The Effect of 400 µg Inhaled Salbutamol on 3 km Time Trial Performance in a Low Humidity Environment

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Abstract
The Objectives of the study were to investigate whether 400 µg inhaled salbutamol influences 3 km running time-trial performance and lung function in a low-voluntary hyperpnea positive (EVH+ve) and negative (EVH-ve) individuals. Fourteen male participants (22.4 ± 1.6ys; 76.4 ± 8.7kg; 1.80 ± 0.07 m); (7 EVH+ve; 7 EVH-ve) were recruited following written informed consent. All participants undertook an EVH challenge to identify either EVH+ve (↓ FEV1>10%) or EVH-ve (↓ FEV1<10%). Participants performed three separate 3 km running time-trials in a low-humidity (20-25%) environment on a non-motorized treadmill, 15 minutes following inhalation of salbutamol (400 µg), placebo (non-active inhalant) or control (no inhalant), in a randomized, single-blind, repeated measures design. Forced vital capacity maneuvers were performed at baseline, 10 minutes post inhalation and post time-trial. Time to complete 3 km and lung function data were analyzed using mixed model repeated measures ANOVA. Significance was assumed at p < 0.05. All EVH+ve participants had FEV1 falls from baseline between 10-25% post-challenge. There was no difference in performance time between trial conditions in EVH+ve (1012.7 ± 129.6s; 1002.4 ± 123.1s; 1015.9 ± 113.0s) (p = 0.074) and EVH-ve (962.1 ± 99.2s; 962.0 ± 76.2s; 950.8 ± 84.9s) (p = 0.401) groups for salbutamol, placebo and control trials, respectively. Exercising heart rate was significantly higher (p = 0.05) in the salbutamol trial (183 ± 8 beats min⁻¹) compared to control (180 ± 9 beats min⁻¹) with a trend towards significance (p=0.06) in the placebo trial (179 ± 9 beats min⁻¹) for the pooled groups, no differences were seen between trials in groups individually. There was an increase in FEV1 in both EVH+ve (4.01 ± 0.8L; 4.26 ± 0.7L; 4.25 ± 0.5L) and EVH-ve (4.81 ± 0.4L; 5.1 ± 0.4L; 5.1 ± 0.5L) groups which was significant post-inhalation (p = 0.01; p = 0.02), but not post-time-trial (p = 0.27; p = 0.06), respectively, following salbutamol. EVH+ve participants did not demonstrate significant falls (>10% from baseline) in FEV1 following any time-trial. Administration of 400µg inhaled salbutamol does not improve 3 km time-trial performance in either mild EVH+ve or EVH-ve individuals despite significantly increased HR and FEV1.

Key words: Asthma, exercise, bronchoconstriction, ergogenic, bronchoprovocation

Introduction
Athletes are more susceptible to exercise induced bronchoconstriction (EIB) than the general population, with those affected being permitted to use up to 1600 µg (max of 800 µg in a 12 hour period) of inhaled salbutamol per day on an as needed basis for the relief of symptoms (Dickinson et al., 2006; 2011; Molphy et al., 2014; WADA, 2017). Inhaled salbutamol is the most common therapy used by athletes to provide acute prevention and reversibility for EIB (Fitch, 2006).

The eucapnic voluntary hyperventilation (EVH) challenge is recognized as a sensitive and specific indirect airway challenge to assist in the diagnosis of EIB in athletic populations (Parsons et al., 2013). When EVH challenges are used as part of a screening program for EIB in athletes, some may present with an EVH positive challenge (EVH+ve) without having any previous history of EIB (Dickinson et al., 2006; Molphy et al., 2014). Our groups previous work has demonstrated that some athletes with a positive EVH challenge do not present with EIB following a field based exercise challenge (Dickinson et al., 2006). Recently Price et al., (2015) demonstrated that mild EVH challenge responses are not repeatable, demonstrating the transient nature of mild EIB. Moreover, the environment in which sporting performance takes place can be a contributing factor for EIB, perhaps individuals with mild EVH+ve challenges would exhibit with EIB in a more bronchoprovocative environment, such as that of low humidity (Sue-Chu et al., 2012).

Limited data exist to suggest whether exercise performance is affected in athletes with no history of EIB, who present with a mild EVH+ve challenge (10% - 25% fall in FEV1; Price et al., 2014). Performance in time trials to exhaustion can improve considerably (50%) when asthmatic patients receive conventional inhaled corticosteroid therapy, largely due to an improvement in lung function and protection against bronchoconstriction (Haverkamp et al., 2007). It is therefore reasonable to assume that athletes with a mild EVH+ve challenge will experience improved endurance performance if they inhale salbutamol prior to exercise. However, Koch et al., (2015a; 2015b) reported inhalation of 400 µg salbutamol prior to 10 km cycling did not influence performance in EVH+ve cyclists. The 10 km cycling trial was completed in laboratory conditions, which has been shown to be an environment that is not particularly provocative for EIB (Dickinson et al., 2006) and perhaps in a more bronchoprovocative environment the studies by Koch et al., (2015a; 2015b) would have seen a performance decrement in EVH+ve cyclists. Accordingly, the purpose of this study was to investigate the effect of 400 µg of inhaled salbutamol on 3 km running time-trial performance in an EIB provocative environment (humidity 20-25% - the minimum humidity attainable in the environmental
Low humidity asthmatic exercise performance

(chamber) in EVH+ve and EVH negative (EVH-ve) individuals, in line with the notion outlined by Sue-Chu et al., (2012) that dry air is more provocative for EIB.

Methods

Participants
Following ethical approval from Liverpool John Moores University research ethics committee (Ethics No. P13SP041), 14 male participants (age: 22.4 ± 1.6 years; weight: 76.4 ± 8.7 kg; height: 1.80 ± 0.07 m) volunteered to participate in the study providing their written informed consent. All participants were in good health, non-smokers and took part in recreational sport and exercise activities for at least 3 hours per week. No participant had previously been diagnosed with asthma and/or EIB, all participants were free from chest infection for at least two weeks prior to testing. Participants were informed about the nature and the risks of the experimental procedures before their informed consent was obtained.

Participants completed an EVH challenge to identify them as either EVH+ve or EVH-ve. Following two familiarization sessions participants completed 3 km running time trials on three occasions over three consecutive weeks, to allow sufficient wash-out and recovery. Prior to each 3 km time trial participants either inhaled 400 µg salbutamol, a placebo (inactive inhalant) or nothing (control); the 3km time-trials were randomized using a Latin square design.

Eucapnic Voluntary Hyperpnoea (EVH) Challenge
All participants undertook maximal flow-volume maneuvers using a spirometer (Microlab ML3500, Cardinal Health, Basingstoke, UK). Flow-volume measures recorded from each maximal flow-volume loop were; Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), FEV1/FVC ratio (FEV1/FVC%), Peak Expiratory Flow (PEF) and forced expiratory flow between 25% and 75% of FVC (FEF25–75%). Three maximal flow-volume loops were measured to gain baseline measures and were accepted in accordance with European Respiratory Society and American Thoracic Society criteria (Miller et al., 2005).

If FEV1 was above 70% of the predicted value, participants completed an EVH challenge (Anderson et al., 2001). The EVH challenge required participants to maintain target minute ventilation (\(\dot{V}_E\)) of 85% of their predicted maximal voluntary ventilation rate (MVV) for 6 minutes, calculated by multiplying their resting FEV1 by 30. Participants inhaled air from a compressed gas cylinder (19 °C and 2% humidity) containing 21% Oxygen, 5% Carbon Dioxide and 74% Nitrogen, via a two way valve. Expired air passed through a dry gas meter to enable \(\dot{V}_E\) to be calculated. Following the completion of the EVH challenge maximal flow volume loops were measured in duplicate at 3, 5, 7, 10 and 15 minutes with the best FEV1 for each time point being recorded. If participants FEV1 fell ≥10% from baseline on two consecutive time points following the EVH challenge they were deemed EVH+ve. Once consecutive falls of 10% or more in FEV1 from the resting value were observed, participants inhaled 200 µg salbutamol, with spirometry measured 10 minutes post inhalation to confirm bronchoconstriction was reversible. Participants who did not experience a ≥10% fall in FEV1 were placed in the EVH-ve group.

3 km Running Time-Trial
Following two familiarization sessions, each participant completed a 3 km time-trial, on a Woodway Curve non-motorized treadmill (Woodway, Wisconsin), on three occasions in a randomized, single blind (salbutamol and placebo trials only), repeated measures design with a minimum of 7 days between trials (see Figure 1), a-priori power calculations for the 3 km running time-trial predicted that for an expected completion time of 1000 seconds with a standard deviation of (2%) 20 seconds, a sample of size of 6 would significantly (p<0.05) predict a (2.5%) 25 second change in performance with 80% power. The 3 km time-trials were performed in an environmental chamber (Sporting Edge, UK) at 18 °C, 20.9% O2, 20%-25% humidity.

Prior to each 3 km time-trial participants completed resting maximal flow-volume loops, performed in triplicate. Participants then inhaled (via pocket chamber) either four x 100 µg Salbutamol (400 µg), four inhalations of non-active inhalant (placebo), or control (nothing inhaled). Ten minutes post-inhalation spirometry was repeated, before the completion of a standardized warm-up (5 minutes on a motorized treadmill at 10 kph); participants then began the performance time-trial on the curve non-motorized treadmill. Every 0.5 km of the 3 km time trial, heart rate (HR), oxygen consumption (\(\dot{V}O_2\)), carbon dioxide production (\(\dot{V}CO_2\)), respiratory exchange ratio (RER) and minute ventilation (\(\dot{V}_E\)) were recorded using

![Figure 1. Schematic of the protocol used for each laboratory visit.](image-url)
the Oxycon online gas analysis system (Oxycon, Carefusion, Kent, UK), as well as rating of perceived exertion (RPE) using the Borg Scale (Borg, 1982). The Oxycon sensor was connected to a rubber mouthpiece and participants exercised whilst wearing a nose clip, this was to avoid a humid microclimate that may have occurred within a facemask. During each time trial the only feedback available to the participant was the distance covered. Blood lactate was analyzed via finger-tip capillary blood sample taken immediately post time-trial (Lactate Pro, Arkay Inc, Finland), followed by the measurement of maximal flow volume loops in triplicate at 5 minutes post time-trial. The measurement of the social sciences (SPSS v21, IBM, New York) was used to compare between groups post salbutamol administration. Significance was set at p < 0.05 for all analyses. All data were reported as mean (±SD) unless otherwise stated. Statistical analysis incorporated a two-way repeated measures analysis of variance (ANOVA) to compare change in lung function with 80% power.

**Statistical analysis**

Statistical analysis incorporated a two-way repeated measures analysis of variance (ANOVA) to compare completion times, HR, \( \dot{V}_E \) and RPE between groups and trial conditions during time-trial performance and blood lactate levels post-exercise, a bonferroni correction was applied to correct for multiple comparisons. Spirometry measurements were analyzed using a mixed model repeated measures ANOVA to compare between groups, between conditions and between time-points. Two-way repeated measures ANOVA was used to compare \( \dot{V}_E \) between groups post salbutamol administration. Significance was set at p < 0.05 for all analyses. All data were reported as mean (±SD) unless otherwise stated. Statistical analysis was performed using the statistical package for the social sciences (SPSS v21, IBM, New York).

**Results**

Fourteen participants (7 EVH+ve; 7 EVH-ve) successfully completed all trials, participant demographics and lung function are shown in Table 1. Predictions for maximum voluntary ventilation (MVV) were 124.1 L and 148.2 L (FEV1 x 30), with \( \dot{V}_E \) attained during \( \dot{V}_O_2 \)peak tests measuring 121.1 L and 150.4 L, for EVH+ve and EVH-ve groups respectively, the \( \dot{V}_E \) attained during performance time-trials are displayed in Figure 2.

**Lung function values**

Post-inhalation FEV1 was greater in the salbutamol trial (4.26 ± 0.69 L; 5.05 ± 0.45 L) when compared with both the placebo trial (4.10 ± 0.7 L p = 0.04; 4.83 ± 0.53 L p = 0.03) and control trial (4.03 ± 0.69 L p = 0.013; 4.84 ± 0.44 L p = 0.003) for the EVH+ve group and the EVH-ve group, respectively. There was an increase in FEV1 in both EVH+ve (4.01 ± 0.8 L; 4.26 ± 0.7 L p = 0.01; 4.25 ± 0.5 L p = 0.27) and EVH-ve (4.81 ± 0.4 L; 5.1 ± 0.4 L p = 0.02; 5.1 ± 0.5 L p = 0.06) groups for baseline, post-inhalation and post-time-trial, respectively following inhaled salbutamol, which was significant post-inhalation, but this significance was not sustained post time-trial. There was a strong trend towards significant differences in baseline FEV1 between EVH+ve and EVH-ve participants for the salbutamol trial (4.01 ± 0.86; 4.81 ± 0.45 p = 0.05) and the placebo trial (4.06 ± 0.80; 4.82 ± 0.55 p = 0.06) with a significant difference at baseline in the control trial (4.0 ± 0.73; 4.84 ± 0.46 p = 0.03). There was no fall in FEV1 from post-inhalation to post-time-trial in any of 3 km time trials (Figure 3).

**Performance variables**

There were no differences in 3 km completion time between EVH+ve and EVH-ve participants across any of the trials (Figure 4). There were no significant differences between post-exercise lactate values, \( V_E \) or \( \dot{V}_O_2 \) during performance for any trial condition (Figure 2).

When the groups were pooled there was a strong trend towards significant difference in mean HR between the salbutamol trial (183 ± 8 beats min⁻¹) and both the placebo trial (180 ± 9 beats min⁻¹; p = 0.06) and the control trial (180 ± 9 beats min⁻¹; p = 0.05). However this difference was not apparent for the EVH+ve (183 ± 8; 182 ± 8; 180 ± 10; beats min⁻¹) and the EVH-ve groups (184 ± 8; 176 ± 9; 180 ± 8 beats min⁻¹) for the salbutamol trial, the placebo trial and the control trial, respectively. There were no differences in ratings of perceived exertion (RPE) between groups or trial conditions (Figure 4).

**Table 1. Mean (±SD) Participant Demographics and Lung Function Values Pre- and Post-EVH Challenge for: EVH Positive Individuals (EVH+ve) and EVH Negative Individuals (EVH-ve), alongside individual participant responses to EVH challenge.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>Age (yrs)</th>
<th>FEV1* (L)</th>
<th>% Predicted FEV1*</th>
<th>FEV1+ (L)</th>
<th>% Predicted FEV1+</th>
<th>% Change in FEV1*</th>
<th>VO2peak † (mL·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVH+ve (n=7)</td>
<td>1.75 (.06)</td>
<td>71.7 (6.6)</td>
<td>22.7 (1.9)</td>
<td>4.13 (.83)</td>
<td>92.9 (13.1)</td>
<td>3.55 (.77)</td>
<td>81.92 (12.9)</td>
<td>-14.4 (4.0)</td>
<td>43.8 (5.7)</td>
</tr>
<tr>
<td>EVH-ve (n=7)</td>
<td>1.84 (.04)</td>
<td>81.1 (8.1)</td>
<td>22.1 (1.1)</td>
<td>4.94 (.45)</td>
<td>102.6 (6.3)</td>
<td>4.64 (.41)</td>
<td>98.8 (6.6)</td>
<td>-6 (2.4)</td>
<td>50.0 (5.6)</td>
</tr>
</tbody>
</table>

* FEV1 - Forced Expiratory Volume in 1 Second; † EVH - Eucapnic Voluntary Hyperpnoea; † VO2peak – Peak volume of oxygen consumed per kilogram body mass per minute
Discussion

Therapeutic doses (i.e. 400 µg) of inhaled salbutamol do not improve 3 km time-trial performance in either EVH+ve or EVH-ve participants despite significantly increasing FEV₁ and a strong trend towards increased exercising HR. The 3 km running time-trial performed in an EIB provocative environment failed to induce a fall in FEV₁ in the EVH+ve group in either the control or placebo conditions. Our findings are similar to Koch et al., (2015a; 2015b) who conducted investigations into the effect of inhaled salbutamol on 10 km cycling time trial performance in a laboratory environment. Koch et al., (2015a; 2015b) reported increases in FEV₁ in EVH+ve and EVH-ve cyclists post-bronchodilator but this did not translate to improved 10 km cycling performance in either males or females.

We did not observe bronchoconstriction in our study following placebo and control 3 km time-trials, this may have been due to the fact that our EVH+ve participants were only mild responders and were not susceptible to bronchoconstriction induced by exercise. In fact, 5 out of 7 of the EVH+ve group had a post EVH challenge FEV₁ fall from baseline only between 10% and 15%
Molphy et al.

Figure 4. Comparisons between trial conditions for a) EVH+ve Group Heart Rate Values b) EVH+ve Group Ratings of Perceived Exertion c) EVH-ve Group Heart Rate Values d) EVH-ve Group Ratings of Perceived Exertion.

Figure 5. Individual percentage changes in FEV₁ from baseline following EVH challenge and low humidity running time-trial in the control condition.

(Table 1). Price et al. (2015) have demonstrated the transient nature of EIB in athletes with FEV₁ falls between 10 and 20% following EVH challenges. Whereas, Williams et al. (2015) have demonstrated that repeatability in the EVH challenge response occurs when FEV₁ falls greater than 20% from baseline. The individual lung function changes following EVH challenge and the individual lung function changes following the low humidity time-trial have been presented in Figure 5, showing a markedly reduced bronchial hypersensitivity following the time-trial in the mild EVH+ve group. Furthermore, Price et al. (2016) have recently suggested that a cut-off criterion of 15% fall in FEV₁ post EVH is more appropriate to confirm EIB diagnosis. Therefore if our study had recruited participants with EVH challenge falls >20% from baseline we may have observed different responses in FEV₁ post placebo and control 3 km time trials. Interestingly, only one of the EVH+ve participants exhibited with a >12% increase in FEV₁ following bronchodilator, further suggesting that although a sufficient fall was seen post-EVH, not all criteria were met (Pellegrino et al., 2005).

We have demonstrated that individuals with a mild
positive EVH challenge who exercise in an EIB provocative environment do not experience any decrements in airway function without salbutamol or any improvements in exercise performance when both EVH+ve and EVH-ve individuals exercise following inhaled 400µg salbutamol. However, we have not measured any markers of airway injury/inflammation to indicate the protective effect that inhaled salbutamol may have. We know that athletes who regularly exercise in provocative environments are more susceptible to airway remodeling (Karjalainen et al., 2000). Simpson et al. (2016) have also recently reported that the acute use of terbutaline can reduce airway inflammation and epithelial cell damage. It would therefore be premature to conclude that individuals with no history of EIB who have a mild positive response to the EVH challenge would not benefit from treatment. Future studies should investigate both acute and long term use of appropriate inhaled therapy in EVH+ve athletes whilst measuring markers of inflammation to assess the protective effect of the medication.

The administration of a single acute dose of inhaled short-acting β₂-agonist (SABA) does not appear to affect exercise performance in either healthy individuals or individuals with a mild positive response to Mannitol challenge. Recently, however, a study performed by Kalsen et al. (2014) examined the acute administration of multiple inhaled β₂-agonists simultaneously, at the WADA maximum permitted daily amounts (salbutamol – 1600 µg; salmeterol – 200 µg; formoterol – 36 µg), in healthy and airway hyper-responsive (AHR) individuals. The findings from their study show a significant increase in FEV₁ post-inhalation in both groups and also significantly greater sprint performance and maximal voluntary contraction (MVC), however no consequent improvement in performance was seen in high-intensity exercise performance.

This is in contrast to the findings of Decorte et al. (2013) who found that there was an increased time to fatigue following salbutamol inhalation, with no improvement in MVC. These differences could be explained by the administration of multiple β₂-agonists in the Kalsen et al. (2014) study which could have had a greater effect on the β₂ adrenergic receptors due to greater systemic availability of the drugs. With greater bioavailability there is the possibility of more potent stimulation of skeletal muscle due to structural differences between the different β₂-agonists which can improve the binding potential and allow for a greater saturation, and therefore stimulation, of the adrenergic receptors, leading to greater force of contraction but also the possibility of a higher rate of fatigue of the muscle fibers (Hoffman, 2001).

When considering study limitations, the present study may not have found any ergogenic effect of salbutamol due to the comparatively small doses used, however the doses administered were the recommended therapeutic limit. There remains the possibility that performance improvements may not have been seen because the present investigation focused solely on endurance performance. Recent work (Decorte et al., 2013; Hostrup et al., 2014; Kalsen et al., 2014) has indicated that inhaled β₂-agonists may enhance strength and power performance but not endurance performance. The present study may also have not found a late response in lung function as the post-exercise spirometry was performed at a single time-point 5 minutes post, BTS/ATS criteria (Parsons et al., 2013) state that spirometry should be performed at regular intervals for a minimum of 15 minutes post-challenge. The present study design stipulated that if a fall of 10% or more was seen at the 5 minute stage post-challenge then follow-up spirometry would have been repeated at 10 minutes to confirm bronchoconstriction, yet this did not occur in any individual. Another limitation was the need for a 5 minute warm-up prior to the 3 km running time-trial, this could have induced a refractory period during the time-trial (Parsons et al., 2013) and bronchoconstriction may not have been evident for this reason. However, upon ethical approval and risk assessment, a minimum 5 minute warm-up was deemed necessary in order for individuals to undergo maximal running time-trial performance.

Conclusions

The findings of the present study highlight that there is a significant increase in FEV₁ and heart rate with inhaled Salbutamol in both EVH-ve and EVH+ve individuals. However, these increases do not translate to improved performance during 3 km running time-trial. The low humidity environment (20-25%) did not induce a fall in FEV₁ in mild EVH+ve individuals. Of note, EVH+ve athletes did not report any symptoms of EIB during any of the trials, highlighting that asymptomatic individuals with a mild positive EVH challenge (>10% <25% ↓FEV₁) may not necessarily exhibit EIB. Although a one-off bout of exercise at low humidity may not result in significant bronchoconstriction, future research should examine the long-term impact of exercising in such conditions both with and without appropriate inhaler therapy in EVH+ve athletes with no previous history of asthma or EIB.

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References


**Key points**

- **Athletes with EIB require short-acting β2-agonists for the relief and/or prevention of symptoms during sporting performance which has the potential to be ergogenic.**
- **The present study demonstrates that there is no ergogenic effect from their therapeutic use in healthy active individuals during 3 km running time-trial performance.**
- **Athletes with mild EIB may exhibit hyper-responsiveness in bronchoprovocative environments.**
- **The present study demonstrates that individuals with a mild positive response to EVH challenge do not exhibit with EIB during intense exercise in a low humidity (20-25%) environment.**

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