ fully saturated with vapour contains 44 g H_2O/m^3. Air of temperature $20\,^{\circ}C$ with 40% relative humidity contains 6.9 g H_2O/m^3 and air of $-18\,^{\circ}C$ with 40% relative humidity contains 0.01 g H_2O/m^3. When the ventilation rates increase during exercise, the water loss increases. These findings indicate that the worsening effect on EIB in asthmatics is partly due to increased water loss and partly due to heat loss and support earlier reports on EIB and cold environment.[1,3,7,29,30]

Our findings are also supported by Zeitoun et al.[5] and Koskela et al.[4] Zeitoun et al.[5] concluded that facial cooling combined with either cold or warm air inhalation causes the greatest EIB as compared with the isolated challenge with cold air inhalation. They suggested that vagal mechanisms activated by changes in osmolarity play a major role in exercise and cold-induced bronchoconstriction. Koskela et al.[4] reported that, for certain stable asthmatic subjects, even a moderate level of exercise can cause bronchoconstriction in climatic conditions similar to a Scandinavian winter. They also found that even sitting in $-20\,^{\circ}C$ caused a greater bronchconstriction than moderate exercise in room temperature and stated that this could not be explained by hyperventilation-induced airway drying alone, but that the reflex mechanism is more important than was previously thought.[4] Boulet and Turcotte[30] reported that EIB was influenced by the changes in water content during and after exercise. The recovery period in the present study was in regular, indoor environmental conditions and according to Boulet and Turcotte[30] the best recovery environment to protect against EIB.

The choice of including subjects with relatively large range in age was to reflect the period of life extending from schoolage to adulthood, where human beings are physically active and spending time on physical activity. The results from the present study can contribute to giving this group of asthmatics better advice and treatment before exercising in a cold environment. Previous reports have shown that both asthmatic children and asthmatic adults seem to respond equally upon exercise in a cold environment.[1,2] In the present study, the subjects below 16 years ($n = 6$) had the same reduction in $\dot{V}O_{2\ peak}$ in the cold conditions as the subjects above 16 years ($n = 14$).

In conclusion, exercising in a cold environment decreases exercise capacity as measured by $\dot{V}O_{2\ peak}$ and peak running speed, and increases EIB in subjects suffering from EIB. This has important implications for training procedures in a cold environment for patients and athletes with EIB. Although similar effect of a cold environment upon exercise capacity in healthy subjects cannot be excluded.

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CLINICAL SCIENCES

Clinical Investigations

Bronchial Hyperresponsiveness in Skiers: Field Test versus Methacholine Provocation?

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ABSTRACT

STENSRUD, T., K. V. MYKLAND, K. GABRIELSEN, and K.-H. CARLSEN. Bronchial Hyperresponsiveness in Skiers: Field Test versus Methacholine Provocation? *Med. Sci. Sports Exerc.*, Vol. 39, No. 10, pp. 1681–1686, 2007. **Introduction:** Asthma is frequently reported in endurance athletes, particularly in cross-country skiers. It has been reported that an exercise field test performed with the competitive type of exercise is the better for diagnosing asthma and bronchial hyperresponsiveness in athletes than bronchial provocation with methacholine. **Objective:** The main objective was to compare an exercise field test consisting of a skiing competition with methacholine bronchial provocation in the diagnosis of asthma and bronchial hyperresponsiveness among skiers. **Methods:** Twenty-four elite cross-country skiers from the Norwegian national teams (males/females = 16/8) were included in the study. The cumulative dose of inhaled methacholine causing a 20% fall in forced expiratory volume in 1 s (FEV_1) (PD_{20}) was compared with reduction in lung function (FEV_1) $\geq$ 10% from before to after an exercise field test consisting of a cross-country skiing competition, 10 km (males) and 7 km (females), respectively. **Results:** Nine out of 24 (37.5%) athletes experienced a positive methacholine test ($PD_{20} < 8$ μmol) (2 females and 7 males), whereas only 2 of the 24 subjects (8.3%) had reductions in $FEV_1 \geq 10\%$ after the exercise field test. A significant negative correlation was found between age and bronchial responsiveness, $r = -0.47$, $P = 0.02$. **Conclusion:** The methacholine bronchial provocation test is more sensitive than a sport specific exercise field test for identifying athletes with asthma and/or bronchial hyperresponsiveness. **Key Words:** ASTHMA, EXERCISE-INDUCED BRONCHOCONSTRICTION, ELITE CROSS-COUNTRY SKIERS, TEST CHALLENGE

Asthma represents an increasing problem for athletes competing within endurance sports. The prevalence of exercise-induced asthma (EIA) has increased during the last two decades, especially amongst elite endurance athletes (10,11,17). Heir and coworkers show that in competitive cross-country skiers, the prevalence of doctor-diagnosed asthma increased markedly up to 24% in the age group above 28 yr, in contrast to normally physically active control subjects (17). Larsson et al. (16) have demonstrated an alarmingly high prevalence of asthma and bronchial hyperresponsiveness (BHR) to methacholine in Swedish top-level cross-country skiers. Whereas BHR to methacholine has been regarded as a direct measure of nonspecific BHR, exercise-induced bronchoconstriction (EIB) and eucapnic hyperventilation test are looked on as indirect measures of nonspecific bronchial responsiveness (22). Weiler et al. (29) report a prevalence of EIA of 11% among American athletes participating in the 1984 summer Olympic Games, increasing to more than 20% among the American participants in the 1996 summer Olympic games in Atlanta, GA (28). As stated by Helenius et al., asthma occurs most commonly in athletes engaged in endurance sports such as cross-country skiing, swimming, or long-distance running. This is particular so for skiers (16), possibly because of the cold and dry air exposure during heavy exercise (19). In addition to the type of training, a major risk factor is atopic disposition (1,11).

The use of inhaled β_2-agonists in asthmatic athletes at the Olympic games increased from 1984 and to later Olympic

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games (20). Reports and observations of frequent use of inhaled β_2-agonists among top athletes led the IOC Medical Commission to make restrictions regarding the use of inhaled β_2-agonists. From the Salt Lake City Olympic games, specific requirements were worked out, such as assessment of bronchial responsiveness, EIB, or reversibility to inhaled β_2-agonists. These rules have later been modified. The present-day athletes must provide evidence of one of the following to be allowed to use inhaled β_2-agonists (IBAs):

1) a 12% or greater increase of the predicted or baseline value of FEV_1 after administration of permitted IBAs;
2) a 10% or greater decrease in FEV_1 after challenge with physical stimuli, such as an exercise field test, exercise treadmill test in a laboratory, or eucapnic voluntary hyperventilation test (EVH); and
3) obtaining by methacholine bronchial provocation test a dose of inhaled methacholine causing a reduction in FEV_1 of 20% ($PD_{20\ methacholine}$) less than 2 μmol (400 μg) or a $PC_{20\ methacholine}$ (concentration of methacholine) less than 4 $mg \cdot mL^{-1}$; for those on topical steroids, the methacholine PD_{20} has to be less than 13.2 μmol or the PC_{20} less than 13.2 $mg \cdot mL^{-1}$.

In this way, the IOC medical commission includes several tests for assessment of direct or indirect bronchial responsiveness for the athlete who wants to use inhaled β_2-agonists in sports. Thus, how these tests compare in sensitivity and specificity for detecting BHR or EIB in the athlete with suspected asthma has impact on applications for and use of inhaled β_2-agonists in international sport competitions.

Rundell et al. (24) report that by using real-life competitive events as the provoking agent among American participants in winter Olympic games, 98% of the athletes reporting EIA had positive tests. Also, 48% of the athletes not reporting EIA were found to have positive tests. They conclude that without relevant provoking agents, such as a sport-specific exercise field test (SSEFT), one might risk several false-negative results on screening for EIB or BHR among athletes (24). Ogston and Butcher (21) agree with Rundell et al., concluding that by using a sport-specific protocol, a large number of athletes can be screened objectively for EIB. On the other hand, Dickinson et al. (7) report that an EVH test is a more sensitive challenge in asymptomatic athletes than a sport-specific and laboratory-based challenge. Thus, this controversy is still unsolved. Therefore, the primary objective of the present study was to determine how a SSEFT compares in sensitivity with $PD_{20\ methacholine}$, in the assessment of asthma and BHR in elite cross-country skiers.

METHODS

Design of the study. The present study was an open study, nonrandomized, comparing indirect bronchial responsiveness as obtained by an exercise field test over a total of 7 and 10 km (♀ and ♂, respectively) with direct bronchial responsiveness obtained by a bronchial provocation test with methacholine measuring $PD_{20\ methacholine}$. The present study comprised 2 d.

Day 1: All subjects underwent a methacholine provocation test.
Day 2: All subjects competed in a cross-country skiing competition.

Before the provocation challenges, all athletes refrained from taking any medication that might have confounded the pulmonary function results. Antiasthmatic medications were withheld according to ERS guidelines (8). Inhaled, short-acting β_2-agonists and sodium cromoglycate were withheld for 8 h before testing; inhaled, long-acting β_2-agonists, theophylline, and leukotriene antagonists were withheld for the last 72 h; antihistaminics were withheld for the last 7 d; and orally administered glucocorticosteroids were withheld for the last month.

The study was performed according to the principles stated in the Declaration of Helsinki. The regional medical ethics committee approved the study, and all subjects gave written informed consent.

Subjects. Twenty-four cross-country skiers, all competing at an international top level, participated in the study. All were members of the Norwegian national cross-country skiing team, five in the men's elite all-round team, eight in the women's elite team, six in the men's elite sprint team, and five male skiers in the recruit team. Demographics and baseline lung function (FEV_1 and FEV_1 % predicted) before the exercise field test are given in Table 1. Asthma treatment used before the study is reported in Table 2. The study took place at an altitude of 1100 m above sea level (masl) in the women's competition with a temperature of −2°C and a relative humidity of 36%, and at an altitude of 1250 masl during the men's competition with a temperature of −4°C and a relative humidity of 34%. Exclusion criteria were any acute or chronic illnesses interfering with the possibility to perform the study, in addition to upper–respiratory tract infections (URTI) during the 4 wk before testing.

TABLE 1. Demographic data and baseline lung function (FEV_1 and FEV_1 percent predicted) before the exercise field test of the 24 elite skiers.

N	Age (yr)	Height (cm)	Weight (kg)	FEV_1(L)	FEV_1predicted (%)
Men (16)	25.7 (3.3)	183.7 (5.4)	79.6 (6.1)	5.84 (0.7)	126 (14)
Women (8)	25.7 (5.8)	169.5 (2.7)	59.8 (5.2)	3.92 (0.6)	112 (10)
All (24)	25.7 (4.1)	179.0 (8.3)	73.0 (11.1)	5.20 (1.1)	121 (15)

Values are expressed as mean (standard deviation).

TABLE 2. Gender, age, results of lung function measurements, exercise-induced bronchoconstriction (EIB), assessment of bronchial responsiveness to methacholine if the patients were diagnosed with asthma or atopy before the study, and treatment before and after the tests.

N	Gender	Age (yr)	FEV_1	FEF_{50}	EIB	PD_{20}	Asthma	Atopy	Treatment BT	Treatment AT
1	♀	23	3.99	6.76	0	> 25.00	Yes	No	None	None
2	♀	23	3.60	2.97	0	> 25.00	No	No	None	None
3	♀	25	3.83	4.28	0	> 25.00	No	No	None	None
4	♀	23	4.26	4.38	0	4.54	Yes	Yes	LTA	LTA
5	♀	39	2.89	2.25	2	1.12	Yes	No	ICS + LABA	ICS + LABA
6	♀	24	4.91	6.80	0	> 25.00	No	No	None	None
7	♀	24	3.50	3.27	0	> 25.00	No	No	None	None
8	♀	23	4.39	4.34	4	6.83	Yes	No	ICS + LABA	ICS + LABA
9	♂	32	5.75	4.70	6	3.72	Yes	No	ICS + LABA + LTA	ICS + LABA + LTA
10	♂	22	5.51	5.57	15	9.54	Yes	No	ICS + LABA	ICS + LABA
11	♂	25	5.96	6.64	5	3.42	No	No	None	None
12	♂	23	5.74	6.24	1	8.67	No	No	None	None
13	♂	24	6.63	7.78	5	9.23	No	Yes	None	None
14	♂	27	7.64	8.62	4	> 25.00	No	No	None	None
15	♂	22	5.33	4.76	3	> 25.00	No	No	None	None
16	♂	25	6.25	6.46	0	18.16	No	No	None	None
17	♂	30	5.07	4.03	0	> 25.00	No	No	None	None
18	♂	28	6.62	6.67	0	2.14	No	No	None	None
19	♂	22	5.67	6.62	1	> 25.00	No	No	ICS + LTA	ICS + LTA
20	♂	21	6.04	5.49	0	8.16	No	Yes	None	None
21	♂	30	4.70	3.89	0	> 25.00	Yes	No	ICS + LABA + LTA	ICS + LABA + LTA
22	♂	26	5.71	6.62	1	4.85	No	No	LTA	LTA
23	♂	27	5.79	5.15	15	5.76	Yes	Yes	None	ICS + LABA
24	♂	28	4.98	3.41	0	0.87	Yes	No	None	ICS + LABA

♂ male; ♀ female; FEV_1, forced expiratory volume in 1 s; FEF_{50}, forced expiratory flow at 50% of forced vital capacity; EIB, percent reduction in FEV_1 from before to after the cross-country skiing competition; PD20, provocative inhaled dose of methacholine (μmol) causing a 20% reduction in FEV_1; treatment BT, treatment before testing; treatment AT, treatment after testing; LTA, leukotriene antagonist; ICS, inhaled corticosteroids; LABA, inhaled, long-acting β2-agonist.

Lung function measurements. Lung function was measured by maximum expiratory flow-volume loops according to the European Respiratory Society criteria (23); the best of three trials were recorded by use of Masterscreen Pneumo Jaeger (Würzburg, Germany). The following variables were recorded: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), forced expiratory flow at 50% of vital capacity (FEF_{50}), and peak expiratory flow rate (PEF).

Nonspecific bronchial responsiveness. Nonspecific BHR was measured by bronchial provocation to methacholine. Methacholine was delivered by the inspiration triggered nebulizer Aerosol Provocation System Jäger (Würzburg, Germany) and was inhaled in doubling doses until FEV_1 decreased 20% from baseline, as measured after inhaled nebulized isotonic saline. The dose causing a 20% reduction in FEV_1 (PD_{20}) was determined by linear interpolation on the semilogarithmic dose–response curve. All tests were performed according to current guidelines from the American Thoracic Society (4). After bronchial provocation testing, subjects were given salbutamol inhalations to reverse bronchial obstruction. BHR was defined as PD_{20} methacholine below 8 μmol.

Exercise challenge. Lung function was measured before and after the exercise field test. The field test consisted of a cross-country skiing competition. The men performed a 10-km cross-country skiing competition, and the women performed a 7-km competition. The ambient temperature and the relative humidity during the cross-country skiing competition was measured and recorded. Lung function was measured by maximum expiratory flow-volume loops, as described above, before the start of the competition, immediately after finishing, and 5, 10, and 20 min thereafter. The term *EIA* has been used to denote symptoms and signs of asthma provoked by physical exercise, whereas *EIB* has been used to denote the measured decrease in lung function after an exercise test, as defined jointly by the Task Force on Sports and Asthma of the European Respiratory Society and the European Academy of Allergy and Clinical Immunology (2).

Statistical methods. The present study included the entire Norwegian National team of cross-country skiers, 24 skiers altogether. Hofstra et al. (13) have reported previously that a sample size of 12 subjects is sufficient to assess differences in EIB. It was, therefore, concluded that a sample of 24 skiers was large enough to assess differences in the number of skiers positive to methacholine provocation compared with the number of skiers with positive exercise field tests.

Statistical analyses were performed with Statistical Package of Social Sciences version 11.0. Results are given as means with 95% confidence intervals unless otherwise stated. Demographics are given as mean with standard deviation in parentheses. Differences in categorical data were assessed by the Fisher exact test. Possible associations were assessed by the parametric Pearson's correlation coefficient. P values ≤ 0.05 (5%) was considered statistically significant.

RESULTS

Demographic variables of the cross-country skiers are shown in Table 1. $PD_{20\ \text{methacholine}} < 16$ μmol was found in 13 of 24 skiers (54.2%), in 3 of 8 females, and in 10 of 16

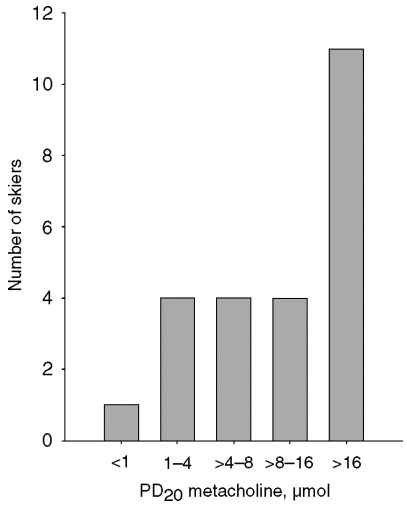

FIGURE 1—Inhaled dose of methacholine causing a 20% reduction in FEV_1 (forced expiratory flow in 1 s compared with the number of skiers within each group (N = 24).

males, whereas 9 skiers (37.5%) had $PD_{20\ methacholine}$ measurements below 8 μmol. The distribution of bronchial responsiveness to methacholine is shown in Figure 1. Two of 24 subjects (8.3%) experienced positive exercise field tests, a maximum reduction in $FEV_1 \geq 10\%$ (Table 2). Both had maximal reductions in FEV_1 at 20 min after exercise. One of the skiers with a positive exercise field test had a positive methacholine provocation, with a $PD_{20\ methacholine}$ of 5.79 μmol, whereas the other had a $PD_{20\ methacholine}$ of 9.55 μmol (Table 2).

Subjects older than 25 yr (N = 9) of age had $PD_{20\ methacholine}$ measurements below 8 μmol significantly more often than did subjects 25 yr and younger (N = 15; P = 0.036). One subject older than 25 yr was atopic, and three of the subjects $\leq$ 25 yr were atopic. All atopic subjects had PD_{20} values of 9.23 and below, and two were below 8 μmol. A significant negative correlation was found between age and log values of $PD_{20\ methacholine}$ ($r = -0.47$, P = 0.02; Fig. 2). No significant difference in BHR to methacholine was found related to gender.

DISCUSSION

The present study suggests that $PD_{20\ methacholine}$ is more sensitive than exercise field testing using the competitive sport to assess BHR in elite cross-country skiers. Nine athletes experienced $PD_{20} < 8$ μmol (37.5%), and 13 (54.2%) were less than 16 μmol. On the contrary, only two subjects experienced positive exercise field tests and maximum reductions in $FEV_1 \geq 10\%$. One of the two did not use any medication (Table 2). A significantly higher percentage of BHR among subjects older than 25 yr compared with younger subjects was found (Fig. 2).

The present study supports the findings of Dickinson et al. (7), concluding that a sport-specific exercise test is not the best challenge for diagnosing EIB or BHR. They suggest that an EVH test provides a more sensitive diagnosis of BHR in elite winter athletes. In accordance with Dickinson et al. (7), our findings do not agree with the findings of Rundell et al. or those of Ogston and Butcher, suggesting that a sport-specific exercise field test is the method of choice in the diagnosis of EIB among top athletes (21,24). Exercise field tests were recommended for the Olympic games in Salt Lake City in 2002 because they were considered effective and more sensitive for identification of EIB in cold-weather athletes when compared with exercise performed under laboratory conditions of temperature and humidity (18,30). It can be assumed that by performing one's usual exercise in the usual environment, the athlete would be in the best position to reproduce his or her respiratory problems. However, in the present study, the sport-specific exercise field test did not reveal any subjects who had not already been recognized by the $PD_{20\ methacholine}$ test, and 11 of 13 subjects with some degree of BHR to methacholine were not detected by the exercise field test (24).

Crimi et al. (5) claim that direct stimuli such as methacholine can allow the identification of asthmatic subjects who do not exhibit EIB because of a low degree of airway inflammation at the time of study but who may eventually become ill if exposed to sensitizing allergens or after virus infections. Langdeau et al. (15) investigated the Canadian Olympic team and found that nearly 50% were positive to methacholine compared with 18% of healthy controls. This is in concordance with the findings of the present study.

A significantly higher percentage of BHR was found among subjects older than 25 yr of age (P = 0.036). This

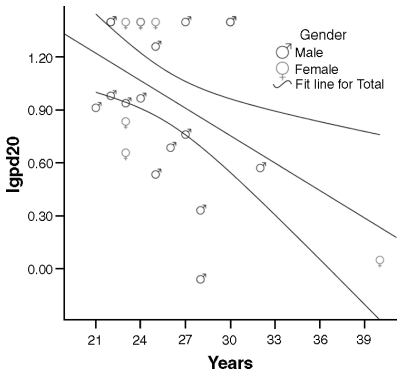

FIGURE 2—Scatterplot of lg $PD_{20\ methacholine}$ vs age; regression line with 95% confidence interval. The lg $PD_{20\ methacholine}$ values of 0.3, 0.6, 0.9, and 1.20 are equivalent to 2.0, 4.0, 8.0, and 16.0 μmol of methacholine, respectively. ♂ male; ♀ female ($r = -0.47$, P = 0.02). N = 24; 9 subjects were older than 25 yr, and 15 subjects were 25 yr or younger.

agrees with the findings of Heir et al., who have demonstrated by their questionnaire study a higher prevalence of doctor-diagnosed asthma among cross-country skiers with increasing age compared with healthy control subjects (10). One possible explanation may be that the continued stress to the airways over many years caused by high ventilation rates during exercise, often in dry, cold environments, increases BHR over time by increasing airway inflammation. This was also found by Sue-Chu et al. (25) and Karjalainen et al. (14) in their bronchial biopsy study of young skiers from a skiing high school. This was also confirmed in a study on exercising mice with increasing epithelial damage with continued exercise (3). Others speculate that immune suppression may contribute in the development of BHR, at least in endurance athletes (6,9,26). Heir et al. (9) found that training with an URTI induced a long-lasting ($\geq$ 6 wk) increase in BHR to histamine, whereas this did not occur in subjects who did not train actively during the infection.

No significant gender difference was found in the present study with 6 of 16 men, and 3 of 8 women displayed BHR to methacholine with PD_{20} values below 8 μmol. Former studies have implied a slightly higher prevalence in female athletes (16,30), and women have been shown to have a slightly higher prevalence of BHR to methacholine (12,27). The number of athletes participating in the present study may have been too low to assess significant differences to gender.

The present study fully demonstrates the discrepancy between direct and indirect tests of bronchial hyperresponsiveness. Furthermore, it should be emphasized that asthma is a clinical diagnosis based on reports of recurring episodes of bronchial obstruction. Both direct and indirect BHR change over time because of changing exposure to allergens and other environmental agents, as well as being dependent on antiinflammatory treatment. The diagnosis of asthma in athletes should be based on a combination of clinical history and clinical signs with the use of supplementary objective tests as used in the present study. For practical purposes, this is important also in relation to the rules given by the IOC medical commission and the World Anti-Doping Association for the use of asthma drugs in sports.

In conclusion, the present study demonstrates that measurement of BHR to methacholine is more sensitive than exercise field testing in confirming the diagnosis of BHR or EIB in elite cross-country skiers.

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REGIONAL KOMITE FOR MEDISINSK FORSKNINGSETIKK

Helseregion Sør

KOPI

Professor dr.med.
Kai Håkon Carlsen
Voksentoppen
Ullveien 14
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Deres ref.: 20.08.00 **Vår ref.:** S-00220 **Dato:** 31.10.00

Klimaets innvirkning på sykdomsaktiviteten hos barn med astma. "Astma og Klima"
Prosjektleder: Avdelingsoverlege, professor dr.med. Kai Håkon Carlsen, Voksentoppen

Komiteen behandlet prosjektet i sitt møte torsdag 19. oktober 2000 og gjorde slikt vedtak:

"Det må stå uttrykkelig i informasjonsskrivet av det er frivillig å delta.

I samtykkeerklæringen vil komiteen om at formuleringen "Vi har forstått hva prosjektet innebærer av fordeler og ulemper for oss" strykes, da et informasjonsskriv bør være av en slik kvalitet at spørsmål om innholdet er forstått ansees som unødvendig.

Det må utarbeides egen informasjon til kontrollgruppen dersom det skal tas ekstra prøver av hensyn til studien. Den sendes komiteen til orientering.

Under denne forutsetningen tilrår komiteen at prosjektet gjennomføres."

Vi ønsker lykke til med prosjektet.

Med vennlig hilsen

Sigurd Nitter-Hauge (sign)
professor dr.med.
leder

Ola P. Hole
avdelingsleder
sekretær

Kopi: Cand.med Arne Vilberg, Voksentoppen

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