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The effect of diurnal variation in exercise-induced bronchoconstriction

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ABSTRACT

Objective: Exercise-induced bronchoconstriction (EIB) is an acute, transient narrowing of the airway as a result of exercise. Diurnal variation in asthma is well-established, however, few studies have investigated diurnal variability in EIB; no study has used eucapnic voluntary hyperpnea (EVH). The aim of this study was to examine circadian variability in EIB using EVH.

Methods: Fourteen recreationally-active males with mild to moderate asthma and nine healthy controls were randomized to first complete either an AM (07:00–08:00) or PM (17:00–18:00) EVH challenge, followed by the alternate test 34 h to 7 days later. The EVH protocol comprised of six-minutes of hyperventilation of a 5% CO₂ gas at a minimum ventilation rate of $21 \times \text{FEV}_1 \text{ min}^{-1}$. The primary outcome measure was FEV₁ pre- and post-EVH.

Results: We observed no diurnal effect on EIB in the asthma group. The minimum observed post-EVH FEV₁ in the asthma cohort was $3.58 \pm 0.95 \text{ L}$ in AM and $3.62 \pm 0.87 \text{ L}$ in PM tests, corresponding to a $15.0 \pm 15.3\%$ vs. $14.9 \pm 14.7\%$ reduction from baseline, respectively. The asthma group showed similar baseline FEV₁ before AM ($4.21 \pm 0.79 \text{ L}$) and PM ($4.25 \pm 0.65 \text{ L}$) tests. No difference was observed in minute ventilation between AM ($26.1 \pm 3.4 \times \text{FEV}_1 \text{ min}^{-1}$) and PM ($25.6 \pm 3.8 \times \text{FEV}_1 \text{ min}^{-1}$) tests for the asthma cohort. Controls displayed no significant changes in FEV₁ or minute ventilation between tests.

Conclusions: When baseline pulmonary function is similar, this study suggests that time-of-day has no effect on EIB in mild to moderate asthma.

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Asthma; circadian rhythm; eucapnic voluntary hyperpnea; exercise-induced asthma; exercise-induced bronchospasm; time-of-day

Introduction

EIB is defined as an acute transient airway narrowing as a result of exercise and is present in up to 90% of asthmatics (1). A significant global burden of disease is attributable to asthma; the World Health Organization estimates that 4.3% of the world's adult population have been diagnosed with asthma (2), while over 10% of the Irish population are affected, the fourth highest global prevalence (3). EIB is also present in individuals without asthma, affecting between 5 and 20% of the general population (4) and up to 34% of previously-undiagnosed elite athletes (5).

Symptoms of EIB include shortness of breath, chest tightness, cough during and after exercise and a reduction in performance. As a result, EIB represents a significant barrier to exercise in some individuals with asthma, who report increased anxiety about and decreased participation in exercise, especially in intense exercise bouts (6). Indeed, significantly lower quality of life scores have been reported in children who report symptoms of EIB (7). Paradoxically, long-

term aerobic exercise is generally recommended for individuals with asthma (8) and has been shown to reduce airway inflammation and bronchial hyper-responsiveness (9).

Diurnal variation in pulmonary function is a well-defined physiological phenomenon, with peak-flow varying throughout the day, being lowest at night (~04:00), and highest in the afternoon (~16:00) (10). Although more pronounced in individuals with asthma, it is also present in those without asthma. Individuals with diurnally-active asthma are more likely to experience deteriorations in pulmonary function at night and early morning (11). While EIB is known to be influenced by factors such as; environmental conditions, type of exercise and pre-exercise treatment (12,13); evidence regarding diurnal variation in EIB is sparse and contradictory. A previous study reported no difference in EIB at 2-hourly intervals between 08:00 and 16:00 (14), while a more recent publication observed more significant bronchoconstriction at 18:00 compared to 07:00 (15).

Exercise challenge tests (ECT) and eucapnic voluntary hyperpnea (EVH) are commonly used objective testing methods for EIB. Compared to an ECT, which involves six minutes of high-intensity exercise, EVH uses the maximal hyperventilation of a dehumidified 5% CO₂ gas mixture to provoke bronchoconstriction. Either test is deemed positive if a reduction in FEV₁ of 10% is observed post-test. While exercise testing is specific (16), it lacks sensitivity and is more difficult to standardize (17), resulting in the International Olympic Committee (IOC) recommending EVH over laboratory ECTs in the diagnosis of EIB (18). Disagreement regarding the 'gold-standard' test for EIB remains in the literature however (19). Of note, previous studies investigating diurnal variation in EIB have used an ECT in their study designs; to this author's knowledge no study has been performed using EVH.

Diurnal variation in pulmonary function is well established in asthma, however, the effect of time-of-day on EIB is less well studied. Recreational and elite athletes with asthma exercise at different times throughout the day to accommodate exercise into busy and demanding lifestyles. Given the potential benefits of exercise in individuals with asthma, and the negative effects of even mild bronchoconstriction on training and performance, there is a need to establish what, if any, effect diurnal variation has on EIB. The primary aim of the current study was to examine the impact of diurnal variation on EIB in a cohort of recreational male athletes with mild to moderate asthma using EVH.

Methods

Participants

Participants ($n = 23$) were recruited from local college sports clubs and from the Asthma Society of Ireland, following advertisement. All participants were male, healthy, non-elite athletes aged 18–45 years involved in regular training of 8–12 h per week and all were competing in senior competition across a broad spectrum of sports. Asthma was defined by a registered medical practitioner diagnosis and regular use of a short-acting inhaled bronchodilator with exercise. Exclusion criteria were: uncontrolled asthma based on Global Initiative for Asthma (GINA) guidelines (20), an asthma exacerbation requiring hospitalization, oral steroids or treatment with a nebulizer in the past 5 years, the use of a long-acting bronchodilator or montelukast, a history of smoking anytime or any disease which it was deemed would prevent participation

in an EVH test. In addition, male recreational athletes aged 18–45 years, with no history or symptoms of asthma and who reported no exclusion criteria were recruited as controls.

Following an explanation of the study and documentation of written informed consent, 14 males with asthma and 9 male controls completed both EVH tests. The Trinity College Dublin Research Ethics Committee granted ethical approval for the study in December 2017.

Protocol

Each participant completed 3 sessions at the Human Performance Laboratory, Trinity College Dublin; an induction visit, an AM EVH test and a PM EVH test.

Induction visit

On induction all participants completed a standard laboratory general medical screening questionnaire, a targeted asthma questionnaire and received a physical examination from a medical professional to ensure eligibility. Baseline spirometry data was recorded in a seated position using a portable spirometer (Micro Medical, Kent, UK) in accordance with American Thoracic Society (ATS) guidelines (21). The measured variable was FEV₁ (L min⁻¹). Each participant then performed a 30-s maximal ventilatory effort to familiarize themselves with the EVH apparatus.

Testing visits

Participants were randomized to complete EVH testing in either an AM/PM or PM/AM sequence of visits. Morning visits commenced between 07:00 and 08:00 while evening tests commenced between 17:00 and 18:00. Participants completed the second EVH test a minimum of 34 h and a maximum of 7 days after the first test. In accordance with ATS guidelines (13), the asthma cohort withheld short-acting bronchodilators for at least 6 h prior, and inhaled corticosteroids for 24 h prior to testing. All participants resumed their normal medications on completion of each test visit. Participants were also required to; avoid vigorous exercise and caffeine consumption within 6 h of testing and have an adequate night's sleep prior to all tests. Pretest spirometry was performed on each visit to assess for diurnal variability and provide pretest reference data.

EVH protocol

A single-stage EVH protocol was used based on the method developed by Argyros et al. (22). Participants

were fitted with a uniport facemask (Hans Rudolph, Kansas, USA), and connected to a medically certified gas cylinder (BOC, Guildford, UK) containing 21% O₂, 5% CO₂ and 74% N₂ via a multi-stage pressure regulator (Gas Arc, Diss, UK), plastic tubing, 100 L Douglas bag and a two-way breathing valve (Hans Rudolph, Kansas, USA). Ventilation volume was measured using a heated (37 °C) Fleisch pneumotachograph (Hans Rudolph, Kansas, USA), amplified with a bio-amplifier (AD Instruments, Oxford, UK) and displayed with the software program LabChart (Version 7.2, AD Instruments, Oxford, UK).

Participants were asked to maintain maximum ventilatory effort for a period of 6 min, using a fast, deep oral breathing technique, emphasizing depth and rate. A multiple of FEV₁ min⁻¹ was used to predict maximal voluntary ventilation (MVV). Participants were asked to aim for a target minute ventilation of 30 × FEV₁ min⁻¹ (85% MVV); however, a minimum of 21 × FEV₁ min⁻¹ (60% MVV) was accepted as a valid test (22). The FEV₁ data measured during the induction visit was used to calculate the ventilation target and all subsequent ventilation data was expressed as a multiple of this figure. The target ventilation volume for the second test was the total volume achieved in test one. Participants were provided with verbal updates every 30 s on the ventilation achieved and instructions were given to alter their ventilation accordingly. On completion of the test, spirometry was performed at 2, 5, 10, 15 and 30 min post-test. A positive EVH test was defined as a 10% reduction in FEV₁ at any measurement post-EVH compared to pretest FEV₁.

Statistical analysis

An alpha-priori test using G*POWER 3.1 (Universitat Kiel, Germany) with an effect size of 0.25, α -error probability of 0.05 and power of 0.80 revealed a total required sample size of 12 participants per group.

A 2-way repeated measure ANOVA was performed to establish diurnal differences in response to EVH for each trial group. The two independent variables were time-of-day and time-of-spirometry measurement. The outcome measures analyzed were absolute FEV₁ and percentage change in FEV₁ from baseline. A 2-way mixed factorial ANOVA, with time-of-day as a repeated measure, and group as a non-repeated measure, was performed to assess for differences in minute ventilation between AM and PM tests and the asthma and control cohort. Any detected differences were quantified using *post-hoc* Tukey tests. Statistical

Table 1. Participant's anthropometric data, baseline pulmonary function and medication usage.

	Asthma (n = 14)	Control (n = 9)	P value
Age (yrs)	26 ± 5	27 ± 4	0.51
Height (cm)	180.3 ± 7.5	178.1 ± 5.0	0.39
BMI (kg m ⁻²)	23.3 ± 2.1	23.3 ± 1.4	0.81
FEV ₁ (L)	4.38 ± 0.79	4.6 ± 0.51	0.50
Predicted FEV ₁ (%)	95.4 ± 13.6	104 ± 9.4	0.11
FEV ₁ :FVC	0.80 ± 0.08	0.86 ± 0.06	0.06
Use of a SABA	14 (100%)	–	–
Use of an ICS	6 (42%)	–	–

Values represented are mean ± SD. $P < 0.05$ indicates statistical significance. BMI = body mass index, FEV₁ = forced expiratory volume in one second, FVC = forced vital capacity, SABA = short-acting bronchodilator, ICS = inhaled corticosteroid.

significance was set at $P < 0.05$. All statistical analyses were performed using SigmaStat 4.0 (Systat, CA, USA).

Results

Participant's anthropometric data, baseline pulmonary function and medication requirements are presented in Table 1. In the asthma cohort, five were classified as having mild intermittent disease, six mild persistent and three moderate persistent according to ATS criteria (23). All 23 participants ($n = 14$ asthma, $n = 9$ control) completed AM and PM EVH tests, at the required minute ventilation of 21 × FEV₁ min⁻¹; and all were able to provide post-EVH challenge spirometry measures without the need for rescue medication.

In the asthma group, no global effect of time-of-day (AM vs PM) was observed on absolute FEV₁ post-EVH ($P = 0.77$), indicating that there was no significant diurnal effect on EIB (Figure 1). Similarly, no significant differences between AM and PM FEV₁ outcomes post-EVH challenge were recorded in controls ($P = 0.29$) (Figure 2).

When FEV₁ was expressed as a % change from baseline, again no significant effect of time of day (AM vs PM) was observed in both asthma ($P = 0.81$) (Figure 3) and control groups ($P = 0.54$). As expected, both cohorts demonstrated a statistically significant effect of time-of-spirometry measurement ($P < 0.001$). In asthmatics, *post-hoc* analysis revealed significant reductions from baseline in both AM and PM values at minute 2, 5, 10 and 15 but not at minute 30 (Figure 1); and, in controls, significant reductions were observed at minute 2 in both AM and PM tests and minute 5 in the PM test (Figure 2). The largest reductions in FEV₁ were observed at minute 2 for the AM asthma and both control tests; the asthma PM group had the biggest drop at minute 5.

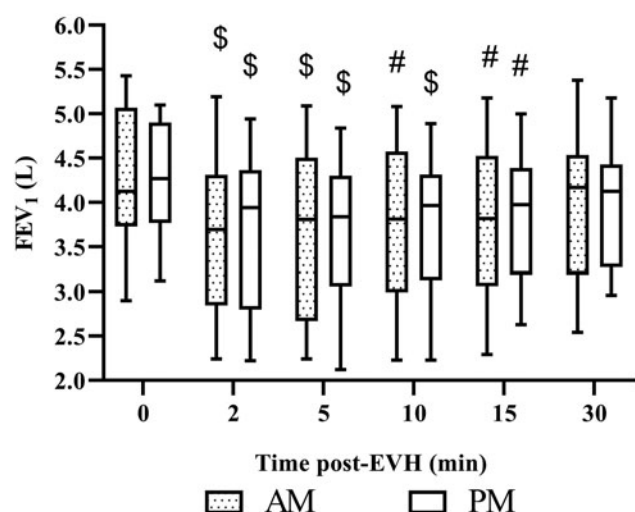


Figure 1. FEV₁ (L) pre- and post-EVH measured in the asthma cohort (AS) ($n=14$) in AM and PM EVH tests. Box denotes interquartile range; bar denotes median value; and whiskers denote range. Symbols indicate significant difference from data measured at 0 min, # infers $P<0.01$ and \$ infers $P<0.001$.

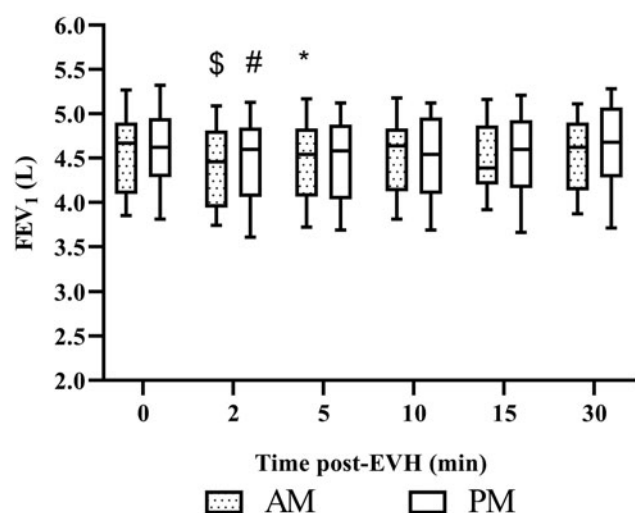


Figure 2. Mean FEV₁ (L) pre- and post-EVH measured in controls (CON) ($n=9$) in AM and PM EVH tests. Box denotes interquartile range; bar denotes median value; and whiskers denote range. Symbols indicate significant difference from data measured at 0 min, * infers $P<0.05$, # infers $P<0.01$ and \$ infers $P<0.001$.

Nine (~64%) of the asthma cohort displayed clinically significant EIB (>10% drop in FEV₁) in at least one EVH test; four were positive on both occasions, four were positive in the evening only and one participant had a single AM positive test (Figure 4). When negative EVH responders were excluded, no significant differences in maximum reduction in FEV₁ from baseline were observed between AM and PM ($P=0.69$). No positive tests were recorded in controls, with the maximum reduction in FEV₁ observed recorded at 7.6%.

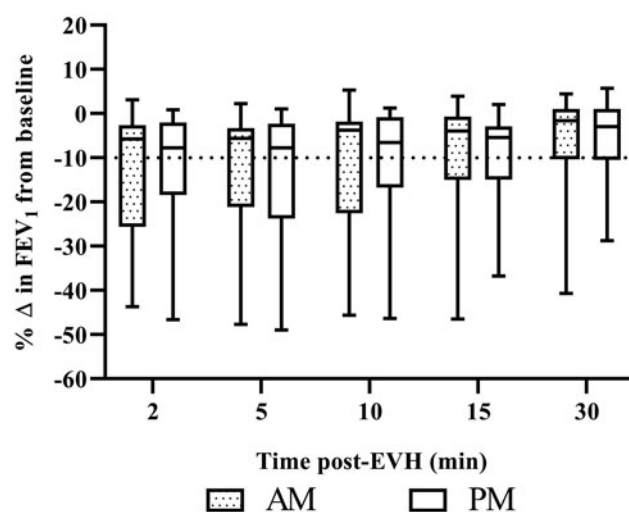


Figure 3. % change in FEV₁ from baseline over time in the asthma cohort ($n=14$) in AM and PM EVH tests. Box denotes interquartile range; bar denotes median value; and whiskers denote range. Whiskers represent the maximum and minimum. Dotted line represents cutoff for a positive EVH test.

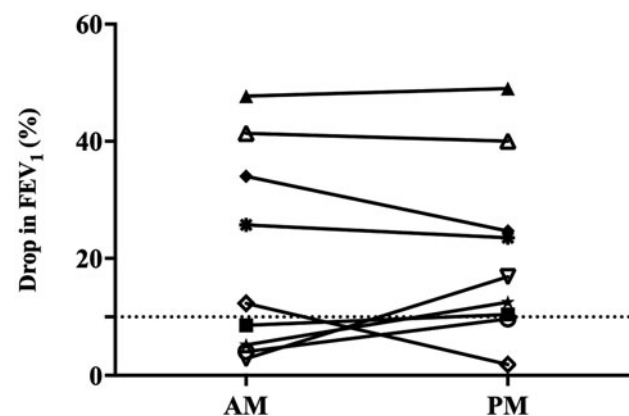


Figure 4. Maximum drop in FEV₁ (%) from pretest value in AM and PM EVH tests in the asthma cohort with at least one positive EVH test ($n=9$). Dotted line indicates cutoff for a positive EVH test. Negative EVH responders excluded for clarity of data presentation.

Comparison of AM/PM baseline FEV₁ data in the asthma cohort failed to indicate significant differences ($P=0.67$) (Table 2), indicating diurnal variation was not prominent at baseline. The range in minute ventilation between AM and PM EVH tests was -7.5 to 11.5 L min^{-1} for the asthma group (Figure 5) and -10.5 to 17.0 L min^{-1} for controls (Figure 5). There was no significant difference noted in minute ventilation achieved between AM and PM tests for both the asthma and control groups ($P=0.31$) (Table 2) indicating the participants received the same stimulus on both occasions. No differences were observed in minute ventilation achieved between the asthma and control groups ($P=0.20$) (Table 2).

Table 2. Pre- and post-EVH spirometry data and minute ventilation recorded in the asthma ($n=14$) and control ($n=9$) cohorts.

	AM	PM	<i>P</i> value
Asthma ($n=14$)			
Baseline FEV ₁ (L)	4.21 ± 0.79	4.25 ± 0.65	0.68
Minimum FEV ₁ post-EVH (L)	3.58 ± 0.95	3.62 ± 0.87	0.75
Maximum % change in FEV ₁	-15.0 ± 15.3	-14.9 ± 14.7	0.97
Minute ventilation (× FEV ₁ min ⁻¹)	26.1 ± 3.4	25.6 ± 3.8	0.19
Control ($n=9$)			
Baseline FEV ₁ (L)	4.55 ± 0.48	4.60 ± 0.46	0.37
Minimum FEV ₁ post-EVH (L)	4.38 ± 0.47	4.44 ± 0.49	0.22
Maximum % change in FEV ₁	-3.7 ± 1.8	-3.2 ± 2.1	0.29
Minute ventilation (× FEV ₁ min ⁻¹)	26.5 ± 4.2	26.4 ± 3.7	0.93

Values represented are mean ± SD. $P < 0.05$ indicates statistical significance. FEV₁ = forced expiratory volume in 1 s, EVH = eucapnic voluntary hyperpnea.

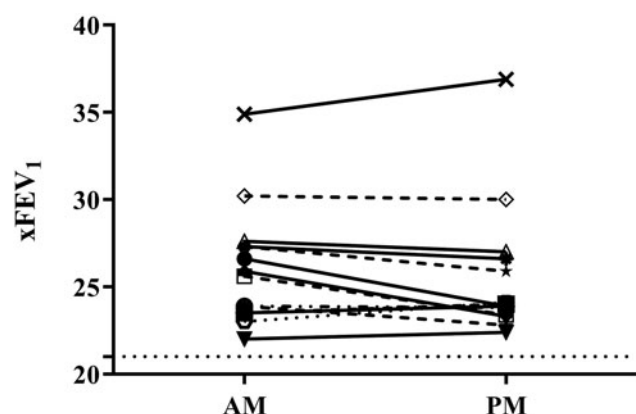


Figure 5. Minute Ventilation (× FEV₁ min⁻¹) in AM and PM EVH tests in the asthma cohort ($n=14$). Dotted line indicates the cutoff for a valid EVH test.

Discussion

The results of the current study suggested that time-of-day had no significant effect on EIB in individuals with mild to moderate asthma. No significant differences in absolute FEV₁ or % drop in FEV₁ were observed in the asthma cohort between morning and evening; indeed, the outcome measures were consistently similar across all time-points. The expected baseline diurnal variation in pulmonary function consistent with asthma was not observed in this cohort. As anticipated, no diurnal variation was recorded in controls. Statistically significant findings were reductions in FEV₁ from baseline post-EVH in the both cohorts, however clinically significant reductions were observed only in the asthma cohort.

To date, no consensus has been formed regarding diurnal variation in EIB (14,24). In keeping with the current study, Eggleston and Guarrant (14) reported no difference in EIB in athletes with asthma completing five ECTs at two-hourly intervals from 08:00 to 16:00 across a single day. The population used was comparable to the current cohort in that participants

had a mild burden of disease, were aged 18–36 years and displayed no baseline variation in pulmonary function. While the study design did not account for EIB refractoriness, the data nevertheless suggests that in this population diurnal variation in EIB is minimal.

By contrast, Vianna et al. (15) reported that the magnitude of EIB was greater in ECTs performed at 18:00 compared to 07:00. While the testing schedule was comparable to the current study, participants had a moderate burden of disease and recorded a baseline diurnal variation in pulmonary function. The authors proposed that differences in airway caliber may influence the EIB response, with a higher baseline FEV₁ producing greater bronchoconstriction.

It appears that circadian variation in baseline pulmonary function is a necessary factor for diurnal variation in EIB (15,25); when this is absent no diurnal differences are observed (14,24). As no baseline circadian variation was present in the current study, our results are consistent with this theory. The absence of circadian variability in our cohort is likely a reflection of a lower disease burden, and considering the above data, it is possible that recruitment of individuals with more severe asthma may have yielded different results.

The current study is novel in its use of EVH to assess for diurnal variability in EIB and differences in the bronchial challenge method could account for differing study outcomes. The most comparable method used to date was the hyperventilation of cold dry air (HVCDA) used by Sly and Landau (25), who noted the greatest response to HVCDA at 16:00 and least at 04:00, however, the use of a pediatric cohort and protocol differences limit comparison to the current study. This contrasts to direct and indirect bronchial challenges, which have consistently reported that bronchial hyperresponsiveness to histamine, methacholine and hypertonic saline inhalation was greatest at 04:00 (26–28). Although EVH replicates the desiccation of airways considered to promote EIB during exercise (19), differences between EVH and ECTs may account for variation in diurnal variation observed.

A possible explanation for diurnal variation in EIB is different ventilation rates across the day. Vianna et al. (15) reported higher minute ventilation in morning ECTs compared to evening challenges, however, the author stated ventilation rates were a less likely explanation of the increased evening EIB as minute ventilation increased while EIB decreased. The use of EVH in the current study allowed the stimulus received by the airway to be tightly controlled so that no difference was observed between tests. Our data

suggested that when minute ventilation is comparable, no diurnal effect in EIB is observed. It is possible, that had participants been instructed to give maximal effort on both tests, diurnal differences in minute ventilation and EIB would have emerged.

The current study involved an AM test which started at 07:00 to 08:00, while the PM test commenced at 17:00 to 18:00. While this is comparable to previous studies (14,15), it does not capture the maximum reported window of variation from 04:00 to 16:00 (10). The possibility must be considered that if EVH had been performed at 04:00, or at additional time-points during the day, it may have resulted in significant diurnal variation being observed. In any case, the practical applications of this data would have been limited as athletes rarely exercise at night.

EVH has primarily been used in elite athletes (29) and few authors have reported on EVH use in recreationally active individuals previously diagnosed with asthma. Our data demonstrated that EVH is a viable test in this population; all participants achieved the target minute ventilation of $21 \times \text{FEV}_1$ on both occasions. The current study observed a positive EVH test in 64% ($n=9$) of the asthmatic group on at least one occasion, in agreement Vianna et al. (15) and a previous study which used EVH (30). Concerns have been expressed about the potential of EVH to provoke large drops in FEV_1 (31). Despite recruitment targeting participants with mild asthma, the maximum observed reduction in FEV_1 was 49%, with a severe response ($>30\%$ drop) recorded in four individuals. All participants tolerated this reduction in pulmonary function well without the need for administration of salbutamol.

Despite the experimental hypothesis being rejected, the data is nevertheless of clinical interest to those health care professionals who manage asthma. This study reassures individuals with mild asthma that they are neither at a higher risk of EIB, nor at a competitive disadvantage, based on what time they exercise. While environmental factors may influence EIB, the data suggests that time-of-day alone has no effect on EIB. Additionally, the results also suggest that there is no optimal time to perform EVH to detect EIB. We did however note severe bronchoconstriction in 29% ($n=4$) of participants with mild asthma. Although EVH is highly provocative and similar reductions may not be observed exercising in an outdoor environment, the data highlights the potential for significant EIB in mild asthma and emphasizes the necessity of compliance with prescribed inhalers prior to exercise.

Limitations of the study included the use of two measurements which did not capture the

maximum reported variation in pulmonary function and a male-only cohort. The inclusion criteria of a physician diagnosis of asthma and regular SABA represents a limitation; ideally an objective measure would have confirmed a diagnosis, but this was outside the scope of the study. The target cohort of the study were those mild asthmatics whose diagnosis is made by a family physician and not confirmed by hospital-based testing: a large proportion of non-elite athletes who train regularly with asthma. The possibility that those ($n=5$) who had two negative EVH tests did not have asthma exists. However, when these participants were excluded, no significant diurnal variation in EIB was observed in those with an EVH-diagnosis of asthma ($n=9$). While our data does suggest the lack of diurnal variation in EIB, unfortunately, due to sample size limitations, particularly in the control group, we acknowledge the possibility of a type-2 error based on a study power of 0.8. Participant recruitment was limited by cost and the time available for the study and resources were prioritized for the asthma group.

Additionally, results may have been influenced by the seasonal variability of asthma, medications and individual differences in circadian rhythms. EVH uses a highly-provocative dehumidified gas and results observed may not translate to exercise in an ambient environment. Further studies should focus on an asthma cohort with significant baseline diurnal variation, a female population and the larger scale use of EVH in recreationally-active athletes with asthma.

In conclusion, our study suggests that when baseline pulmonary function is similar, and the airway receives a challenge of similar magnitude, time-of-day has no effect on EIB in mild to moderate asthma. Given the well-described benefits of exercise, the best time-of-day for athletes with asthma to exercise is the time-of-day they are most likely to exercise.

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Declaration of interest

The authors report no conflicts of interest.

References

1. Goodman M, Hays S. Asthma and swimming: a meta-analysis. *J Asthma* 2008;45:639–647. doi:10.1080/02770900802165980.

2. To T, Stanojevic S, Moores G, Gershon A, Bateman E, Cruz A, Boulet L-P. Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health* 2012;12:204. doi:10.1186/1471-2458-12-204.
3. Holohan J. Asthma check. Chronic disease watch - Asthma. 2012. Available from: <https://hse.ie/eng/services/publications/Clinical-Strategy-and-Programmes/Asthma-Check-Chronic-Disease-Watch.pdf> [accessed 6 March 2019].
4. Weiler JM, Bonini S, Coifman R, Craig T, Delgado L, Capão-Filipe M, Passali D, Randolph C, Storms W. American Academy of Allergy, Asthma & Immunology work group report: Exercise-induced asthma. *J Allergy Clin Immunol* 2007;119:1349–1358. doi:10.1016/j.jaci.2007.02.041.
5. Dickinson J, McConnell A, Whyte G. Diagnosis of exercise-induced bronchoconstriction: eucapnic voluntary hyperpnoea challenges identify previously undiagnosed elite athletes with exercise-induced bronchoconstriction. *Br J Sports Med* 2011;45:1126–1131. doi:10.1136/bjsm.2010.072520.
6. Dimitrakaki V, Porpodis K, Bebetos E, Zarogoulidis P, Papaiwannou A, Tsiouda T, Tsioulis H, Zarogoulidis K. Attitudes of asthmatic and nonasthmatic children to physical exercise. *Patient Prefer Adherence* 2013;7:81–88.
7. Kojima N, Ohya Y, Futamura M, Akashi M, Odajima H, Adachi Y, Kobayashi F, Akasawa A. Exercise-induced asthma is associated with impaired quality of life among children with asthma in Japan. *Allergol Int* 2009;58:187–192. doi:10.2332/allergolint.08-OA-0034.
8. Global Initiative for Asthma. Global strategy for asthma management and prevention, 2017. http://ginasthma.org/wp-content/uploads/2017/02/wmsGINA-2017-main-report-final_V2.pdf [accessed 25 July 2018]
9. França-Pinto A, Mendes F, de Carvalho-Pinto R, Agondi R, Cukier A, Stelmach R, Saraiva-Romanholo B, Kalil J, Martins MA, Giavina-Bianchi P, et al. Aerobic training decreases bronchial hyperresponsiveness and systemic inflammation in patients with moderate or severe asthma: a randomised controlled trial. *Thorax* 2015;70:732–739. doi:10.1136/thoraxjnl-2014-206070.
10. Clark TJ, Hetzel MR. Diurnal variation of asthma. *Br J Dis Chest* 1977;71:87–92.
11. Durrington HJ, Farrow SN, Loudon AS, Ray DW. The circadian clock and asthma. *Thorax* 2014;69:90–92. doi:10.1136/thoraxjnl-2013-203482.
12. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma is *J Allergy Clin Immunol* 2000;106:453–459. doi:10.1067/mai.2000.109822.
13. Parsons JP, Hallstrand TS, Mastronarde JG, Kaminsky DA, Rundell KW, Hull JH, Storms WW, Weiler JM, Cheek FM, Wilson KC, et al. An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction. *Am J Respir Crit Care Med* 2013;187:1016–1027. doi:10.1164/rccm.201303-0437ST.
14. Eggleston PA, Guerrant JL. A standardized method of evaluating exercise-induced asthma. *J Allergy Clin Immunol* 1976;58:414–425. doi:10.1016/0091-6749(76)90122-6.
15. Vianna EO, Boaventura LC, Terra-Filho J, Nakama GY, Martinez JA, Martin RJ. Morning-to-evening variation in exercise-induced bronchospasm. *J Allergy Clin Immunol* 2002;110:236–240.
16. Anderson SD, Fitch K, Perry CP, Sue-Chu M, Crapo R, McKenzie D, Magnussen H. Responses to bronchial challenge submitted for approval to use inhaled beta2-agonists before an event at the 2002 Winter Olympics. *J Allergy Clin Immunol* 2003;111:45–50.
17. Rundell KW, Anderson SD, Spiering BA, Judelson DA. Field exercise vs laboratory eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite cold weather athletes. *Chest* 2004;125:909–915. doi:10.1378/chest.125.3.909.
18. Fitch KD, Sue-Chu M, Anderson SD, Boulet LP, Hancox RJ, McKenzie DC, Backer V, Rundell KW, Alonso JM, Kippelen P, et al. Asthma and the elite athlete: Summary of the International Olympic Committee's Consensus Conference; 2008 Jan 22–24; Lausanne, Switzerland.
19. Hull JH, Ansley L, Price OJ, Dickinson JW, Bonini M. Eucapnic voluntary hyperpnea: gold standard for diagnosing exercise-induced bronchoconstriction in athletes? *Sports Med* 2016;46:1083–1093. doi:10.1007/s40279-016-0491-3.
20. Reddel H, Bateman ED, Becker A, Boulet LP, Cruz AA, Drazen JM, Haahtela T, Hurd SS, Inoue H, De Jongste JC, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015;46:622–639. doi:10.1183/13993003.00853-2015.
21. Miller MR, Hankinson V, Brusasco F, Burgos F, Casaburi R, Coates A, Crapo R, Enright PV, Van der Grinten CP, Gustafsson P, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319–338. doi:10.1183/09031936.05.00034805.
22. Argyros GJ, Roach JM, Hurwitz KM, Eliasson AH, Phillips YY. Eucapnic voluntary hyperventilation as a bronchoprovocation technique: development of a standardized dosing schedule in asthmatics. *Chest* 1996;109:1520–1524. doi:10.1378/chest.109.6.1520.
23. Colice GL, Burgt JV, Song J, Stampone P, Thompson PJ. Categorizing asthma severity. *Am J Respir Crit Care Med* 1999;160:1962–1967. doi:10.1164/ajrccm.160.6.9902112.
24. Bungaard A, Andersen KK. Diurnal variation in exercise-induced asthma. *Eur J Resp Dis* 1983;64:424–426.
25. Sly PD, Landau LI. Diurnal variation in bronchial responsiveness in asthmatic children. *Pediatr Pulmonol* 1986;2:344–352. doi:10.1002/ppul.1950020606.
26. de Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. *Int Arch Allergy Appl Immunol* 1962;20:93–101. doi:10.1159/000229248.
27. Martin RJ, Cicutto LC, Ballard RD. Factors related to the nocturnal worsening of asthma. *Am Rev Respir Dis* 1990;141:33–38. doi:10.1164/ajrccm/141.1.33.

28. Ferraz E, Borges MC, Terra-Filho J, Martinez JA, Vianna EO. Comparison of 4 AM and 4 PM bronchial responsiveness to hypertonic saline in asthma. *Lung* 2006;184:341–346. doi:[10.1007/s00408-006-0017-0](https://doi.org/10.1007/s00408-006-0017-0).
29. Dickinson JW, Whyte GP, McConnell AK, Harries MG. Screening elite winter athletes for exercise induced asthma: a comparison of three challenge methods. *Br J Sports Med* 2006;40:179–182. doi:[10.1136/bjsm.2005.022764](https://doi.org/10.1136/bjsm.2005.022764).
30. Price OJ, Ansley L, Hull JH. Diagnosing exercise-induced bronchoconstriction with eucapnic voluntary hyperpnea: is one test enough? *J Allergy Clin Immunol Pract* 2015;3:243–249. doi:[10.1016/j.jaip.2014.10.012](https://doi.org/10.1016/j.jaip.2014.10.012).
31. Brannan JD, Porsbjerg C, Anderson SD. Inhaled mannitol as a test for bronchial hyper-responsiveness. *Expert Rev Respir Med* 2009;3:457–468. doi:[10.1586/ers.09.38](https://doi.org/10.1586/ers.09.38).