REPRODUCIBILITY OF EUCAPNIC VOLUNTARY HYPERVENTILATION FOR EXERCISE-INDUCED BRONCHOSPASM DIAGNOSIS IN ASTHMATIC CHILDREN AND ADOLESCENTS.

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Abstract

BACKGROUND: Exercise-induced respiratory symptoms are frequently reported by asthmatics and exercise-induced bronchospasm (EIB) is a frequent cause that requires objective testing for diagnosis. Eucapnic voluntary hyperventilation (EVH) is recommended as an exercise surrogate stimulus for this purpose, but its short-term reproducibility is not yet established in young asthmatics. OBJECTIVE: To evaluate the short-term test-retest agreement and reproducibility of FEV1 changes after EVH in young asthmatics. METHODS: Asthmatics aged between 10 and 20 years underwent EVH for EIB diagnosis on two occasions 2-4 days apart at a specialized university clinic. FEV1 was measured 5, 15 and 30 minutes after EVH with a target ventilation rate 21 times baseline FEV1. EIB was diagnosed as a decrease >10% in FEV1 from baseline. RESULTS: Twenty-six of 62 recruited individuals tested positive for EIB on both visits (positive group) and 17 on one visit only (divergent group); and 19 tested negative on both visits (negative group). The overall agreement was 72.5% (95%CI 61.6%, 83.6%) and positive and negative agreement was 41.9% and 30.6% respectively. Despite overall low bias in FEV1 response between test days (0.87%), the limits of agreement were wide (+20.72%). There were no differences in pre-challenge FEV1 or achieved ventilation rate, between visits either between groups (p=0.097 and p=0.461) or within groups, (p=0.828 and p=0.780). No test was interrupted by symptoms and there were no safety issues. CONCLUSIONS: More than one EVH test should be performed in young asthmatics with a negative test to exclude EIB and minimize misdiagnosis and mistreatment.

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ABSTRACT

BACKGROUND: Exercise-induced respiratory symptoms are frequently reported by asthmatics and exercise-induced bronchospasm (EIB) is a frequent cause that requires objective testing for diagnosis. Eucapnic voluntary hyperventilation (EVH) is recommended as an exercise surrogate stimulus for this purpose, but its short-term reproducibility is not yet established in young asthmatics. OBJECTIVE: To evaluate the short-term test-retest agreement and reproducibility of FEV_1changes after EVH in young asthmatics. METHODS: Asthmatics aged between 10 and 20 years underwent EVH for EIB diagnosis on two occasions 2-4 days apart at a specialized university clinic. FEV_1 was measured 5, 15 and 30 minutes after EVH with a target ventilation rate 21 times baseline FEV_1. EIB was diagnosed as a decrease$>$ 10% in FEV_1 from baseline. RESULTS: Twenty-six of 62 recruited individuals tested positive for EIB on both visits (positive group) and 17 on one visit only (divergent group); and 19 tested negative on both visits (negative group). The overall agreement was 72.5% (95%CI 61.6%, 83.6%) and positive and negative agreement was 41.9% and 30.6% respectively. Despite overall low bias in FEV_1 response between test days (0.87%), the limits of agreement were wide ($+$ 20.72%). There were no differences in pre-challenge FEV_1 or achieved ventilation rate, between visits either between groups (p=0.097 and p=0.461) or within groups, (p=0.828 and p=0.780). No test was interrupted by symptoms and there were no safety issues. CONCLUSIONS: More than one EVH test should be performed in young asthmatics with a negative test to exclude EIB and minimize misdiagnosis and mistreatment.

Key-words: asthma; adolescents; eucapnic voluntary hyperpnea; exercise-induced bronchoconstriction; indirect bronchoprovocation testing; reproducibility.

Abbreviations

ACT = Asthma Control Test

$AUC_{0\text{-}30min}$ = area under the curve up to the thirtieth minute

EIB = exercise-induced bronchospasm

EIRS = Exercise-induced respiratory symptoms

EVH = Eucapnic voluntary hyperventilation

FEV_1 = Forced expiratory volume in the 1st second

Introduction

Exercise-induced respiratory symptoms (EIRS) are frequently reported by asthmatics, with rates ranging from 33% in the Asia-Pacific area to 59% in Central Eastern Europe[1]. In Latin America, this condition has been observed in 37% of such individuals[2]. Many children and adolescents believe their asthma is a factor that prevents them from participating in physical activities and that this is an inherent feature of the disease. This often brings with it the stigma of being less able and thus restricts peer group activities and participation in sports and games[3,4,5].

Although various conditions, including poor pulmonary function, poor physical conditioning, exercise-induced laryngeal obstruction and dysfunctional breathing [6,7], may be associated with EIRS in children and adolescents with asthma, exercise induced bronchospasm (EIB) is probably the most frequent and occurs in approximately 50%[8] of them. EIB is defined as the acute narrowing of the lower airways after exercise and may also contribute significantly to avoidance of participation in physical activities in children and young people with asthma[9,10].

Diagnosis of EIB cannot be made solely on the basis of self-reported respiratory symptoms, as it has poor predictive value compared to objective tests[11,12]. To avoid under- and over-diagnosis and to enable correct treatment strategies to be adopted, EIB must be established by measuring changes in lung function provoked by exercise or a surrogate stimulus. The most common technique is serial measurement of forced expiratory volume in the first second (FEV_1) before and after treadmill running. The criterion for EIB diagnosis is a $>$ 10% drop in FEV_1 after the exercise challenge compared to the baseline[13].

Eucapnic voluntary hyperventilation (EVH) has been recommended as a surrogate stimulus for EIB diagnosis.[13] It is considered safe, enables better control of ventilation rates and inspired air conditions, and dispenses with the need for physical effort[14,15,16,17]. The procedure follows the same physiopathological pathways for EIB as treadmill running, namely dehydration of the airway surface liquid of the bronchial mucosa[13]. In this method, the patient voluntarily hyperventilates by breathing dry air enriched with 5% CO2 (to avoid respiratory alkalosis) at a pre-specified target ventilation rate[13].

One important property of a diagnostic test is reproducibility and knowledge of the inherent variability (agreement and repeatability) has practical implications for evaluation of its clinical usefulness[18]. Anderson et al.[19]found a general agreement for EIB diagnosis of 76% between two exercise challenge tests carried out 1 to 4 days apart in 373 individuals reporting signs or symptoms suggestive of asthma. Lower agreement (30%) between two tests separated by 1 to >35 days for EIB diagnosis was found in a study of asthmatic adults when the diagnostic cutoff was a 15% drop in FEV_1[20].

The few studies that examined the reproducibility of EVH for EIB diagnosis were conducted in adult athletes and included a small number of individuals with asthma[21,22] or compared different EVH provocation protocols[23]. One study reported the results of repeated EVH tests in a small number of non-athlete adult males with asthma (eight individuals) and found a 100% agreement between challenge responses, obtained seven days apart or with an interval of 35 days[24]. We were not able to find studies of the short-term reproducibility of FEV_1response in asthmatic children and adolescents after EVH challenge.

The aim of the present study was to evaluate the short-term test-retest agreement and reproducibility (repeatability) of FEV_1changes after EVH in children and adolescents with medically diagnosed asthma.

Methods

The study was approved by the Institutional Ethics Committee and signed informed consent was obtained from parents/guardians along with the assent of children/adolescents.

Study population

Patients aged 10 to 20 years were recruited from the pediatric allergy and immunology clinic of the Hospital das Clínicas of the Universidade Federal de Pernambuco, in Recife – Brazil between 2017 and 2019. All had received a specialist diagnosis of asthma based on GINA criteria[25].

Individuals were excluded if they had a recent history (less than 6 weeks) of asthma exacerbation or acute respiratory infection symptoms, were regularly using inhaled steroids, were smokers or ex-smokers, had any other known pulmonary diseases, were unable to perform adequate forced expiratory maneuvers, or had a baseline $FEV_1 < 60\%$ of predicted FEV_1.

Procedures

All subjects were required to attend the laboratory on two occasions separated by a period of 2 to 4 days at a similar time of day ($+2$ hours), in the morning. On the first visit, anthropometric measurements were taken and patients responded to the Asthma Control Test (ACT) (Portuguese/Brazilian version)[26]. They then underwent spirometry to determine baseline FEV_1[27] using a daily calibrated spirometer (KitMicro Cosmed, Rome, Italy). The best of three acceptable maneuvers was chosen. If the value was $> 60\%$ of that predicted (for the Brazilian population[28]), individuals proceeded to the EVH challenge. On the second visit, baseline FEV_1 was measured and, if the best value lay within 15% of that of the first day (but higher than 60% of predicted), patients proceeded to the EVH challenge. The laboratory room temperature was controlled and air humidity was measured using a thermo hygrometer (Incoterm, Brazil).

Patients were instructed to abstain from caffeine and exercise on test days and from using short- and long-acting beta-2 agonists for 12 and 48 hours, respectively. It was recommended that patients not use inhaled corticosteroids on the test day[13].

Eucapnic voluntary hyperpnea and spirometry

Patients breathed a mixture of dry room-temperature air with 5% added carbon dioxide (CO2) (White-Martins, Recife, PE - Brazil) collected in a Douglas balloon, through the mouth, with nose clipped using a one-way low-resistance valve (Laerdal, Copenhagen -Denmark). The test lasted 6 min and the target ventilation per minute was set at 21 times that of the personal baseline FEV_1 (equivalent to 60% of predicted maximal voluntary ventilation)[13]. The ventilation rate per minute was monitored using an analog ventilometer (Wright Mark 8 NSPIRE Health, Colorado - USA) and subjects were coached every 30 seconds to maintain the target ventilation level. The ventilation rate was recorded every minute for six minutes and the mean expressed as a percentage of the calculated target ventilation. Spirometry for FEV_1 measurement was performed in duplicate 5, 15 and 30 minutes after EVH and the highest value recorded. The % drop in FEV_1 was calculated using the formula (baseline FEV_1– post-EVH FEV_1/baseline FEV_1.100) and the maximum decrease in FEV_1 at any of the three points in time was recorded (FEV_1fallmax%)[13]. Subjects were considered positive for EIB diagnosis if a decrease of$> 10\%$ in FEV_1 was observed at any evaluation time-point on at least one of the test days.

Individuals were divided into three groups: those with a FEV_1fallmax% $> 10\%$ on both test days, those with FEV_1fallmax% $> 10\%$ on one day only (divergent group) and those with FEV_1fallmax% $<10\%$ on both test days. The severity of EIB was graded as mild, moderate, or severe if the percentage decrease in FEV_1 from the pre-exercise level was $> 10\% < 25\%$,$> 25\% < 50\%$, and$> 50\%$, respectively[13].

Statistical analysis

Data were processed and analyzed using Statistical Package for the Social Sciences (SPSS), version 20.0 and figures produced using GraphPadPrism® version 6.00 for Windows. Normally distributed data (Kolmogorov-Smirnov) are expressed as means + SD and/or 95% confidence interval (CI) and non-normal data as median and interquartile range (IQR). Agreement was evaluated as a binary outcome considering the proportion of positive and negative response on both tests (proportion of agreement). Reproducibility (repeatability) and limits of agreement (LOAs) were assessed for FEV_1fallmax% and $AUC_{0\text{-}30min}$ using the method described by Bland & Altman[29]. Individuals who tested positive on both days with a FEV_1falmax% > 15% and those with > 20% were also analyzed separately for bias and LOAs in these sub-groups. The area under the curve up to the thirtieth minute (AUC $_{0\text{-}30min}$) was calculated by the trapezoidal method using the percentage of FEV_1fallmax% at each point in the evaluation. For the calculation of $AUC_{0\text{-}30min}$, positive variations in FEV_1after EVH were considered zero. Multivariate analysis of variance (MANOVA) was used to compare baseline FEV_1 as a percentage of predicted and the achieved ventilation rate as a percentage of the target calculated by group (with and without BIE) and visit. The Kruskal-Wallis test was performed to assess the differences in ACT between the groups. The intra-class correlation coefficient (ICC) for the FEV_1falmax% and $AUC_{0\text{-}30min}$ was calculated using a two-way mixed-effect model with the mean single measurement reported to evaluate relative reliability. The alpha error probability was set at 0.05.

Results

Baseline characteristics

Sixty-two asthmatic children and adolescents completed the study. Seven did not attend on the second day because of transport difficulties and were excluded. All individuals were able to complete the six-minute EVH and there was no need to interrupt the 30-minute FEV1 evaluation because of respiratory symptoms.

No patient was excluded because of a low predicted FEV_1level. Twelve were prescribed a low-dose (200mcg/day) continuous-use corticosteroid inhaler (beclomethasone, which is distributed free of charge by the government) but none were using it regularly and none had used it for at least one week before testing. No patient was using long-acting beta-2 agonists and all had albuterol for recovery. General data are presented in Table 1.

Mean laboratory temperature was 25.5°C (+ 2.8°C) and relative humidity 58.7% (+ 6.2%), with no differences between test days (p>0.05).

Reproducibility and agreement

Twenty-six of the 62 patients responded positively for EIB after EVH on both days (i.e., had a FEV_1fallmax%> 10%), 17 on one day only (5 on the first day and 12 on the second), and 19 responded negatively on both days. The overall agreement was 72.5% (95% CI 61.6%, 83.6%) and the positive and negative agreement proportions were 41.9% and 30.6% respectively.

No difference was observed in mean FEV_1fallmax% after EVH between visits for the group as a whole, with low bias (mean difference between visits for FEV_1fallmax%) but with wide LOAs. The same was found for the $AUC_{0\text{-}30min}$ (Table 2 and Figure 1). Seventeen out of 26 patients with a positive response on both test days (65.4%) experienced a decrease in FEV_1 > 15%, and, in 10 of these, the reduction was > 20%. In this sub-group of individuals, bias was also small and LOAs were wide for both FEV_1falmax% and $AUC_{0\text{-}30min}$ (Table 2). The mean FEV_1fallmax% in those individuals testing positive for EIB on one visit only (divergent group) was 17.7%+ 13.5% on the positive day (either visit one or two) and was statistically different from that observed in those individuals testing positive for EIB on both days (p = 0.016 for visit 1 / p = 0.021 for visit 2).

For the group as a whole, the intra-class correlation coefficient (ICC) for the FEV_1fallmax% between the two visits was 0.854 (95% CI 0.758, 0.912; p<0.001) and was 0.858 (95% CI 0.764, 0.915; p<0.001) for $AUC_{0\text{-}30min}$.

There was a significant correlation between FEV_1fallmax% and $AUC_{0\text{-}30min}$ for both visits (visit 1: r = 0.91,

$p < 0.001$ and visit 2: $r = 0.89$, $p < 0.001$).

Confounding factors

Potential differences in baseline FEV_1 (as a percentage of predicted) and achieved ventilation rate (as a percentage of the calculated target) between visits were considered confounding factors that could interfere with the FEV_1 response after EVH. As shown in Table 3, no differences between visits were observed in these two parameters either between groups or within groups. A more detailed overview of the individual target ventilation rates achieved on both visits for each group is provided in Figure 2.

Baseline asthma control status measured using the ACT score was also considered a potentially confounding variable but showed no difference between groups (Table 3). There were no differences either between groups in terms of age and BMI (p=0.624 and p=.0957, respectively - Kruskal-Wallis) or sex (p=.0738 - Chi-square).

Severity of EIB and recovery

During visit one, FEV_1 did not return spontaneously to baseline levels by the thirtieth minute in 15 of the individuals testing positive on both days (n=26), and, during visit two, in 14. In those testing positive on one day only, this occurred in 6/17. These individuals were given 400mcg inhaled albuterol and FEV_1returned to baseline values (within 10%) in all of these. Among those testing positive on both days, the severity of FEV_1fallmax% was graded as mild on Visits 1 and 2 in 16 and 13, respectively, as moderate in 8 and 11, and as severe in the same two individuals on both days. Fourteen of the individuals testing positive on one visit only had a mild FEV_1fallmax% response and three had a moderate response. There was no need to interrupt the FEV1 measurements after EVH due to respiratory complaints or oxygen desaturation below 94%.

Discussion

Knowledge of the reproducibility of a test is paramount for understanding its clinical utility for diagnosis or evaluation of changes over time and responses to therapeutic interventions[18]. In a group of 62 children and adolescents with asthma, we found the EVH challenge test to have a short-term FEV_1 reproducibility response of 72.5% for EIB diagnosis, with positive and negative agreement of 41.9% and 30.6%, respectively. It should be noted that, in 12 patients, EIB occurred only on the second challenge. This is relevant for the differential diagnosis of respiratory symptoms upon exercise in young asthmatics, in whom EIB is one of the most common but not the only triggering factor, with consequences for the individual patient and the choice and monitoring of treatment options[6,7,30,31]. Our study fills a gap in the literature, as published studies on the reproducibility of the EVH have been conducted only in athletes[21,22] or in small numbers of asthmatic adults[23,24]. The presence of EIB is defined by a decrease in $FEV_1 > 10\%$ from baseline, either for exercise or EVH challenges[13,17]. For research purposes, the dimensionless area under the FEV_1fallmax% curve and ICC may contribute to general understanding of the bronchial response and repeatability but are difficult to interpret on clinical grounds. Despite a high ICC and low bias, we found wide limits of agreement (LOAs), both for FEV_1fallmax% and $AUC_{0\text{-}30min}$ (Figure 1). As shown in Table 2, the overall LOAs were similar among those individuals with a positive response on two visits, irrespective of the magnitude of the FEV_1fallmax%. In an earlier study comparing EIB response between EVH and treadmill running challenges in a similar population, we also observed wide LOAs between tests ($+19.7\%$)[32].

The overall LOAs are wider than those found by Anderson et al.[19] in exercise tests in their group of children ($+$ 13.4%). This may be explained by the lower rate of negative agreement found in our study (30.6% compared to 56.8%) and may be a consequence of the difference in study populations, as Anderson's study group had symptoms compatible with asthma, but no medical diagnosis. Despite these differences, the overall agreement for EIB diagnosis in our study varies little from that observed by these authors, who found 76% agreement between two exercise tests conducted one to four days apart. Price et al.[21] found a 75% agreement between EIB responses to EVH tests on two occasions (14-21 days apart) in 32 adult athletes (19% with asthma) with a high proportion of negative agreement (55%). These authors considered the limits of agreement to be wide ($+$ 10.1%), although they were approximately 50% lower than in our study. This

too may be explained by differences in study population.

Our population comprised young individuals receiving an asthma diagnosis at a tertiary university specialty care facility, most (95%) classified as having controlled or partially controlled asthma, with a score >18 on the ACT (Brazilian version[26]). There were no differences in asthma control score between EVH response groups (Table 3). No patient was regularly using inhaled corticosteroids capable of interfering with the EIB response[33].

The recommended target ventilation rate during the six-minute EVH test for elite athletes is 85% of maximum voluntary ventilation (MVV), i.e., 30 times the baseline FEV_1. For non-athletes, 60% of MVV is considered adequate (21 times baseline FEV_1)[13,34]. We adopted this as the target ventilation rate to be achieved during the EVH challenge. Overall, there were no differences in the mean ventilation rate achieved between visits or between the three groups (Table 3). Most individuals (48/62) were able to achieve more than 80% of the target ventilation, but fourteen (22.5%) achieved values between 60% and 80%. This may be a weakness of our study. Detailed examination of these individuals reveals that four and eight were in the positive and negative EIB groups on both visits respectively, and two tested positive on one day only ($p = 0.119$, Chi Square).

No correlation was found between target ventilation and the FEV_1falmax% for any visit (Visit 1: $r = -0.20$, $p = 0.110$, Visit 2: $r = -0.12$, $p = 0.358$ – Spearman's rho). These findings are consistent with those of Brummel et al.[16] and Stadelmann et al.[22], who found the ventilation rate not to be related to FEV_1fallmax% after EVH. More studies need to be conducted to evaluate this relationship. Unlike these authors, we found no differences in ventilation levels achieved during EVH between males and females (p=0.430).

Published guidelines recommend a baseline FEV_1 of over 75% of predicted as a safety measure to avoid severe bronchospastic responses[35,36]. Brannan and Kippelen[34] recommend that individuals known to be asthmatic be excluded from performing EVH. Parsons et all.[13] do not provide specific safety recommendations. The experience in our laboratory, as reported by other publications[14,16,17,23,] is that a safe EVH test can be conducted in asthmatics with a baseline FEV_1 as low as 60% of predicted. Of course, the test must be conducted by trained personnel with resources available for a possible emergency event. On Visits 1 and 2, 11 and 14 patients respectively had baseline FEV_1 <75% but >60% of predicted; only one presented a severe EVH response (FEV_1fallmax% > 50%) but had no severe respiratory symptoms or hemoglobin oxygen saturation below 94%.

Different from Hurwitz et al.[37], who observed a weak but statistically significant correlation between baseline FEV_1 and the FEV_1fallmax%, we were unable to detect such a correlation on either of the two visits (Visit 1 $r = 0.165$, $p = 0.201$; Visit 2 $r = 0.178$, $p = 0.166$. Spearman's rho).

The limits of agreement between repeated tests allow us to evaluate the magnitude of response that can be considered to lie outside expected variations in the parameter[29]. For a treatment to be considered of benefit in individuals with a FEV_1falmax% greater than 20%, using the LOAs for this study population with a mean drop of 37.5%, the FEV_1fallmax% after administration of the drug would need to be less than 18%. On the other hand, the narrow LOAs observed for those individuals with negative response on both visits (Table 2) agree with the results of Burman et all.[17] that found a 10% fall in FEV_1as a good cutoff value for EIB diagnosis after EVH.

Bias in a test repeatability evaluation may be related to the execution of the test itself (measurement error), changes in environmental conditions or in individual factors, such as medication use, severity of disease, and exposure to triggering factors. The patients in our study were a homogeneous group in terms of asthma diagnosis, disease control level and medication use. The EVH tests were repeated within a short period of time to avoid any time-related changes in airway responsiveness, disease conditions or environmental exposure. No statistical differences were observed in baseline FEV_1% of predicted between visits. Although patients were coached to achieve the target ventilation rate (60% of MVV), some did not reach this level. However, comparison of individuals with divergent EIB responses to EVH on separate days revealed no differences in the mean ventilation rates achieved suggesting that this relationship deserves more investigation. Although

undetected factors may have interfered with the short-term reproducibility of response to the EVH stimulus in 17 patients that presented a different EIB response on each visit, our previous findings[33], like those of Price et al.[21] and Anderson et al.[19], suggest that there may be an inherent individual variability in bronchial response to EVH or exercise test, even between short term evaluations.

Our study was conducted in young asthmatics, most with well controlled disease and with a short interval between tests. The findings cannot, thus, be extrapolated to other populations or to larger intervals between tests, for which specifically designed studies should be conducted. It remains intriguing, however, that the same bronchial stimulus elicits different bronchial responses in different individuals with the same condition and, in the same individuals, on different occasions.

In conclusion, it is clear that, for the moment, there are no diagnostic tests that can be considered a "gold standard" for EIB diagnosis, either in athletes[38] or in asthmatic individuals[19]. Without a repeated EVH test, 28% of our patients with EIB diagnosis would have been missed. There is a need for cautious interpretation of a negative EVH challenge for EIB diagnosis and two or more tests should be performed in those patients in whom EIB is to be excluded. Keen clinical judgment needs to be exercised, especially in cases of diagnosis and therapeutic intervention in patients with exercise-related respiratory complaints and mild or no decrease in FEV_1 after EVH challenge.

Table 1. Baseline characteristics of patients

N	62
Sex	
Male (%)	25 (40%)
Age (Years)	
Mean (SD)	13.2 (2.7)
Range	10 – 20
Height (cm)	
Mean (SD)	152.8 (11)
Range	134 - 181
Weight (Kg)	
Mean (SD)	47.9 (13.2)
Range	24.7 – 72.0
BMI	
Mean (SD)	20.2 (4.0)
Range	13.6 - 29.9
FEV_1 ($L.sec^{-1}$)	
Mean (SD)	2.44 (0.72)
Range	1.19 - 4.18
FEV_1 % Pred	
Mean (SD)	89.0 (16.6)
Range	60.5 – 124.0
ACT	
Median	23
IQR	20 – 25
Range	16 – 25

BMI- Body mass index. FEV_1 = Forced expiratory volume in the first second. ACT = Asthma control test. SD = Standard deviation. IQR = Interquartile range.

Table 2 - Mean highest decrease of FEV_1 as percentage of basal values and area under the curve of FEV_1 decrease from basal (as percentage) between zero and the thirtieth minute of evaluation after EVH for both visits and bias and limits of agreement (LOAs) for each category.

	N	FEV_1 Mean FEV_1 fall-max% (SD) Visit 1	FEV_1 Mean FEV_1 fall-max% (SD) Visit 2	FEV_1 p	FEV_1 Bias* (SD)	FEV_1 LOAs	$AUC_{0\text{-}30min}$ Mean $AUC_{0\text{-}30min}$ (SD) Visit 1	$AUC_{0\text{-}30min}$ Mean $AUC_{0\text{-}30min}$ (SD) Visit 2	$AUC_{0\text{-}30min}$ p	$AUC_{0\text{-}30min}$ Bias** (SD)
All patients	62	14.91 (14.50)	15.77 (13.71)	0.520	0.87 (10.57)	+ 21.14	327.18 (295.26)	291.72 (328.42)	0.165	19.90 (198.68)
2 tests fal >10%	26	26.68 (13.46)	26.38 (13.49)	0.701	0.29 (10.73)	+ 22.06	516.00 (273.00)	521.06 (285.13)	0.604	5.05 (218.92)
2 tests fall >15%	17	32.47 (13.07)	31.44 (13.12)	0.717	1.03 (11.5)	+ 24.26	619.22 (274.99)	632.41 (262.02)	0.786	13.19 (210.82)
2 tests fall >20%	10	37.46 (12.23)	38.65 (11.92)	0.666	1.18 (8.37)	+ 19.08	726.21 (291.84)	751.64 (213.34)	0.763	25.43 (176.47)
1 test fall > 10%	17	10.74 (8.94)	12.63 (7.84)	0.607	1.90 (14.91)	+ 31.46	211.61 (225.96)	173.19 (74.88)	0.562	38.42 (267.50)
2 tests fall <10%	19	2.53 (3.43)	4.07 (3.66)	0.061	1.54 (3.35)	+ 7.01	82.66 (64.68)	99.84 (62.94)	0.083	17.18 (40.76)

LOAs = Limits of agreement. $AUC_{0\text{-}30min}$ = Area under the curve up to the thirtieth minute. Mean FEV_1fallmax% = mean percentage maximum decrease in FEV_1 compared to baseline. Bias* = Mean difference (SD) in FEV_1fallmax% between 2 days. Bias** = Mean difference in $AUC_{0\text{-}30min}$(SD) between 2 days.

Table 3 –Analysis of associated factors possibly influencing FEV_1 response after eucapnic voluntary hyperventilation

	All (n=62)	All (n=62)	Groups	Groups	Groups	Groups	Groups	Groups				
			FEV_1 decrease > 10% on 2 days (n=26)	FEV_1 decrease > 10% on 2 days (n=26)	FEV_1 decrease > 10% on 1 day (n=17)	FEV_1 decrease > 10% on 1 day (n=17)	FEV_1 decrease < 10% on 2 days (n=19)	FEV_1 decrease < 10% on 2 days (n=19)	MANOVA analysis	MANOVA analysis	MANOVA analysis	MANOVA a
	V 1	V 2	V 1	V 2	V 1	V 2	V 1	V 2	Visits	Visits	Groups	G

	All (n=62)	All (n=62)	Groups	Groups	Groups	Groups	Groups	Groups				
Basal FEV_1 % pred									F	p	F	p
Mean	88.9	87.94	88.18	86.48	85.27	84.61	92.03	92.56	0.047	0.8285	2.379	0
(SD)	15.92	14.9	16.01	14.79	15.42	18.05	13.50	14.48				
Achieved ventilation as % of target calculated												
Mean	96.45	94.81	99.73	98.48	90.88	96.81	92.47	94.55	0.3017	0.5839	0.7801	0
(SD)	24.62	20.64	23.93	20.12	14.58	25.75	25.32	23.13				
ACT* V1									Kruskal–Wallis test	Kruskal–Wallis test	Kruskal–Wallis test	K W t
Median	23	-	23	-	22	-	24	-		p	p	
IQR	20-25	-	19.5-25	-	19.5-24	-	22-25	-		0.2450	0.2450	

FEV_1 % pred. = Forced expiratory volume in the first second expressed as percentage of predicted value. ACT = Asthma control test. V = Visit. MANOVA= multivariate analysis of variance. SD= Stand deviation. IQR = interquartile range

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Figure 1. Bland-Altman plot of the maximum decrease in FEV_1 (upper figure) and area under the curve from baseline to thirtieth minute (lower figure) after EVH, comparing visits. Filled circles indicate individuals with EIB on both visits, empty circles individuals with EIB on one visit only (divergent response), and split circles individuals with no EIB.

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Figure 2. Ventilation achieved (as % of calculated) on Visits 1 and 2 (Day 1, Day 2). A = individuals with EIB on both visits, B = individuals without EIB, C = individuals with EIB on one visit only (divergent response).

References

1. Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, Weiss ST. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol. 2004 ;114(1):40-7.
2. Neffen H, Fritscher C, Cuevas Schacht F, Levy G, Chiarella P, Soriano JB, et al. Asthma control in Latin America: the Asthma Insights and Reality in Latin America (AIRLA) survey. Rev Panam Salud Publica. 2005;17(3):191–7.
3. Mancuso CA, Sayles W, Robbins L, Phillips EG, Ravenell K, Duffy C, Wenderoth S, Charlson ME. Barriers and facilitators to healthy physical activity in asthma patients. J Asthma. 2006 Mar; 43(2):137-43.
4. Velsor-Friedrich B, Vlasses F, Moberley J, Coover L. Talking with teens about asthma management. J Sch Nurs. 2004;20(3):140-148.
5. Winn CON, Mackintosh KA, Eddolls WTB, et al. Perceptions of asthma and exercise in adolescents with and without asthma. J Asthma. 2018;55(8):868-876.
6. Abu-Hasan M, Tannous B, Weinberger M. Exercise-induced dyspnea in children and adolescents: if not asthma then what? Ann Allergy Asthma Immunol. 2005;94(3):366-371.
7. Depiazzi J, Everard ML. Dysfunctional breathing and reaching one's physiological limit as causes of exercise-induced dyspnoea. *Breathe (Sheff)* . 2016;12(2):120-129.
8. de Aguiar KB, Anzolin M, Zhang L. Pediatr Pulmonol. Global prevalence of exercise-induced bronchoconstriction in childhood: A meta-analysis. Pediatr Pulmonol. 2018; 53(4):412-425.
9. Williams B, Powell A, Hoskins G, Neville R. Exploring and explaining low participation in physical activity among children and young people with asthma: a review. BMC Fam Pract. 2008;9:40. Published 2008 Jun 30.
10. Schwartz LB, Delgado L, Craig T, et al. Exercise-induced hypersensitivity syndromes in recreational and competitive athletes: a PRACTALL consensus report (what the general practitioner should know about sports and allergy). Allergy. 2008;63(8):953-961.
11. Hallstrand TS, Curtis JR, Koepsell TD, et al. Effectiveness of screening examinations to detect unrecognized exercise-induced bronchoconstriction. J Pediatr. 2002;141(3):343-348.
12. Correia MA Jr, Rizzo JA, Sarinho SW, Cavalcanti Sarinho ES, Medeiros D, Assis F. Effect of exercise-induced bronchospasm and parental beliefs on physical activity of asthmatic adolescents from a tropical region. Ann Allergy Asthma Immunol. 2012;108(4):249-253.
13. Parsons JP, Hallstrand TS, Mastronarde JG, et al. An official American Thoracic Society clinical practice guideline: exercise-induced bronchoconstriction. Am J Respir Crit Care Med. 2013;187(9):1016-1027.
14. Nicolai T, Mutius EV, Reitmeir P, Wjst M. Reactivity to cold-air hyperventilation in normal and in asthmatic children in a survey of 5,697 schoolchildren in southern Bavaria. Am Rev Respir Dis. 1993;147(3):565-572.
15. Anderson SD, Argyros GJ, Magnussen H, Holzer K. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. *Br J Sports Med* . 2001;35(5):344-347.
16. Brummel NE, Mastronarde JG, Rittinger D, Philips G, Parsons JP. The clinical utility of eucapnic voluntary hyperventilation testing for the diagnosis of exercise-induced bronchospasm. *J Asthma* . 2009;46(7):683-686.
17. Burman J, Elenius V, Lukkarinen H, et al. Cut-off values to evaluate exercise-induced asthma in eucapnic voluntary hyperventilation test for children [published online ahead of print, 2020 Jun 3]. Clin Physiol Funct Imaging. 2020;10.1111/cpf.12647. doi:10.1111/cpf.12647.
18. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound Obstet Gynecol* . 2008;31(4):466-475.
19. Anderson SD, Pearlman DS, Rundell KW, et al. Reproducibility of the airway response to an exercise

protocol standardized for intensity, duration, and inspired air conditions, in subjects with symptoms suggestive of asthma. Respir Res. 2010;11(1):120. Published 2010 Sep 1.
20. Dahlén B, O'Byrne PM, Watson RM, Roquet A, Larsen F, Inman MD. The reproducibility and sample size requirements of exercise-induced bronchoconstriction measurements. *Eur Respir J* . 2001;17(4):581-588.
21. 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(2):243-249.
22. Stadelmann K, Stensrud T, Carlsen KH. Respiratory symptoms and bronchial responsiveness in competitive swimmers. Med Sci Sports Exerc. 2011;43(3):375-381.
23. Argyros GJ, Roach JM, Hurwitz KM, Eliasson AH, Phillips YY. Eucapnic voluntary hyperventilation as a bronchoprovocation technique: development of a standarized dosing schedule in asthmatics. *Chest* . 1996;109(6):1520-1524.
24. Williams NC, Johnson MA, Hunter KA, Sharpe GR. Reproducibility of the bronchoconstrictive response to eucapnic voluntary hyperpnoea. *Respir Med* . 2015;109(10):1262-1267.
25. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Retrieved from http://www.ginasthma.org
26. Roxo JP, Ponte EV, Ramos DC, Pimentel L, D'Oliveira Júnior A, Cruz AA. Validação do Teste de Controle da Asma em português para uso no Brasil: validation for use in Brazil [Portuguese-language version of the Asthma Control Test]. J Bras Pneumol. 2010;36(2):159-166.
27. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* . 2005;26(2):319-338.
28. Pereira CAC, Neder JA. Sociedade Brasileira de Pneumologia. Diretrizes para Testes de Função Pulmonar. J Pneumol 2002;28(suppl. 3):1-82.
29. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-310.
30. Winn CON, Mackintosh KA, Eddolls WTB, et al. Perceptions of asthma and exercise in adolescents with and without asthma. J Asthma. 2018;55(8):868-876.
31. Brannan JD, Koskela H, Anderson SD. Monitoring asthma therapy using indirect bronchial provocation tests. Clin Respir J. 2007;1(1):3-15.
32. Chateaubriand do Nascimento Silva Filho MJ, Gonçalves AV, Tavares Viana M, Peixoto DM, Cavalcanti Sarinho ES, Rizzo JÂ. Exercise-induced bronchoconstriction diagnosis in asthmatic children: comparison of treadmill running and eucapnic voluntary hyperventilation challenges. Ann Allergy Asthma Immunol. 2015;115(4):277-281
33. Subbarao P, Duong M, Adelroth E, et al. Effect of ciclesonide dose and duration of therapy on exercise-induced bronchoconstriction in patients with asthma. J Allergy Clin Immunol. 2006;117(5):1008-1013.
34. Brannan JD, Kippelen P. Bronchial Provocation Testing for the Identification of Exercise-Induced Bronchoconstriction. *J Allergy Clin Immunol Pract* . 2020;8(7):2156-2164.
35. Weiler JM, Brannan JD, Randolph CC, et al. Exercise-induced bronchoconstriction update-2016. J Allergy Clin Immunol. 2016;138(5):1292-1295.e36.
36. Hallstrand TS, Leuppi JD, Joos G, et al. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. Eur Respir J. 2018;52(5):1801033. Published 2018 Nov 15.
37. Hurwitz KM, Argyros GJ, Roach JM, Eliasson AH, Phillips YY. Interpretation of eucapnic voluntary hyperventilation in the diagnosis of asthma. Chest. 1995;108(5):1240-1245.
38. 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(8):1083-1093.