Cold air exercise screening for exercise induced bronchoconstriction in cold weather athletes

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ABSTRACT

Exercise Induced Bronchoconstriction (EIB) prevalence in cold weather athletes is high. Currently, no standardized cold air exercise provocation test exists. Thus we aimed to determine EIB prevalence using a Cold Air Test (CAT; 5 km outdoor running; $-15\,°C$) compared to the most common EIB screen the Eucapnic Voluntary Hyperpnea (EVH) test in cold weather athletes.

Sixteen (9 male; 20–35 years old) cold weather athletes completed EVH 72 h before CAT. Spirometry, Fractional Expired Nitric Oxide (FENO), respiratory symptoms were measured and atopy status was determined. Five and 7 participants were EIB + on the EVH and CAT, respectively. Level of agreement was 50% between tests. FEV_1 recovery was significantly prolonged and Peak Expiratory Flow was decreased after CAT compared to EVH. Predictive characteristics of EIB + included FENO > 12 ppb, FEV_1/FVC ratio (< 0.75) and BMI < 20. EVH does not always reflect EIB triggered by cold weather exercise. More research is required to understand the best EIB screens for cold weather athletes.

1. Introduction

Acute airway narrowing that occurs as a result of exercise is defined as Exercise Induced Bronchoconstriction (EIB) and is found in both asthmatic and non-asthmatic individuals (Parsons et al., 2013). It is the most common medical condition in both summer and winter sport athletes where prolonged high ventilation sport activity (training and competition) and "environmentally unfavourable" conditions both contribute to occurrence and severity (Kippelen et al., 2012). Unfavourable environmental conditions include chronic exposure to cold dry air (Carlsen, 2012) during exercise, typical of cold weather regions (Kennedy et al., 2016a,b). Long-term exposure to such conditions may lead to respiratory dysfunction such as chronic cough, wheeze and dyspnea (Koskela, 2007). In addition, the prevalence of EIB and respiratory dysfunction is higher in athletes who specifically compete in cold air endurance activities (Rundell et al., 2000) (e.g. Nordic skiing events).

High respiratory volumes in these environments causes water loss from the airways, which increases the osmolarity of airway surface lining (Koskela, 2007). This results in the contraction of bronchial epithelial cells and release of a number of pro-inflammatory mediators (e.g. nitric oxide (NO)), leading to airway smooth muscle constriction

(Ricciardolo, 2003). However, it is unclear what the NO exercise response in cold weather acclimatized athletes is and whether this could be predictive, or a more sensitive tool for diagnosis of EIB in these athletes. Thus, continued investigation of cold weather athlete's airway response to the environment they are required to train and compete in is important to provide accurate health recommendations for management of lung health and performance.

To diagnose EIB a number of airway provocation challenge tests exist (Randolph, 2011). These include the widely utilized Eucapnic Voluntary Hyperpnea (EVH) recommended by the IOC to assist in asthma/EIB diagnosis in elite athletes (Fitch et al., 2008). Laboratory exercise challenges (Rundell and Slee, 2008) have also been used to understand EIB in a variety of temperatures. The recommended protocols for EVH and laboratory exercise challenge tests are similar (high ventilation of dry air for 6 min in EVH test and high ventilation of dry to low humidity air for 6–8 minutes in an exercise challenge at room temperature). However, these tests are not strictly representative of the environmental demands of cold-weather exercise and competition. Typical field tests on the other hand have utilized a wide range of durations, modes of activity, exercise intensities and ambient humidity designed to provoke the airway (Helenius et al., 1998; Rundell and Slee, 2008; Carey et al., 2010). These types of challenges also lack the

specificity of a cold-dry air challenge and may contribute to low sensitivity of EVH testing to identify EIB in cold weather athletes (Rundell et al., 2000; Mannix et al., 1999).

Thus we aimed to examine a cold weather field test that was the same temperature for all participants, at a temperature ($-15\,°C$) previously known to induce bronchoconstriction (Kennedy and Faulhaber, 2018) and then compare those results to the EVH lab test. Through this exploratory examination of different EIB protocols we hope to provide improved understanding of screening for EIB in cold weather athletes, especially given the additional mechanisms involved in cold air exercise compared dry air inhalation found in lab based tests. In addition, we intended to understand if baseline characteristics might contribute to EIB in cold weather athletes by examining atopy profile (Bonini et al., 2009), common respiratory symptoms reported (Koskela, 2007) as well as anthropometrics (William et al., 2016).

We hypothesized that a cold weather field test would provide sufficient provocation to induce EIB as would the EVH test and that the level of agreement between the two tests would be high. We hypothesized that EIB positive participants would also have greater underlying airway inflammation as measured by Fractional Expired Nitric Oxide (FENO), have a stronger atopy profile (as measured by questionnaire), and report more respiratory symptoms.

2. Materials and methods

2.1. Recruitment and participant characteristics

Sixteen aerobically fit participants (9 males) between the ages of 20 and 35 with normal baseline FEV_1 and FVC values for their age, height and sex were recruited (Table 1). Participants whom had a respiratory infection, or had difficulty breathing at rest at the time of recruitment were excluded. Participants were also screened with the Leicester Cough Questionnaire to ascertain no chronic cough or respiratory symptoms associated with poor quality of life (their average score was 20.1 ± 1.0 out of a possible 21 where 21 is free of cough affecting quality of life). We did not exclude participants whom used or had used a bronchodilator or inhaled steroid. Two participants had been diagnosed as having asthma but were not currently using an inhaler or medication. Six other participants reported using a bronchodilator in the past however no participant regularly used a short acting or long acting bronchodilator at the time of the study (see Table 2 for which participants were identified as having asthma or used an inhaler). To be included participants had to be actively training and pursuing competitive goals and accustomed to high ventilation in cold weather during training and competition (Parsons et al., 2007). On average participants

exercised between 8–14 hours per week in the winter months preceding their participation in this study. Approximately 75% of the hours of exercise were outside and 25% indoors. All participants were required to be capable of running a five-kilometre race at near maximal intensity.

2.2. Experimental design

Participants were assessed on two separate visits with at least seventy-two hours between testing days. Testing day one included the gold standard screening lab test for EIB (Eucapnic Voluntary Hyperpnea (EVH) test). A Cold Air Trial (CAT) was completed on day two. To ensure no influence of cold exposure affecting pre-test measures, participants only walked from another building on campus or from a vehicle to the lab (total cold air exposure maximum 3 min) and refrained from extraneous physical activity/exercise (indoors and outdoors) prior to their arrival at the laboratory (Anderson et al., 2001). Participants were asked to refrain from medications that might influence lung function (24 h for short-acting β2-agonists and 72 h for inhaled corticosteroids).

At both visits pre-test assessments (baseline for each test day) for both FENO and spirometry were performed after 5 min of rest. On both visits, spirometry was measured immediately post EVH and CAT at 5, 10, 15, and 20 min. In instances where spirometry had not returned to pre-test values for that day (i.e. baseline) after 20 min, participants completed another measure every 10 min until spirometry measures had returned to baseline; in this project this included measures at 30 (2 participants) and 40 (2 participants) min post provocation. Post EVH and CAT, FENO was measured at 25 min post-test in all participants (including those whom might have had a 30 or 40 min spirometry measure). Follow up questions regarding the occurrence, frequency and severity of respiratory symptoms in the 24 h post CAT were completed online the day after. For data collection to occur, the temperature at the time of time trial was required to be $-15\,°C$ ($\pm 1\,°C$) as measured by the University meteorological weather station tracked on line by the investigators in real time. This approach ensured standardization of air temperature, however relative humidity was recorded but not controlled for in this design (Range 58–80 %).

Participants followed standard preparation guidelines for fluid and nutritional intake prior to heavy intensity exercise. We asked that no exercise was done prior on the same day as a scheduled test, and that no strenuous exercise indoors/outdoors that induced heavy ventilation was done in the 24 h leading into a test. The tests were completed during the months of February and early March. All participants provided written informed consent for experimentation with human

Table 1

Baseline characteristics for participants overall, range for overall sample and male and female participant means $\pm$ SD. Spirometry values were taken as pre-test values after participant had completed all questionnaires on first visit to lab (pre EVH test).

Characteristic	Overall mean $\pm$ SD	Range Overall (min – max)	Male mean $\pm$ SD	Female mean $\pm$ SD
Age, years	26.9 ± 4.8	20.0-35.0	25 ± 4	29 ± 3
Height, cm	173.2 ± 9.2	160.0-188.0	180 ± 5	165 ± 4
Weight, kg	67.1 ± 8.5	51.0-75.0	72 ± 5	60 ± 7
BMI	23.3 ± 2.1	18.6-25.9	22 ± 1	22 ± 2
FEV_1, L·min^{-1}	4.0 ± 0.8	2.7-5.4	4.5 ± 0.5	3.2 ± 0.2
FEV_1, % predicted	109.3 ± 14.4	87.9-137.3	115.4 ± 14.2	101.2 ± 9.9
FVC, L	5.4 ± 1.4	3.4-8.9	6.2 ± 1.3	4.1 ± 0.4
FVC, % predicted	138.9 ± 28.3	104.4-213.6	151.1 ± 31.1	123.1 ± 13.1
FEV_1/FVC ratio	74.9 ± 6.2	60.6-86.3	73.1 ± 6.9	77.2 ± 4.4
FEV_1/FVC ratio, % predicted	79.8 ± 6.6	64.2-92.5	86.7 ± 7.7	92.4 ± 5.8
FEF 25-75, L·min^{-1}	3.2 ± 0.6	2.2-4.3	3.5 ± 0.5	2.8 ± 0.5
FEF 50, L·min^{-1}	3.6 ± 0.7	2.5-4.9	3.9 ± 0.5	3.2 ± 0.6
PEF, L·min^{-1}	8.9 ± 1.9	5.5-11.8	10.0 ± 1.2	6.9 ± 0.9

Data reported as mean $\pm$ SD, (overall n = 16); range reported as minimum – maximum value for each measure; male (n = 9) and female (n = 7) participants reported as mean $\pm$ SD. BMI, Body Mass Index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF 25-75, forced expiratory flow at 25%–75%; FEF50, forced expiratory flow at 50%, PEF, Peak Expiratory Flow.

Table 2

Maximal decrease for FVC, FEV$_1$, FEF$_{25-75}$, FEF$_{50}$, PEF and FeNO measurements post Eucapnic Voluntary Hyperpnea (EVH) test and Cold Air Trial (maximal 5 km run at -15 °C). Delta changes are expressed as percent change from pre-test values. Range, Minimum, Maximum, Mean and Standard Deviations are provided for the entire sample (n = 16). Statistical significance (p) is provided for the pre-post difference for each measure and each condition as well as for comparing the post-test EVH and CAT FENO values based on the corresponding bilateral paired t-test.

Participant	FVC (%) EVH	FVC (%) CAT	FEV1 (%) EVH	FEV1 (%) CAT	FEF25-75 (%) EVH	FEF25-75 (%) CAT	FEF50 (%) EVH	FEF50 (%) CAT	PEF (%) EVH	PEF (%) CAT	FeNO (ppb) Pre-EVH	FeNO (ppb) Post-EVH	FeNO (ppb) Pre-CAT	FeNO (ppb) Post-CAT
1 (M)	2.6	4.4	2.6	32.3	3.1	39.2	0.3	37.3	−11.3	18.9	5.0	6.0	7.0	5.0
2 (M;P)	−5.3	−1.4	−8	*−13.1*	−12.9	−30.8	−8.5	−31.1	−4.1	−7.6	24.0	5.0	17.0	5.0
3 (M;P)	−1.7	−6.1	−5.4	−4.0	−14.2	−4.6	−13.4	−7.0	−3.9	−12.8	18.0	13.0	18.0	8.0
4 (M)	−0.4	−9.8	−2.8	−9.5	−4.1	−11.7	−6.3	−15.4	−7.3	−3.1	13.0	8.0	22.0	5.0
5 (M;P)	2.7	12.9	−0.9	5.0	−11.8	−2.6	−10.7	−6.5	−15.4	1.1	11.0	9.0	11.0	5.0
6 (F)	−11.3	−11.3	*−11.5*	−0.8	−16.1	−7.4	−19.9	−15.0	−43.2	−61.3	15.0	17.0	23.0	22.0
7 (F;P)	−13.6	−6.5	*−14.7*	*−13.4*	−21.4	−26.5	−18.4	−27.5	−15.7	−15.2	23.0	17.0	16.0	18.0
8 (M)	−15.2	−0.4	−1.6	4.8	−6.4	9.8	−18.3	4.9	−15.0	−0.2	10.0	5.0	5.0	9.0
9 (F;P)	−3.4	11.0	−4.9	7.7	−14.1	−41.1	−65.0	−4.8	−33.3	0.6	25.0	27.0	17.0	21.0
10 (F)	−24.2	−3.8	*−25.9*	*−27.8*	−23.5	−33.3	−26.0	−48.1	−50.7	−48.3	9.0	8.0	18.0	14.0
11 (M;A)	−1.7	−13.9	−0.9	*−12.8*	−5.1	−5.2	−7.7	−7.7	−5.1	−7.7	27.0	17.0	34.0	13.0
12 (M;P)	−8.1	0.1	*−11*	0.2	−26.0	6.0	−33.5	−10.5	−14.1	−11.1	5.0	5.0	5.0	5.0
13 (M)	−3.4	−18.8	−7.8	*−22.0*	−18.2	−26.1	−19.7	−24.8	−49.0	−18.8	22.0	17.0	11.0	5.0
14 (F)	0.2	−10.7	−4	*−15.5*	−13.9	−24.9	−12.9	−28.6	−6.7	−11.7	5.0	5.0	5.0	7.0
15 (F)	−3.8	4.2	*−16.2*	3.0	−34.7	2.7	−36.0	−1.9	−24.9	−10.0	62.0	46.0	85.0	64.0
16 (F;P)	−1.3	−0.4	1.1	2.0	4.9	3.0	3.6	−1.2	−2.0	−3.1	14.0	10.0	8.0	13.0
Min	−24.2	−18.9	−26.0	−27.8	−34.7	−41.1	−65.0	−48.1	−50.7	−61.4	5.0	5.0	5.0	5.0
Max	2.7	−13.0	2.6	32.3	4.9	39.2	3.7	37.3	−2.1	18.9	62.0	46.0	85.0	64.0
Mean	−5.5	−3.1	−7.0	−4.0	−13.4	−9.6	−18.3	−11.7	−18.8	−11.9	17.9	13.4	18.8	13.6
SD	7.3	8.8	7.5	14.2	10.4	20.4	16.5	19.0	16.5	19.0	13.9	10.7	19.3	14.7
P (pre –post)	0.00	0.20	0.00	0.30	0.00	0.05	0.00	0.03	0.00	0.02	0.01		0.03	
Effect size	0.753	0.352	0.933	0.281	1.288	0.470	1.109	0.616	1.139	0.626	0.324		0.269	
P between tests (pre -pre)	0.40		0.30		0.40		0.30		0.08		0.70[μ]		0.90[β]	
Effect size	0.294		0.244		0.215		0.369		0.397		0.357		0.298	

(A) = reported asthma in past; (P) = use of bronchodilator/steroid; M = Male; F = Female; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF 25-75, forced expiratory flow at 25%–75%; FEF50, forced expiratory flow at 50%; PEF, peak expiratory flow; FeNO, Fractional exhaled nitric oxide, EVH, Eucapnic Voluntary Hyperpnea; CAT, Cold Air Trial. Participants with a ≥10% fall in FEV$_1$ on either test are bolded and italicized. Pre-post means pre-post for that test (EVH or CAT); pre-pre means baseline between EVH and CAT; [μ] indicates the comparison of FENO pre-EVH to FENO pre-CAT; [β] indicates the comparison of FENO post-EVH to FENO post-CAT.

participants prior to starting the study. The privacy rights were respected at all times for all participants and the study received institutional research ethics: Health Research Board – Biomedical approval (Pro00062261, Approved February 8, 2016).

2.2.1. Description of the CAT

To achieve the goal of race pace intensity and effort, participants were asked to treat the CAT as a race. The start and finish were at the building which housed the laboratory of the investigators. Participants arrived at the lab and completed resting pre-test spirometry and FENO in the same manner as described for the resting measures for pre EVH test. A standardized warm up protocol on a treadmill indoors was performed (5 min walking at 6 km/hr at 1% grade and 20 min running at 1% grade at a Rating of Perceived Exertion (RPE) on the Borg Scale of 11–13 (Fairly Light to Somewhat Hard)). Participants did not cover their mouth during and at the end of the CAT participants proceeded indoors within 30 s to perform post spirometry measures. Session based RPE was also measured in all participants which is a valid measure of physiological intensity to understand whether the level of effort exerted was race pace effort.

2.2.2. Lab-based bronchial provocation test

An EVH test was completed on day one, which has been previously described in detail by Anderson (Anderson et al., 2001) and is considered the gold standard EIB screen in athletes (Holzer and Brukner, 2004). Participants breathed for six minutes at a target of 30 breaths/ minute with a respiratory duty cycle of 1:1 s (metronome) at a target tidal volume equal to baseline FEV$_1$. This approximated 85% of Maximal Voluntary Ventilation. Participants were provided visual feedback from a digital chart recorder (Labchart, ADInstruments, Colorado Springs, USA) to help them maintain desired tidal volume. An inspired

dry gas mixture of 21% O_2, 5% CO_2, balance N_2 was used and participants drew their breath from a 200 L reservoir via a 2 inch hose that was connected to a non-rebreathing mouthpiece (Hans Rudolph, Kansas, USA).

Routine spirometry was completed in the sitting position according to the American Thoracic Society guidelines (Miller et al., 2005). Spirometry was repeated using a validated, portable electronic spirometry device (Spirolab III Portable Desktop Spirometer). The main outcome measures were volume of air forcefully exhaled in 1 s, (FEV$_1$), forced vital capacity (FVC), the ratio of FEV$_1$ to FVC (FEV$_1$/FVC%), forced expiratory flow at 50% of FVC (FEF50) and the average forced expiratory flow during the mid (25–75%) portion of the FVC (FEF 25–75). Pre-post change in spirometry measures were calculated in raw units as well as maximum percentage change ((pre-exercise - minimum post exercise)/(pre-exercise value) x 100) based on previously published protocol (Stensrud et al., 2007). FENO (NIOX Mino, Morrisville, NC, USA) was assessed in accordance with the American Thoracic Society 2011 guidelines.

2.2.3. Questionnaires

Two questions from the European Community Respiratory Health Survey (ECRHS) previously used to determine cough sensitivity to exercise and environmental allergens in athletes were used (Kennedy et al., 2016a,b). The Allergy Questionnaire for Athletes (Bonini et al., 2009) determined atopic status and has been previously validated in athletes (Bonini et al., 2009). The occurrence, frequency and severity of respiratory symptoms were recorded for the following symptoms: cough, wheeze, chest tightness, excessive mucus formation, all which are common after intense exercise in athletes (Rundell et al., 2000) as well as "frog in your throat". Participants also described other respiratory symptoms not identified above.

2.2.4. Statistical analysis

Descriptive analysis is reported as means and standard deviations. Repeated measures ANOVA were used to determine the significance of reductions of in FEV_1, FVC, FEF 25-75, and FEF50 at the repeated post-test intervals relative to baseline separately for the EVH and the CAT test, respectively. Least significance differences pairwise comparisons were used to identify intervals with significant reductions.

The maximum differences between pre-test and post-test values on the EVH and CAT testing day, respectively for all the spirometry and FENO measurements were tested using paired-test using a bilateral hypothesis. The significance of the maximal differences between base-line pre-test and any post-test values on the EVH and CAT testing day were tested using paired-test using a bilateral hypothesis. Pre-post-test FENO values on the EVH and CAT testing day were also tested using a paired-test using a bilateral hypothesis.

The inter-test reliability of the maximum decreases measured in FEV_1 over the post-test recovery period was assessed using an intraclass correlation coefficient (ICC2,1) with a 95% confidence interval. Similarly, the inter-test reliability in the detection of EIB + results was assessed using a Kappa Coefficient with a 95% Confidence interval. To inspect if the magnitude of the differences between the maximum decreases measured in FEV_1 on both test varied as a function of the FEV_1 drop observed, a Bland and Altman plot with bias and 95% limits of agreement was prepared.

Comparing the FENO levels pre-test, height, weight or AQUA questionnaire scores between participants that were EIB + or EIB – on each test was done using independent sample T-tests. Pearson product-moment correlation tests were used to determine the relationship between anthropometric data (height and weight) and pre-test or delta change in spirometry and FENO measurements. Statistical analysis was performed using SPSS version 21. P-values below 0.05 were considered significant.

3. Results

3.1. Descriptive information

Descriptive statistics and pre-test spirometry on each day including predicted mean valves for FEV_1, FVC and FEV_1/FVC ratio baseline values are shown in Table 1. No participant presented values outside of the lower limit of normal on any spirometry measure as predicted based on their age, height and sex (Stanojevic et al., 2010). Baseline pre–test FENO values for the EVH and CAT test as shown in Table 2 were not significantly different.

3.2. Change in spirometry and FENO pre-post provocation

The EVH test caused a significant maximal drop from pre-test to any of the post-tests expressed as a percent of pre-test in FEV_1, FVC, FEF 25-75, FEF50 and PEF (Table 2). FENO was also significantly decreased post EVH test (Table 2). Eleven of the 16 participants had a normal airway response (0 to < 10% decrease in FEV_1), 4 had a mild response ($\geq$ 10 to < 25% decrease in FEV_1) and one had a moderate response to the EVH test ($\geq$ 25 to < 50% decrease in FEV_1) (Price et al., 2014). Peak Expiratory Flow (PEF) was significantly decreased at 5, 10 and 15 min (pairwise comparison; p = 0.001) post EVH test. Comparing to baseline, no other spirometry measures were decreased significantly post EVH test at any time point (results not shown).

The mean time for the 5 km time trial was 20.9 ± 2.8 min with an average session based RPE of 7.1 ± 1.3 (out of 10) which is described as "very hard" and is associated with exercise intensity above Ventilatory Threshold 2 (Seiler and Kjerland, 2006). Maximal FEV_1 and FVC decreases were not significant after CAT however the maximal decreases in FEF 25-75, FEF50 and PEF were significant post CAT (Table 2). The maximal decrease in FENO was also significant post CAT (Table 2). Six participants were EIB positive to CAT. Five had a FEV_1

fall between 10 and 25% and 1 had a moderate response ($FEV_1 \geq$ 25 to < 50% decrease) (Table 2).

In the post provocation time period it was found that, immediately after the 5 km time trial was completed, FEF 50 was significantly increased (Results not shown: ANOVA p = 0.02). No other spirometry measure (FVC, FEV_1, FEF25-75 or PEF) changed significantly after the 5 km time trial at any specific time point (immediately post, 5, 10, 15 and 20 min post exercise). The time point at which participants recorded the greatest drop post provocation was highly variable. Eleven of 16 participants recorded the greatest drop in FEV_1 post provocation within 20 min of completing the 5 km time trial. Three reported their lowest recordings at 30 min post CAT and 2 participants reported their lowest recordings at 40 min post CAT.

3.3. Comparison of provocation test results

Visual inspection of maximum reduction in spirometry values (FVC, FEV_1, FEF 25-75, FEF50, PEF) between the EVH and the CAT test, indicated the EVH test maximum delta was lower for all spirometry measures compared to the CAT. However, statistically the maximum delta's were not significantly different between tests (Table 2).

Inter-test intraclass Correlation Coefficient for FEV_1 max delta was poor (0.4) with a (95% Confidence Interval: 0.0 to 0.8). Level of agreement displayed as Bland Altman plot showed that participants with a smaller combined mean drop had larger drop in the EVH test while patients with the largest combined average max drops had their largest drop on the CAT test (Fig. 1).

The Kappa reflecting the level of agreement for the detection of EIB positive cases using the EVH test and the CAT was 0.0 for FEV_1 max delta (95% Confidence Interval: 0 to 0.2). Only two participants had a sustained fall in FEV_1 greater than 10% on both the EVH and CAT tests (Fig. 1). Using the EVH as the reference test we observed 18.8% of false negative results and we observed 33.3% of false positive results by using the CAT. Five participants with a pre-test baseline FEV_1/FVC ratio of less than 0.75 were found to be EIB negative on the EVH test ($\geq$ 10% drop in max FEV_1) but EIB positive from the CAT (Fig. 2). Two participants whom were EIB positive post EVH test had baseline FEV_1/FVC ratio of less than 0.75 and one of those participants had the greatest decreases in FEV_1 for both EVH and CAT provocation tests (see open blue circle, Fig. 2).

The mean group average for FENO decreased significantly in both tests (paired t-tests p < 0.00, Fig. 3). Decreases were not observed in all participants: only 12/16 participants' FENO decreased post EVH test and 10/16 decreased post CAT with only 8/16 participants decreased after both tests. There was a decrease in FENO in both EIB positive and EIB negative groups on each test day however the decrease was not different between groups (paired t-test: p > 0.05). There were no significant differences in resting pre-test FENO between EIB positive and EIB negative participants on either test day (independent t-tests
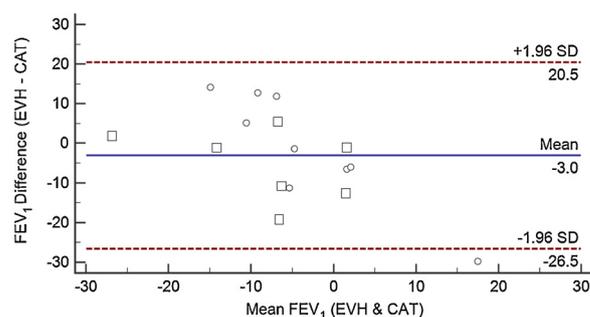

Fig. 1. Level of agreement for max delta change in FEV_1 between EVH and CAT tests using a Bland Altman plot (limits of agreement shown as 95% confidence intervals). Males and females are identified where circles are male and square symbols are female.
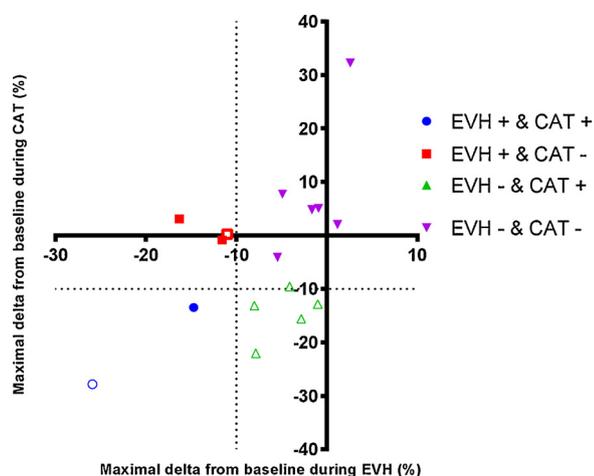
Fig. 2. Max delta decrease in FEV_1 during Eucapnic Voluntary Hyperpnoea (EVH) and Cold Air Trial (CAT) which was an outdoor 5 km running time trial at -15 °C. Data expressed as percent change from pre-test for both test days. FEV_1, Forced Expiratory Volume in 1 s; CAT is Cold Air Trial; "+" indicates participant was Exercise Induced Bronchoconstriction (EIB) positive on that test measured as a fall of $\geq$ 10%; "-"means participant was Exercise Induced Bronchoconstriction (EIB) negative on that test measured as a fall of < 10%. Participants with unfilled symbols had baseline FEV_1/FVC ratio $\leq$ 0.75 pre-test on both test days.

$p > 0.05$, Fig. 3).

Lower height was correlated to the max reduction in FEV_1 on the EVH test ($r = 0.44$; $p = 0.04$) and lower weight was correlated to nadir in FVC, FEF50 and PEF for the EVH test as well ($r = 0.50$, 0.49 and 0.43 respectively; $p < 0.05$). Height and weight were also correlated with change in FENO after CAT (height: $r = -0.63$, weight: $r = -0.63$; $p < 0.01$). Height and weight were significantly less in EVH EIB positive participants compared to EVH EIB negative participants (10.0 kg less and 11.3 cm shorter; independent t-test, $p < 0.05$).

Participants scored on average 7.3 ± 6.4 on the AQUA questionnaire and 56% of all participants had a score > 5 which is predictive of allergy (Table 3). EVH EIB negative participants had a significantly worse atopy score than EIB positive participants (independent t-test; $p < 0.05$). The most common triggers of cough were tobacco smoke (56.3%) and exercise (50% cough within 1 h of exercise cessation) (Table 3). Cough was the most common reported symptom post CAT and of those participants reporting cough (n = 13), cough/coughing bouts occurred on average 18 times in the 24 h post exercise. Excessive mucus was also common (reported expulsion of mucus was 3.2 ± 2.9 times in the 9 reporting mucus issues) and sore throat was felt on average 9.6 ± 14.7 times in those who had a sore throat (5 participants). Most participants (n = 13) reported that they

could exercise at -15 °C for a "few hours" (Table 3).

4. Discussion

EVH testing is recommended as the primary screening test for EIB for high ventilation and cold weather athletes (Fitch et al., 2008). However, we demonstrate low agreement between EVH testing and a standardized cold weather field test. As our field test was more specific to training and competition environments, this data highlights the need for similar specificity for identifying EIB in cold-weather athletes. Further, our results show that a baseline FEV_1/FVC ratio of < 0.75 may be additional criteria to assist in EIB determination whether it is a lab or field bronchial provocation test.

Although the importance of a low FEV_1/FVC (< 0.75) with a normal FEV_1 in athletes remains unclear, it is important to note that all of our cold weather participants (Table 1) were well below the expected FEV_1/FVC ratios of 0.88-0.89 compared to other well trained endurance sport athletes (Durmic et al., 2017). It may be that those with a low ratio have underlying constriction at rest which is exacerbated to a greater degree via provocation. This underlying constriction associated with a low resting FEV_1/FVC ratio also seems to be exacerbated to a greater degree with a cold air exercise provocation test based on our results. Specifically, of 8 participants whom had a baseline < 0.75 FEV_1/FVC ratio, 6 participants were cold air positive compared to one participant whom was EVH positive and one participant whom was positive on both tests. In addition, 5 of these 7 participants whom were CAT positive using a previously published EIB diagnosis criteria of > 12 ppb for adults were correctly predicted to have EIB based on resting FENO (Price et al., 2014). Thus, our results illuminate a potential connection of higher than normal resting FENO and baseline spirometry characteristics (FEV_1/FVC < 0.75) where this combination may increase susceptibility to EIB in cold weather exercise. However, further research on underlying athlete characteristics and response to cold air exercise is required if CAT is to be recommended as a gold standard screen in cold weather athletes.

We think that the CAT was sufficient in length and intensity to ensure that cold air ventilation caused bronchial provocation. The duration of our CAT challenge is longer than previous studies that have used lab and field cold air exercise challenges (Rundell and Slee, 2008; Carey et al., 2010). Relative to other research that involved free running in cold temperatures the occurrence of > 10% drop in FEV_1 was higher in our study (37%) compared to a 2000 m run at -6.6 °C (Helenius et al., 1998) or an 8 min run at different cold temperatures (-9.8 $\pm$ 10.6 °C) (Carey et al., 2010). However, compared to projects whom completed sport specific field tests (other than running) in a variety of temperatures and durations we had similar EIB occurrence compared to figure skaters (Mannix et al., 1999) and healthy cold weather athletes (Rundell et al., 2000). To our knowledge our field based CAT provided the greatest potential exposure to prolonged heavy
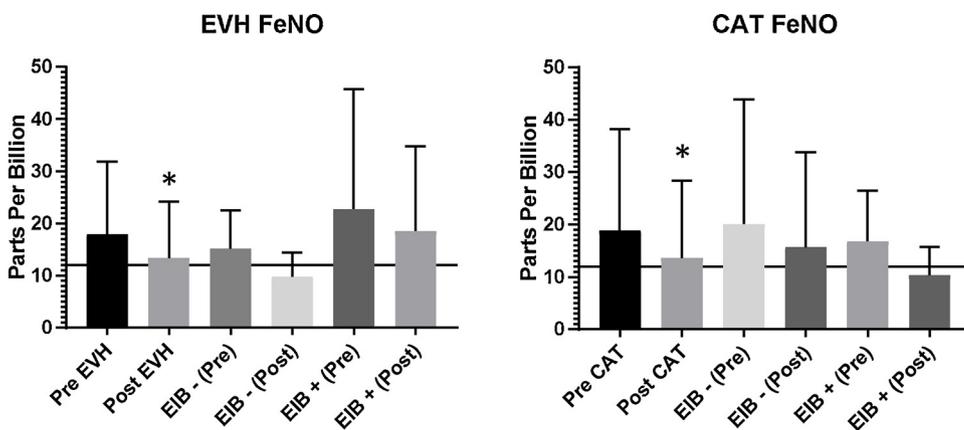

Fig. 3. Fractional exhaled nitric oxide (FeNO) expressed as parts per billion post pre and post provocation for both Eucapnic Voluntary Hyperpnoea (EVH) and Cold Air Trial (CAT) which was an outdoor 5 km running time trial at -15 °C. Data expressed as mean $\pm$ SD. * indicates significant decrease from Pre test value. "+" means participant was Exercise Induced Bronchoconstriction (EIB) positive on that test measured as a fall of $\geq$ 10%; "-" means participant was Exercise Induced Bronchoconstriction (EIB) negative on that test measured as a fall of < 10%; CAT, Cold Air Trial. Solid line indicates 12 ppb which is the threshold for positive predictive power of EIB based on current guidelines.

Table 3

AQUA questionnaire, prevalence of chronic cough from the modified ECRHS questionnaire and reported responses to cold, and respiratory symptoms experienced in the 24 h post Cold Air Trial (5 km maximal intensity run outdoors at – 15 °C).

	Atopy	All Groups		EVH Positive		EVH Negative	
		Prevalence (%)	Average Values (n = 16)	Prevalence (%)	Average Values (n = 5)	Prevalence (%)	Average Valves (n = 11)
	AQUA	56.3	7.3 ± 6.4 (0-18)	20	3.4 ± 3.1 (0-8)	63.6	9.0 ± 6.7 (0-18)
Exercise related	During Exercise	18.8		0		27.3	
	Within 1 Hour after	50.0		20		63.6	
	Between 2 and 8 hours after exercise	25.0		0		36.4	
Time of day	After you get up in the morning	6.3		0		9.1	
	During the night	6.3		0		9.1	
Irritants	When you are exposed to tobacco smoke	56.3		40		63.6	
	Exposed to other irritants/strong odors	25.0		20		27.3	
	Exposed to dusts	31.3		20		36.4	
	Exposed to tree pollens (spring)	18.8		0		27.3	
	Exposed to grass pollens (summer)	12.5		0		18.2	
	Exposed to ragweed (Aug-Sept)	18.8		0		27.3	
	In the presence of domestic animals	6.3		0		9.1	
	Other occasions	50.0		40		54.5	

Reported responses to cold, and respiratory symptoms experienced in the 24 hours post Cold Air Trial (5 km maximal intensity run outdoors at – 15 °C) are found below.

	Prevalence (%)	Average Values (n = 16)	Prevalence (%)	Average Values (n = 5)	Prevalence (%)	Average Valves (n = 11)
Did you feel safe exercising at this temperature? [*] (Yes)	100.0		100.0		100.0	
How long do you think you can exercise at this temperature?	6.5	1 hour	20		0	
	81	A Few Hours	60		91	
	12.5	All Day	20		9	
Cough	81.3 (n = 13)	18.5 ± 22.9 (2-72)	60 (n = 3)	26.7 ± 20.8 (10-50)	90.9 (n = 10)	16.0 ± 24.0 (3-72)
Wheeze	12.5 (n = 2)	1.5 ± 0.7 (1-2)	0.0 (n = 0)	0 ± 0 (0-0)	18.2 (n = 2)	1.5 ± 0.7 (1)
Chest tightness trouble breathing	18.8 (n = 3)	2.7 ± 1.5 (1-10)	20.0 (n = 1)	3.0 ± 0 (3)	18.2 (n = 2)	2.5 ± 2.1 (1-4)
Excessive mucus	56.3 (n = 9)	3.2 ± 2.9 (1-15)	40.0 (n = 2)	4.5 ± 2.1 (3-6)	63.6 (n = 7)	2.9 ± 3.2 (1-10)
Frog in your throat	18.8 (n = 3)	7.3 ± 7.1 (1-35)	40.0 (n = 2)	10.5 ± 9.9 (6-15)	9.1 (n = 1)	1.0 ± 0 (1)
Sore throat	31.3 (n = 5)	9.6 ± 14.7 (1-1)	40.0 (n = 2)	22.5 ± 17.7 (10-35)	18.2 (n = 3)	1.0 ± 0 (1)
Other symptoms #	6.3 (n = 1)	1.0 ± 0 (0)	20.0 (n = 1)	1.0 ± 0 (1)	0 (n = 0)	0 ± 0 (0)

[*] at a temperature of -15 °C; # other symptoms included one participant reporting burning in chest feeling; respiratory symptoms reported as both prevalence of the symptom expressed in percent with raw frequency reported in brackets (prevalence column) and as average values (frequency averaged only in those with a respiratory response post 24 h of the CAT with range reported in brackets below).

ventilation (exposure was at least 17 min at maximal exertion) in cold dry air although others have completed lab based CAT's at exactly or at colder temperatures than −15 °C (Kennedy and Faulhaber, 2018). Paradoxically, despite this large cold air ventilation stimulus approximately 70% of our participants had a normal airway response and those whom took longer to finish the 5 km did not invoke EIB to a greater degree. This supports previous research that has shown the length of the field based CAT does not significantly change the FEV_1 decrease post exercise trial (6 to > 25 min duration) (Rundell et al., 2000). Thus, it is likely that as long as intensity induces heavy ventilation a duration of at least 6 min is sufficient to provoke EIB in cold true winter sport athletes (Rundell and Slee, 2008) or active individuals whom are exposed to cold weather training and competition environments (Carey et al., 2010).

The typical time course of recovery of FEV_1 post exercise challenge in EIB positive individuals has been cited as reaching a minimum at 10 min and then improving to pre-test baseline by 30 min (Rundell and Slee, 2008). Our participants post 5 km time trial reached their maximum decrease in FEV_1 at 16.2 ± 12.7 min (range 0–40 min) and those whom were EIB positive for the 5 km time trial had their maximum decrease at 21.7 ± 16.4 min (individual responses as follows: 40 min (2), 30 min (1), 20 min (1), and immediately post (2)). In comparison, the EVH test maximum FEV_1 decrease occurred on average at 8.8 ± 6.2 min indicating that cold air may affect the time course of maximal provocation compared to EVH testing. Furthermore, there was no significant correlations between the "time point at which the max delta was achieved" for the EVH test and the CAT for any spirometry

measure. The latter may indicate that the same person exposed to different provocation stimuli has different time courses of maximal provocation detection. In other words different provocation tests may induce different provocation responses in the same person making someone positive in one test and not in the other test. Furthermore these results encourage following individuals for longer than 20 min post provocation to ensure the low point is determined.

Applied to repetitive high intensity cold weather sports such as ice hockey or a team sprint competition in Nordic skiing, it may be that those with slower recovery may be at a disadvantage in the latter portions of a race or game. This is a working hypothesis that should be explored in future investigations with direct implications to athlete welfare and performance. Our participants were also required to return to a warm air environment as soon as possible after the exercise trial which is not typical of cold weather competitions (i.e. competitors don't immediately return to indoors post-race and typically do a cool down outside before returning indoors). Paradoxically return to indoor air temperature has been shown to exacerbate drying of the airway after exercise in cold air previously defined as the "burden of rewarming"(Farley et al., 1988). Furthermore, this burden is greater post cold air exercise compared to a non-exercise lab based hyperventilation challenge, thus the overall longer time course to recovery post exercise trial might be explained by this phenomenon. Our observed frequency of cough in the 24 h post exercise (range: 2–72 instances of cough) and time to nadir post CAT were significantly correlated for FVC, FEV_1, FEF25-75 and FEF 50 (r = 0.73, 0.47, 0.57, 0.58 respectively). This finding implies that the length of time that lung

function is altered post cold exercise is a factor associated with cough in the days after cold air exercise. Based on our results and high incidence in post cross country ski race cough previously reported (Kennedy et al., 2016a,b) more research investigating the influence of ambient temperature on lung function recovery and cough post heavy intensity cold air exercise is encouraged.

The measurement of FENO as "an indirect marker for up-regulation of airway inflammation" (Dweik et al., 2011) was useful to understanding if baseline airway inflammation was a contributing factor to the EIB response to either provocation test. To our knowledge those EIB positive participants with significantly higher FENO pre-test may be due to greater chronic epithelial damage (Carbonnelle et al., 2002) leading to additional dehydration during high ventilation and subsequent smooth muscle constriction causing EIB (Anderson and Kippelen, 2008). In addition, it has been suggested that less airway surface area due to greater airway constriction may reduce FENO post provocation (Terada et al., 2001). Our results don't reflect this idea (our EIB positive participants had significantly larger post provocation values in both tests and the variability in the resting FENO is very important (see Fig. 3) possibly limiting the interpretability of the change scores). However, our findings reflect previous cold weather exercise challenges (running at $-10\,°C$) where FENO overall is reduced post exercise in well trained males (Therminarias et al., 1998). It has been recommended that FENO testing before and after a standardized provocation test may more accurately diagnose individuals whom are prone to EIB (Price et al., 2014). Our findings would support this recommendation, however it is suggested that both lab and field lab test in combination with pre-post test FENO might improve screening for EIB especially in cold weather athletes.

Our participants atopy profiles based on the AQUA questionnaire indicated that most of our EVH positive participants did not have a score that is indicative of atopy (average score was 3.4 where a score of > 5 indicates potential atopy; 2/5 EVH positive participants were > 5). This finding is contrary to other research which has found that AQUA scores of > 5 are associated with airway hyper responsiveness in athletes (Helenius and Haahtela, 2000). In addition, it was the EVH negative participants whom scored as more atopic, meaning despite greater sensitization to aeroallergens, their response to EVH (the reference provocation test) (Fitch et al., 2008) was not positive.

Furthermore EVH negative participants reported more environmental irritants and greater prevalence of cough post exercise (Table 3), which means prevalence of symptoms and severity of atopy is not necessarily connected to being EIB positive with a standardized provocation test. Furthermore, those with the greatest AQUA scores of 18, 13, 12, and 10 were CAT positive suggesting that environmental stress and atopy are more aligned in cold weather athletes. In keeping with the hypothesis that cold air exposure is a long term hazard of winter sport (Helenius et al., 1998), these 4 participants also had the most significant cold exposure over their sporting careers (male elite cyclist/ski mountaineer, world class female alpine ski racer and 2 elite cross country skiers) all with a minimum of 15 years exercise training and competition in cold environments.

EIB EVH positive participants included the 3 lightest and shortest participants in the study (all female with mean BMI of 20.1 and FVC of 3.0 L). We hypothesized that their anthropometrics caused additional shear stress as shown previously in female high ventilation athletes (McClaran et al., 1985). This additional shear stress has been attributed to small stature athletes. The authors defined this ventilation limitation during exercise as operating at the maximal limit of their "exercise tidal flow-volume loops" compared to larger stature athletes (Verges et al., 2005). This means anyone with a ventilation limitation has greater potential airway stress than those without ventilation limitation (Verges et al., 2005). Thus in our study small stature and subsequent small lungs may have also affected underlying airway inflammation because these 3 participants also had elevated resting FENO values of 15, 23 and 62 ppb pre EVH test.

The utility of the EVH test for screening in previously undiagnosed EIB athletes has been shown (Dickinson et al., 2011), but questions about reliability, sensitivity and specificity have also been raised recently (Hull et al., 2016) including our own study which found EVH inter-day reliability in swimmers to be poor (Kennedy et al., 2016a,b). These results were aimed at determining whether cold weather EIB would produce the same diagnosis as the common gold standard EVH EIB screen, and we found low agreement as previously described. We did find that the interval to observing the maximal drop in FEV_1 is extended post CAT compared to warm air lab test. In CAT EIB positive participants we found more of a cluster of previously reported predictive features: a) high pre-test FENO levels with b) < 0.75 FEV_1/FVC ratio and c) identified allergic disease based on atopy questionnaire. This suggests that a CAT test may provide a more specific response to EIB in cold weather than an EVH test.

In conclusion, -15 °C was likely a sufficient stimulus for bronchial provocation because air water content is very small at -15 °C (Cole, 1953). This means the combinative effect of the cold and dry air ventilation likely provoked the airway (Kennedy and Faulhaber, 2018). However some limitations of this study include the fact that we only evaluated one cold air temperature for the CAT. Thus, we can't infer that CAT tests at other temperatures (colder or warmer than -15 C) would provide similar EIB responses to a 5 km running trial. Furthermore, we used distance instead of time for the CAT which meant that participants had different lengths of cold air ventilation. Although, our results don't reflect that more time to complete the 5 km (i.e. more cold air exercise exposure) exacerbated EIB post CAT, the lack of standardized cold air time is a limitation of the study. We also did not make a comparison between male and female participants because we did not have sufficient number of male and female participants to draw any appropriate statistical differences between genders. Thus, these results provide preliminary data that requires further investigation of CAT free running at different lengths (shorter), different temperatures (both colder and warmer) and how different CAT's influences male and female cold weather athletes.

Declaration of Competing Interest

None.

Acknowledgements

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