Non-invasive evaluation of lower airway inflammation in hyper-responsive elite cross-country skiers and asthmatics

M. SUE-CHU, A. H. HENRIKSEN AND L. BJERMER

Department of Lung Medicine, University Hospital, Trondheim, Norway

Asthma-like symptoms and bronchial hyper-responsiveness (BHR) to methacholine are prevalent in competitive cross-country skiers. Whether these symptoms (ski asthma) in these athletes are caused by asthma remains uncertain.

Bronchial responsiveness to adenosine 5'-monophosphate (AMP) and nitric oxide (NO) concentration in exhaled air, both indirect markers of asthmatic airway inflammation, were investigated in two non-smoking study populations of skiers and asthmatics.

Of 18 skiers with ski asthma, 15 non-steroid and 14 steroid-treated asthmatics, BHR to AMP was present in five (28%), six (40%) and 10 (71%) subjects respectively. Although the groups were not significantly different in responsiveness to methacholine, responsiveness to AMP increased in order of magnitude from ski asthma < non-steroid-treated < steroid-treated asthma. Exhaled NO in 44 (nine with ski asthma) skiers was not significantly different from 82 healthy non-atopic controls [median [interquartile range (IQR)] 6.5 (4.1-9.9) vs. 5.2 (4.2-6.5) ppb]. Exhaled NO in 29 subjects with mild intermittent asthma was three-fold greater [median (IQR) 19.2 (5.1-25.6) ppb, P < 0.01] than in skiers. Exhaled NO was two- and four-fold greater in atopic than non-atopic subjects in the skier (P < 0.001) and asthmatic (P < 0.01) groups, respectively, and was correlated to methacholine responsiveness in atopic asthmatics (n = 22, rho = 0.55, P < 0.01).

Exhaled NO was not elevated in ski asthma and may be more useful as a marker of atopic status than inflammation in the lower airway in skiers. Few skiers were hyper-responsive to AMP, indicating that pre-activated mucosal mast cells are not a predominant feature in ski asthma.

Introduction

Exercise can trigger acute bronchoconstriction in asthmatics. This effect of exercise is potentiated by simultaneous exposure to cold air (1). Highly trained athletes are reported to have a high prevalence of bronchial hyper-responsiveness to methacholine and exercise, asthma-like symptoms, use of anti-asthmatic medication and asthma (2,3,4). Moreover, cross-country skiers commonly report obstructive airway symptoms with co-existent hyper-responsiveness to methacholine (5,6).

Although associated with airway inflammation in asthmatics, bronchial hyper-responsiveness to methacholine is not specific to asthma, as it can also be present in healthy subjects during viral infections and in patients with other respiratory diseases (7,8,9). However, inhalation of adenosine 5'-monophosphate (AMP) also provokes bronchoconstriction in asthmatic subjects. This effect is believed to be mediated through release of mediators from pre-activated mast cells situated in the bronchial mucosa (10). Thus, hyper-responsiveness to AMP is believed to be more specific (11,12) and a more sensitive marker for assessing airway inflammation in asthma (13,14) compared to methacholine. Another promising, indirect marker of airway inflammation is the concentration of nitric oxide (NO) in exhaled air. The measurement of exhaled NO is easy to perform and is non-invasive. Increased levels have been reported in normal individuals with upper respiratory tract infections (15) and in patients with asthma, especially those with allergic asthma (16,17). A further increase can be seen in relation to asthma exacerbation, with normalization often occurring either spontaneously or after steroid treatment (18,19). As uncertainty exists as to whether asthma is present in athletes with bronchial hyper-responsiveness to methacholine and obstructive symptoms (20) the aims of this two-part study were to investigate the bronchial responsiveness to AMP and the concentration of exhaled NO in skiers with ski asthma.
TABLE 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>AMP study</th>
<th>Nitric oxide study</th>
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<tbody>
<tr>
<td></td>
<td>Ski asthma</td>
<td>Asthmatics</td>
</tr>
<tr>
<td></td>
<td>Asthmatics</td>
<td>Controls</td>
</tr>
<tr>
<td>n (males)</td>
<td>18 (10)</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Age-mean (range) years</td>
<td>18.5 (16-27)</td>
<td>17.5 (16-31)</td>
</tr>
<tr>
<td>Atopy (%)</td>
<td>7 (39%)</td>
<td>10 (67%)</td>
</tr>
<tr>
<td>BHR methacholine (%)</td>
<td>18 (100%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Asthma medication (%)</td>
<td>7 (39%)</td>
<td>9 (60%)</td>
</tr>
<tr>
<td>Topical steroid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>14 (2)</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Baseline FEV₁ (%) predicted</td>
<td></td>
<td>100.4 (9.9)</td>
</tr>
<tr>
<td>Methacholine—mean (SD)</td>
<td>106.2 (11.2)</td>
<td>94.9 (11.3)</td>
</tr>
<tr>
<td>AMP—mean (SD)</td>
<td>97.5 (9.6)</td>
<td>96.2 (9.6)</td>
</tr>
</tbody>
</table>

Steroid −: non-steroid treated asthmatics; steroid +: steroid-treated asthmatics. *P<0.05 vs. Controls. **P<0.01, skiers vs. Asthmatics. ***P<0.001 vs. Controls vs. Asthmatics (one-way ANOVA). #P<0.05 vs. Steroid − asthmatics (one-way ANOVA). AMP: adenosine 5’monophosphate. BHR: bronchial hyper-responsiveness, NA: not applicable.

Methods

SUBJECTS

Competitive cross-country skiers, asthmatics and healthy controls were recruited to the study. All subjects were non-smokers and had no history of an upper respiratory tract infection within the 3 weeks prior to participation in the study. All subjects, as well as the parents of those subjects less than 18 years of age, gave written informed consent to the study, which was approved by the Regional Ethics Committee in Trondheim.

Two separate study populations were investigated (Table 1). The study population for assessment of bronchial responsiveness to AMP consisted of 18 skiers and 29 asthmatics. Of the asthmatics, 15 subjects had a history of mild intermittent asthma and the remainder had mild to moderate persistent asthma that was well controlled with daily doses of 400–2000 μg inhaled corticosteroids. All skiers and intermittent asthmatics had reported asthmatic symptoms within the previous year in a self-completed questionnaire, and all subjects had bronchial hyper-responsiveness to methacholine. For the exhaled NO study, the study population consisted of 44 skiers, 29 asthmatics and 82 controls. Of the skiers, 9 subjects had bronchial hyper-responsiveness to methacholine and asthmatic symptoms. All asthmatic subjects had mild intermittent asthma, with asthma symptoms within the previous year, and bronchial hyper-responsiveness to methacholine. Control subjects did not have a past history of allergic symptoms, wheeze or abnormal breathlessness, or use of anti-asthmatic medication.

CLINICAL METHODS

Asthma symptoms were defined in skiers as wheeze and abnormal breathlessness or chest tightness, which was present on exertion, exposure to irritants or at rest, and in asthmatics as wheeze or abnormal breathlessness with or without exposure to pollen, household pets or house dust. Ski asthma in skiers was defined as the presence of asthma symptoms and bronchial hyper-responsiveness to methacholine.

Lung function was assessed by flow-volume spirometry performed with a pneumotachograph (Master Scope™, Erich Jaeger GmbH&Co.KG, Würzburg, Germany) or a turbine spirometer (Microlab 3300 Mk2 spirometer, Micro Medical Ltd, Gillingham, Kent, England). The better of two measurements of forced expiratory volume in 1 s (FEV₁) with less than 5% variation was recorded at baseline and during bronchial provocation testing. Baseline values were expressed as a percentage of predicted normal values (European Coal and Steel Community/Zapletal reference values).

Bronchial responsiveness to methacholine and AMP was performed with a controlled tidal volume breathing technique and an inhalation synchronized dosimetric jet nebulizer. Provocation testing with methacholine was performed in all subjects in both study populations. For skiers in both studies, and asthmatics in the AMP study, the protocol consisted of a cumulative dose of 9.1 μmol (1800 μg) methacholine administered with the Spira Elektrio 2 nebulizer (Respiratory Care Centre, Hameenlinna, Finland), as previously described (2). For asthmatic and control subjects in the exhaled NO study, the protocol
consisted of a cumulative dose of 10.1 \mu mol (2000 \mu g) methacholine delivered in five increments with the MedicAid jet nebuliser and the Aerosol Provocation System (APS, Jüger). Subjects in the AMP study were tested on 2 separate days over a period of 2 weeks, with methacholine provocation on the first day. Provocation with AMP (Sigma Chemicals, St. Louis, MO, U.S.A.) was performed with solutions in concentrations of 25 and 250 mg ml\(^{-1}\) freshly prepared in saline. The protocol consisted of a cumulative dose of 145.4 \mu mol (50 500 \mu g), delivered in six increments with the Spira Elektro 2 nebulizer. Prior to bronchial provocation testing, short-acting \beta_2\text{-agonists} were withheld for at least 8 h and long-acting \beta_2\text{-agonists}, cromolyns and theophylline were withheld for at least 24 h. Short-acting systematic anti-histamines were withheld for at least 24 h prior to AMP provocation. The challenge was discontinued if FEV\(_1\) declined by 20% or more during the protocol. Bronchial hyper-responsiveness was defined as the cumulative dose that caused a decline in FEV\(_1\) by 20% or more (PD\(_{20}\)FEV\(_1\)). The PD\(_{20}\)FEV\(_1\) was determined by interpolation of the last two points on the log dose-response plot. The PD\(_{20}\)FEV\(_1\) methacholine was defined as \leq 9.1 \mu mol (1800 \mu g) with the Spira Elektro 2 nebulizer and \leq 10.1 \mu mol (2000 \mu g) with the MedicAid nebulizer, and PD\(_{20}\)FEV\(_1\) AMP was defined as \leq 145.4 \mu mol (50 500 \mu g). Bronchial responsiveness was expressed as the dose-response ratio (DRR), which is the % fall in FEV\(_1\) per \mu mol\(^{-1}\) of the provocation agent (22).

Serum was screened for the presence of specific IgE antibodies to eight common inhalant allergens (grass and birch pollen, mugwort, moulds, cat, dog and horse dander, and house dust mite) with the AlaTOP microtitre plate method (Diagnostics Products Corp., Los Angeles, CA, U.S.A.).

**EXHALED NITRIC OXIDE (ENO) MEASUREMENT**

NO concentration in the exhaled air was measured, prior to methacholine provocation, by the chemiluminescence method with LR 2000 nitric oxide gas analyser (Logan Research Ltd., Rochester, U.K.), as previously described (23). After calibration and autozeroing, subjects performed standardized exhalation from total lung capacity to residual volume against a target resistance of 4–5 cm H\(_2\)O and at an expiratory flow rate of 250 ml sec\(^{-1}\), with the help of a biofeedback monitor. The level of exhaled NO was based on analysis of the plateau portion of the exhaled NO curve and the average value of duplicate measurements was used for analysis.

**STATISTICAL ANALYSIS**

Data on lung function are presented as mean \pm SD values and analysed for statistical significance with one-way ANOVA and Bonferroni's multiple comparison test. Bronchial responsiveness and hyper-responsiveness, and exhaled NO levels are presented as median values with interquartile ranges (IQR) and analysed for statistical significance with Mann–Whitney U-test or Kruskal Wallis test with Dunn's multiple comparison test, as appropriate. Correlation coefficients were calculated using Spearman's rank method. Statistical significance was defined as a \(P\) value of less than 0.05.

**Results**

**AMP STUDY**

Neither the PD\(_{20}\) FEV\(_1\) nor the dose response ratio for methacholine was significantly different in the three groups (Figs 1 and 2). In contrast, only five of 18 skiers were hyper-responsive to AMP, compared to six of 15 non-steroid-treated and 10 of 14 steroid-treated asthmatics. Of the skiers who tested positive, three were atopic on screening. The responsiveness to AMP was increased in order of magnitude from ski asthma < non-steroid-treated < steroid-treated asthma (Fig. 2).

Baseline FEV\(_1\) (% predicted) was normal in all three groups. FEV\(_1\) was lower in the steroid-treated than in the non-steroid-treated asthmatics \((P=0.05)\) prior to bronchial provocation (Table 1).

**EXHALED NO STUDY**

Exhaled NO concentrations in skiers were not significantly different to those in controls [median (IQR) 6.5 (4.1–9.9) vs. 5.2 (4.2–6.5) ppb]. In asthmatics, the exhaled NO was three-fold greater than in skiers [median (IQR) 19.2 (5.1–25.6) ppb, \(P<0.01\) (Fig. 3)].

Exhaled NO was three-fold greater in asthmatics than in skiers with ski asthma (Fig. 4). There were no significant differences between skiers with and without ski asthma [median (IQR) 5.3 (2.8–6.9) vs. 7.2 (4.1–9.9) ppb]. Exhaled NO was two-fold greater in atopic than non-atopic skiers.
Fig. 2 Dose response ratio for (a) methacholine and (b) AMP in skiers and asthmatics. Abbreviations as in Fig. 1.

Fig. 3 Exhaled NO concentration in controls, skiers and asthmatics. Data presented as box and whiskers, Kruskal Wallis test with Dunn's multiple comparison test.

Fig. 4 Exhaled NO concentration in skiers with ski asthma and asthmatics. Horizontal bars : median values; Mann–Whitney U-test for statistical significance.

and four-fold greater in atopic than non-atopic asthmatics (Fig. 5).

Baseline FEV₁ (% predicted) was normal in all groups and was lower in skiers than in asthmatics and controls (Table 1). Nine (21%) skiers and all asthmatics were hyperresponsive to methacholine with a median (IQR) PD₂₀ FEV₁ of 3.5 (1.1–6.1) and 0.9 (0.3–3.1) μmol, respectively. The maximum individual PD₂₀ FEV₁ was 6.7 μmol in skiers and 7.81 μmol in asthmatics. The DRR for methacholine was lower in skiers than in asthmatics [median (IQR) 0.7 (0.4–1.3) vs. 15.8 (6.3–48.1), P < 0.001] and was not different from in controls [0.9 (0.6–1.4)]. Within the skier and asthmatic groups, DRR was not significantly different between subjects with and without atopy.

Of the three groups, exhaled NO concentration was significantly correlated to the dose response ratio (DRR) for methacholine in asthmatics only (N = 29, rho = 0.57, P < 0.01). Within the asthmatic group, the correlation between these two variables was significant in atopic subjects only (Fig. 6).

Discussion

In the present study, we have investigated two indirect markers of asthmatic airway inflammation in competitive cross-country skiers with ski asthma. Concomitant hyperresponsiveness to AMP was present in 28% of skiers with ski asthma and 55% of asthmatics. While exhaled NO was significantly increased in asthmatics only, we were unable to demonstrate any significant difference in exhaled NO concentration between skiers, with or without ski asthma, from that in healthy controls.

AMP is rapidly converted after inhalation to adenosine. In both atopic and non-atopic asthmatics, adenosine reacts with purine receptors located on the surface of pre-activated mast cells with an increased release of mediators...
such as histamine, prostanoids and leukotrienes (10,24,25,26). Hyper-responsiveness to AMP is considered to be specific for asthmatic subjects (27), but bronchoconstriction has been observed in non-asthmatic subjects with atopy after administration of a 25-fold greater dose of AMP than methacholine on a molar basis (28). While seven of 18 skiers (39%) were atopic, three of five AMP responders had atopy. A higher prevalence of atopy was observed in both asthmatic groups (69%). As the maximal administered dose of AMP in the present study was only 16-fold greater than methacholine on a molar basis, it is unlikely that the hyper-responsiveness in these skiers was due to an excessive dose of AMP. If the mechanism underlying the bronchoconstriction of AMP in skiers is similar to that in asthmatics, the present study would suggest that a minority of skiers with ski asthma have pre-activated airway mast cells.

The present study also suggests that additional information may be obtained with AMP challenge. Although we were not able to demonstrate any difference in bronchial responsiveness to methacholine in the three groups, we did demonstrate differences in responsiveness to AMP between the groups. Skiers had the lowest bronchial responsiveness to AMP, suggesting that the degree or type of airway inflammation in ski asthma is different from that present in mild intermittent asthma. It has been shown that inhaled budesonide has a greater effect on AMP-induced, than on methacholine-induced, bronchoconstriction in asthma, probably by reducing mast cell numbers and/or function (29). We have recently performed a placebo-controlled treatment study with inhaled budesonide. In that study we were unable to demonstrate any beneficial effect of inhaled budesonide treatment in skiers with ski asthma (submitted for publication). Whether responsiveness to AMP may define a subgroup of ski asthma subjects that may benefit from treatment remains to be clarified. However, even though the responsiveness of asthmatics to AMP has been shown to be reduced by inhaled corticosteroid treatment, it is of interest to note that the steroid-treated asthmatic group in the present study also had the greatest responsiveness to AMP. The reason for this is unknown, as these asthmatics were clinically apparently well controlled, and their lung function was within normal limits.

To our knowledge, hyper-responsiveness to this agent has not been reported in endurance athletes with asthma symptoms and methacholine hyper-responsiveness. Demonstration of hyper-responsiveness to AMP in 28% of skiers with ski asthma further supports our previous observation of a tendency to a greater degree of bronchial inflammation at bronchoscopy, in those skiers with ski asthma, and of an increased mast cell count in the bronchoalveolar lavage fluid of skiers compared to controls (30).

In contrast, although confirming previous observations of increased exhaled NO levels in asthmatics, the present
study suggests that NO appears to be of less value as a surrogate marker of inflammation in ski asthma, than it is in allergic asthma. Exhaled NO was consistently greater in atopic than non-atopic subjects in both groups, and a significant correlation with bronchial responsiveness was observed in atopic asthmatics only. Of the six skiers and 15 asthmatics with exhaled NO in excess of 15 ppb (Fig. 4), all were atopic, with five skiers and all asthmatics sensitized to perennial allergens, and with the remaining skier sensitised to grass pollen. The finding of an elevated exhaled NO concentration in atopic subjects in the present study is in accordance with other studies of asthmatic and non-asthmatic subjects with atopy (31,32). As allergic symptoms are less prevalent in skiers with atopic symptoms and methacholine hyper-responsiveness (21) measurement of exhaled NO has a limited place in the diagnosis and monitoring of the effect of treatment.

In addition to inflammatory changes at bronchoscopy, we have observed an inflammatory infiltrate in the mucosa of the proximal airways in a biopsy study of skiers (submitted for publication). An elevated NO concentration in asthmatics is associated with increased expression of inducible NO synthase (33). The absence of an elevated exhaled NO concentration in skiers raises the possibility that the airway inflammation in these athletes may be mediated by mechanisms other than those believed to be operating in allergic asthma.

In summary, bronchial challenge with AMP indicate a difference between skiers with ski asthma and atopic asthma, with the presence of pre-activated mast cells being a less prominent feature in ski asthma. Exhaled NO was not elevated in ski asthma, and appears to be more useful as a marker of atopic status than as a marker of inflammation in skiers.

References


