**Is there cardiac autonomic dysfunction in children and adolescents with exercise-induced bronchoconstriction?**

**Abstract**

**Background:** The pulmonary impairment in patients with bronchoconstriction induced by eucapnic voluntary hyperpnea(EVH) goes beyond the respiratory system, also impairing autonomic nervous modulation. This study aimed to evaluate the behaviour of cardiac autonomic modulation in young asthmatics with and without EIB after the EVH test. **Research design and methods:** A cross-sectional study design using 54 asthmatics(51.9% female), aged between 10 and 19 years, investigated with the EVH test. Forced expiratory volume in one second(FEV1) was measured at 5, 10, 15, and 30 min after EVH. Heart rate variability(HRV) measures of time were assessed pre and 30 min-post EVH. The diagnosis of Exercise-Induced bronchoconstriction with underlying clinical asthma(EIBA) was confirmed by a fall in FEV1≥10% compared to baseline. **Results:** Thirty(55.5%) asthmatics had EIBA. Subjects with EIBA have reduced mean of the R-R intervals in relation to baseline until 15 minutes after EVH. Individuals without EIBA had increased parasympathetic activity compared to baseline(rMSSD) from 5 min after EVH(p<0.05). This parasympathetic activity increase in relation to baseline was seen in individuals with EIBA after 25 minutes (rMSSD=49.9±5.3 *vs* 63.5±7.2, p<0.05). **Conclusion:** Young asthmatics with EIBA present a delay in the increase of the parasympathetic component after EVH when compared to asthmatics without EIBA.

**Keywords:** Exercise-Induced bronchoconstriction; Heart Rate Variability; Asthma; Adolescent; Eucapnic voluntary hyperpnea

**1.0 Introduction**

Exercise-Induced bronchoconstriction (EIB) is characterized as an acute airflow obstruction of the lower airways that occurs during or after vigorous physical exercise, occurring in 50 to 90% of children and adolescents with asthma[1–3]. Recent guidelines recommend distinguishing EIB in individuals with underlying clinical asthma (EIBA) from that occurring in those without symptoms and signs of asthma (EIBwA) [1,2,4]. Although regular physical activity is considered an important component in the treatment of asthma, exercise can trigger bronchoconstriction and, when not identified and properly controlled, can result in lower participation rates of youth thus increasing the risk for chronic non-communicable diseases in the medium and long term, as well as affecting quality of life and socialization[2,5–7].

The pathophysiology of EIB is directly related to hyperventilation caused by moderate to intense physical exercises that results in dehydration of the lower airway mucosa, mainly in the periciliary fluid region, generating an osmotic gradient in relation to the epithelial cells that respond with water transfer and consequent release of mediators and nerve terminals activation that ends up in bronchoconstriction[2,8]. Respiratory symptoms such as dyspnea, chest tightness and cough may be present during EIB, however they cannot be considered sufficient for an accurate diagnosis, requiring more specific respiratory function tests to obtain an accurate diagnosis[2,4,9–11].

A reduction greater than 10% in forced expiratory volume in one second (FEV1) after running on a treadmill is the most used test for diagnosing EIB in clinical practice. The protocol requires an increase in exercise intensity over 2 minutes to reach a heart rate between 80 and 90% of the predicted maximum heart rate. This intensity is then maintained for an additional 4 to 6 minutes[2,4,9–11]. Surrogate tests for the diagnosis of EIB that do not require exercise and impose a similar osmotic stress on the airway epithelium include eucapnic voluntary hyperpnea (EVH) or inhalation of hyperosmolar aerosols, such as 4.5% saline, or dry powder manitol [2,4,9–11].

EVH is a validated and, widely used method[2,4,12–14], is considered safe and allows better control of ventilation rates and inspired air conditions, having the same pathophysiological pathway for EIB as the exercise challenge protocol outlined above, that is, the effect of dehydration on the bronchial mucosa[2,4]. In an EVH test, the patient hyperventilates for 6 minutes breathing dry air enriched with 5% CO2 (to avoid alkalosis) at a recommended ventilation rate (VR) of 21 times the individual's FEV1 for non-athletes and 30 times the FEV1 for athletes , corresponding to 60% and 85% of maximum voluntary ventilation (MVV), respectively [2,4,12–14].

Heart rate variability (HRV) has been used to assess autonomic modulations of the heart in physiological processes and pathological conditions, in individuals of different ages during rest or exercise. Change in HRV pattern provides an early and sensitive indicator of compromised health[15] and is considered an easily applicable and promising clinical tool for the assessment of physiological adaptability and alterations in autonomic function control[15–19]. Autonomic modulation, particularly sympathetic hyperactivity and parasympathetic hypoactivity, has been reported as an indication of increased risk of chronic diseases, aging, in addition to being a predictor of mortality[20,21].

A recent systematic review with meta-analysis drew attention to the fact that children and adolescents with asthma had lower heart rate variability and problems in autonomic modulation[22]. This draws attention to the fact that a chronic disease linked to the airways may be related to cardiac autonomic imbalance and consequently to other organs regulated by the ANS. During physical exercises, parasympathetic activity decreases (vagal withdrawal) and sympathetic activity increases and this may be altered in some way in asthmatics especially with EIB[22]. Despite the little evidence, it has been suggested that autonomic dysfunction is associated with a pathological response to exercise in patients with asthma [22,23] and in addition to the standard measurement at rest, measuring HRV in response to exercise may be alternative approach to assess ANS function in patients with asthma and EIB[16,24,25]. In this way, identifying and treating these patients with EIB can help not only with pulmonary improvement, but also with autonomic issues, potentially improving the overall health of an individual.

Most of the studies that evaluated some type of physical exertion and autonomic modulation in asthmatics did not present data on subjects with and without a diagnosis of EIB in their results[22,26,27]. Only one study was found[16]that evaluated individuals with and without a diagnosis of EIB after a bronchoprovocation exercise protocol. However, this research was carried out more than 20 years ago and the results presented do not allow for more current and in-depth comparisons regarding the technology and methods used[16]. In general, it is known that there is ANS dysfunction, especially the parasympathetic in asthma, and this would be associated with bronchial hyperresponsiveness and inflammation[22]. Regarding the EIB, the little existing knowledge does not allow a more detailed assessment on the subject [16]. Therefore, it is interesting to know whether individuals with asthma, who already present alterations in autonomic modulation[16,22,26,27], have other alterations in the functioning of the ANS when they have EIB.

A study on the responses of the ANS to a bronchoconstrictor stimulus that simulates vigorous physical exercise (i.e. Eucapnic voluntary hyperpnea) may help our understanding on triggers of EIB and its pathophysiology, in the search for a better treatment interventions. This is especially useful in understanding how an osmolar stimulus can trigger bronchoprovocation in about half of individuals with asthma[13,28,29] and in tracing the course of autonomic modulation in young asthmatics who have EIBA when compared to their peers that do not. In this context, this is an exploratory study that aimed at evaluating the behaviour of cardiac autonomic modulation in young asthmatics with and without bronchoconstriction induced by Eucapnic voluntary hyperpnea (EVH). Our hypothesis is that pulmonary impairment in patients with bronchoconstriction induced by EVH extrapolates the lung, also impairing autonomic nervous modulation.

**2.0 Methods**

*2.1 Type of study*

This was an analytical cross-sectional study, carried out from December 2018 to February 2020, at the Pulmonology Service of the Federal University of Pernambuco, Pernambuco, Brazil. The study was approved by the institution's ethics and research committee (Opinion No: 2.796.049). All parents or guardians signed the informed consent form, and the adolescents signed the consent form as requested by the regulatory agency.

*2.2 Population and sample*

Participants who were diagnosed with asthma and classified according to the criteria of the Global Initiative for Asthma[30] were referred from the allergy and clinical immunology, pulmonology, and paediatric clinics of the same hospital to participate in the study. Patients were new to the clinic(s), were in the initial clinical evaluation process, and still had not had treatment adjustments to achieve adequate control of symptoms.

Inclusion criteria were children and adolescents of both sexes, aged between 10 and 19 years old with classification of controlled asthma according to the Asthma Control Test (ACT)[31]. The exclusion criteria were as follows: baseline FEV1<60% of the predicted value for Brazilians[32], individuals reporting asthma exacerbation or acute respiratory infection in the last six weeks, inability to suspend medication for the tests, use of systemic corticosteroids, were smokers or ex-smokers, or those who were unable to perform the necessary maneuvers for spirometry or EVH.

All volunteers were instructed not to perform physical activity 24 hours before the scheduled day for data collection, not to drink stimulant drinks (e.g. caffeine containing drinks) 12 hours before collection, and to stop using medication, 12 hours for short-acting bronchodilators and 48 hours for long-acting bronchodilators[2,5]. All patients had a positive prick test for at least one of the tested aeroallergen mites (D. pteronyssinus, B. tropicalis) and some also for the other tested allergens (A. alternata, A. fumigatus, dog and cat dander).

*2.3 Procedures*

The study flow diagram is shown in Figure 1.

*2.4 Specific tests*

*2.4.1 Anthropometrics and Body Composition*

Body weight was measured on a digital scale with an accuracy of 0.01kg (Fillizola, Brazil). Height was measured with a portable 2m, 0.1cm graduation stadiometer (Sanny, Brazil). The body mass index was calculated according to the World Health Organization.

*2.4.2 Spirometry*

The pulmonary function test to measure FEV1 was performed using a calibrated MicroQuark spirometer equipment (COSMED, Rome, Italy) and in accordance with international standards[33]. Values for FEV1 were expressed as a percentage of predicted values for Brazilians[32]. The assessment of FEV1 was repeated at 5, 10, 15 and 30 minutes after the EVH[2].

*2.4.3 Eucapnic voluntary hyperpnea (EVH)*

The bronchial provocation test performed was EVH and this was carried out according to international criteria[2,5]. Participants breathed a mixture of dry air plus 5% carbon dioxide (White Martins, Recife, Brazil) to avoid alkalosis, from the Douglas balloon, through a mouthpiece and a low-resistance unidirectional valve (Laerdal, Copenhagen, Denmark). The test lasted six minutes and the target ventilation per minute was 21 times greater than the baseline FEV1 (recommendations for non-athletes). The ventilation rate (VR) was monitored using an analog ventilometer (Wright Mark 1098NSPIRE Health, Colorado, USA), and subjects were coached every 30 s to maintain the target ventilation. The VR was recorded every minute for six minutes.

The diagnosis of EIBA was confirmed by a fall in FEV1≥10% compared to baseline at any of the evaluated times[2]. If this reduction persisted until the last measurement, a bronchodilator was administered by inhalation (albuterol-spray, 400 mcg), followed by new FEV1 evaluations until a return to baseline[2] (nine patients needed to use rescue medication and none had to interrupt the measurement points during the test). All participants underwent pulse oximetry before and after exercise. The classification of EIBA intensity was given according to the reduction in FEV1 compared to baseline values, being mild (>10%, <25%), moderate (≥ 25%, <50%) or severe (>50%)[2].

*2.4.4 Heart rate variability (HRV)*

To capture the recordings of R-R intervals was used heart rate monitor (RS800CXTM, Polar Electro Co.Ltd.Kempele, Oulu, Finland) at a sampling rate of 1000 Hz with the transmitter located anatomically over the lower sternum. This device has been shown to provide valid recordings of beat-to-beat heart rate during rest[34] and exercise[35,36], in addition to good reproducibility indicators [34–38].

Following an initial 10-minute period of seated rest for acclimatization, patients were directed to stay seated for an additional 10 minutes, during which resting HRV data was collected. Throughout this period, participants were instructed to refrain from speaking, avoid using electronic devices, and minimize movement. Subsequent to bronchoprovocation through EVH, R-R intervals were measured during the time intervals of 0-5, 5-10, 10-15, and 25-30 minutes. These periods correspond exactly to the sequenced evaluations in FEV1 after the EVH protocol[2,5].

The captured R-R intervals were transferred to a computer through an infrared signal and recorded using the Polar-Pro-Trainer5TM® software. The next step consisted of transforming these data into files in txt.doc format, which were later analyzed using an HRV software (Kubios® version 3.3.1, Kuopio, Finland).

After visually inspecting the recordings, ectopic beats (those differing by more than 20% from the previous interval) were eliminated using an automated filter within the Kubios software. This process adhered to the recommendation of not exceeding 3% of the signal. The Task Force for HRV[39] also recommends at least 5 minutes to assess short-term HRV. Therefore, the signals lasted for at least 5 minutes of stationary signals. If any signal is less than 5 minutes this data would need to be deleted (no data was excluded). After these procedures, the time-domain variables of HRV, such as standard-deviation of all RR intervals (SDNN), root mean square of the squared differences between adjacent normal RR intervals (RMSSD), and the percentage of adjacent intervals over 50ms (PNN50) were obtained.

The tests were always performed in the morning and the laboratory room was air conditioned with an average temperature and relative humidity of 21.7±0.7C° and 50.4± 3.1%, respectively.

*2.5 Statistical analysis*

Mean and standard deviation were used to present continuous variables, while categorical data were presented using absolute values and relative frequencies. Statistical analysis was performed using the Statistical Package for Social Science (SPSS), version 21. The Shapiro Wilk test was applied to test the normality assumption.

Chi-square test was used to assess the differences between proportions, while the comparison between the means in the different groups was performed by the t-tests.

To compare the effects between the HRV variables and FEV1 according to the EIBA, during the evaluated times, and their interactions (group x time x interaction), Generalized Estimating Equations (GEE) were used, followed by Bonferroni's post hoc test with correction for multiple comparisons. Bilateral p values were calculated, and the significance level adopted was 5%.

**3.0 Results**

The study included 54 asthmatic individuals (51.9% female; 48.1% male), with a mean age of 12.5±3.6 years (range 10-19 years). Thirty (55.5%) participants were diagnosed with EIBA, of which 20 (66.7%) were classified as mild, 9 (30%) moderate and 1 (3.3%) severe. The characterization of the sample according to the diagnosis of EIBA is shown in table 1. There was no statistically significant difference between groups for sex, anthropometric data, and initial spirometry. As expected, asthmatics with EIBA showed a greater decrease in FEV1 after EVH compared to those without EIBA. The ages categorized between 10 to 13 years, 14 to 16 years and 17 to 19 years were similar between the groups with and without EIBA (p=0.890, chi square - data not shown in table).

Table 2 shows the values of the HRV indices of the groups according to EIBA diagnosis, during rest and after EVH in the time intervals of 0-5, 5-10, 10-15 and 25-30 minutes. Subjects with EIBA decreased the mean of R-R intervals in relation to the baseline until 15 minutes after EVH and increased the mean of HR up to 10 minutes. Individuals without EIBA had increased parasympathetic activity (rMSSD) from 5 min after EVH. This parasympathetic activity increase was seen in individuals with EIBA only after 25 minutes (rMSSD=49.9±5.3 vs 63.5±7.2, p interation <0.05). These differences are between the same groups compared with the baseline considering the interaction after the Bonferroni post hoc test.

Figure 2A shows the time course of FEV1 post EVH [i.e. (postFEV1 - preFEV1) / preFEV1 \* 100]. There were significant differences in FEV1 reduction between the groups with values for subject with EIBA being lower than without EIBA at all times evaluated (p interation < 0.001). Only those indexes that showed significant differences (p interation < 0.05) in the HRV presented in Table 2 are shown again in the form of figures (Figures 2B, and 2C).

**4.0 Discussion**

This research showed that participants with EIBA present a delay in the increase of the parasympathetic component (rMSSD) assessed by cardiac modulation after EVH when compared to asthmatics without EIBA. Thus, in addition to the bronchial impairment evidenced by the fall in FEV1 after EVH, individuals with EIBA also have alterations in cardiac autonomic modulation.

The recovery of HR after exertion is an important indicator of parasympathetic reactivity in the first minutes, there is a gradual withdrawal of the sympathetic at the same time with the continuation of vagal reactivity[40]. It was expected that soon after EVH there would be an increase in the parasympathetic rate (rMSSD), however in asthmatics without EIBA this occurred after 5min and in those with EIBA only after 25 minutes.

In normal breathing, parasympathetic efferent nerves are present in the regulation of bronchial smooth muscle tone, acting on constriction via the vagal cholinergic pathway and bronchodilation via the non-adrenergic non-cholinergic pathway (NANC)[23,41]. In the face of physical exertion, the ANS responds to stimuli sent by the cardiorespiratory system for the withdrawal of the parasympathetic activity, resulting in an increase in the diameter of the airways to improve gas exchange in response to the increase in metabolic demand[42], returning to homeostasis, under normal conditions, soon after cessation of the stimulus. However, young asthmatics had a delay in the return of parasympathetic indicators assessed by HRV that accompanied bronchial hyperresponsiveness assessed by FEV1 in response to EVH.

Terada et al.[43] showed a decrease in the fraction of exhaled nitric oxide (FENO) five and 10 minutes after exercise in atopic asthmatic individuals with EIB, the author explains that this apparently occurred through neural mechanisms, suggesting that patients with EIB have impaired nitric oxide (NO) production in response to exercise[41,43]. It was expected that there would be a prominent neural bronchodilator response from the non-adrenergic non-cholinergic inhibitory system (i-NANC), which is the neural relaxant response in the human airways, in addition, the presence of endogenous NO may inhibit contractile cholinergic responses[44]. However, the studies are not clear about which reasons would lead to this decrease in FENO in asthmatics who have EIB, since those without EIB did not present alteration[41,43]. Regarding our study, an important question would be whether this dysfunction seen in FENO in the previous study[43] would be a possible response to the delay of the parasympathetic activity seen in the heart, since the rapid return of the parasympathetic activity in a bronchus that is contracted could cause damage even greater in lung function. These facts lack scientific evidence[41,43] and highlight the innovative nature of our current research.

Altered parasympathetic activity is an important indicator of health and even mortality, it is usually affected in older individuals and in chronic diseases[20,21], but it can be compromised in children in the case of asthmatics, especially with EIB[22]. This respiratory impairment induced by a stimulus similar to exercise showed that, in addition to bronchoconstriction at the pulmonary level, assessed by FEV1, there was also an impact on cardiac autonomic modulation. As the autonomic balance is a fundamental health mechanism, if there is an anomaly in the rapid control of the ANS, this may be affecting several systems, since most organs are able to response quickly, as well as cardiac chronotropic and inotropic activities, blood pressure blood pressure and even glycemic control[20,21].

In this sense, a delay of a few seconds in the parasympathetic system already means a loss of autonomic function and control of several organs[20,21,39]. Thus, the impairment identified by the HRV measurements caused by a pulmonary stimulus serves as a warning that there may be a problem in the rapid response of other organs. With this, the early identification and treatment of EIB can help not only in the engagement in physical activities and socialization of these young people, but also in a possible adjustment of the autonomic homeostatic return after exercise of individuals with EIB and asthma.

Pichon et al.[45] evaluated the relationship between bronchial hyperresponsiveness and the cardiac parasympathetic component in 53 adults undergoing bronchial challenge with methacholine. A synthetic derivative of the neurotransmitter acetylcholine, methacholine is used to assess airway hyperresponsiveness (AHR) through direct aerosol administration to the smooth muscles of the bronchi, leading to constriction by stimulation of muscarinic M3 receptors[2,5]. The authors[45]reported a significant increase in parasympathetic tone at rest in the 24 (45.3%) test-positive individuals, in addition to showing an increase in SD1 (parasympathetic) after cholinergic stimulation when compared to baseline values up to five minutes of examination. In our research, we used the EVH test in young asthmatics and in contrast to the findings of Pichon et al.[45] no differences in parasympathetic activity were found in rest and in the first five minutes post EVH. The increased parasympathetic response occurred only at 25 minutes in subjects with a bronchoconstriction response to the test (compared to rest in the same group) and 5 minutes for those without (compared to rest in the same group), both of which correspond to an improvement in the fall in FEV1.

It is clear that the direct cholinergic stimulus[45] in the bronchi causes a different response than EVH and exercise. Methacholine inhalation is a test for evaluating direct AHR, not for EIB. There is significant discordance between AHR to methacholine and the severity of EIB[5,46,47]. Despite supporting the potential diagnosis of EIB, a positive methacholine challenge test does not rule in EIB, and a negative methacholine challenge does not entirely exclude EIB[5]. Exercise challenge is the direct method to determine whether exercise-related respiratory effects are EIB[2,4,5]. Several substitutes for exercise testing have been developed that may be easier to implement than challenge exercise and ensure the same physiological effects[2,4,5]. Among these substitutes, the most widely recognized is EVH, but there is also inhalation of hyperosmolar aerosols, such as 4.5% saline, or dry powder mannitol[2,4,5].

The data presented here provide an important question about the type of assessment and method to be used to identify the EIBA, with regard to physical exercise and its repercussions on the ANS. Silva et al.[26], when evaluating the autonomic dysfunction through the HR recovery after the functional capacity test (shuttle test), reported that youth with asthma had a delayed HR recovery compared to the group without asthma, suggesting ANS imbalance in this population. Gomes et al.[27] analysed 18 asthmatic and six healthy children after the walk test and the step test and found a significantly higher baseline HR in asthmatics, suggesting that resting tachycardia in asthmatic children may be caused by a change in autonomic modulation of the heart. Different from our study, Silva et al.[26] and, Gomes et al.[27] compared groups of asthmatics and non-asthmatics and did not assess the repercussions of exercise in individuals with and without a diagnosis of EIB with standardized protocols.

Fujii et al.[16] performed an analysis of LF, HF and LF/HF after bronchoprovocation challenge on a treadmill in 12 asthmatic children without EIBA, three with EIBA and seven control children. Despite having been conducted more than twenty years ago, this study was the only one that evaluated lung function and HRV before and at intervals of 0, 5, 10, 15 and 30minutes after a treadmill challenge in young population. It is important to note that the method used for EIBA diagnosis (diagnosed in only three patients) performed at the time was different from the one adopted in the current research, in addition to the fact that the authors[16] did not evaluate the time domain in their analyses and have used a statistical approach that evaluated the groups separately, without analysing the interaction between them, which may have increased the chances of type I error. These findings still need further investigation, but when added to other research they can add to the pathophysiological understanding of this clinical manifestation such that health professionals can identify and treat EIB in order that these young individuals do not limit their physical activities[2,4,7,26,48].

A possible limitation of our study was the non-use of frequency domain parameters due to the absence of respiration rate control (e.g., any respiratory issue including cough) during the collection of RR intervals. Another issue was not having followed these individuals for more than 30 minutes to better assess the pulmonary and HRV response, however, as we strictly followed the protocols, it was necessary to perform beta adrenergic rescue medication in some patients after 30 minutes of EVH. Future studies may extend the evaluation time until all patients return to the baseline, in addition to including better control of the respiratory rate and comparing other techniques such as the treadmill challenge.

The current research adds that the increase in the parasympathetic component after EVH occurred differently in patients with EIB and without EIB. New research may attempt to clarify whether this finding may be related to bronchoconstriction (caused by EVH) or whether an autonomic dysfunction may be influencing these patients to remain with the bronchi contracted.

**5.0 Conclusion**

It is concluded that the bronchoconstriction induced by eucapnic voluntary hyperpnea in asthmatic children and adolescents delayed the increase in the parasympathetic (25 minutes in subjects with bronchoconstriction and 5 minutes for those without, both when compared to baseline in the same group), suggesting, in addition to the bronchoconstriction, there is an altered autonomic function reflected by changes in HRV.

**6.0 References**

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**Image Legends**

**Figure 1:** Study flowchart. Black rectangle indicates the period of rest before collection and gray the protocol for eucapnic voluntary hyperpnea. Unfilled rectangle indicates capture of data on Heart Rate variability and unfilled circle indicates forced expiratory volume in one second. BD = Bronchodilator

**Figure 2:** Data were expressed as mean ± Standard deviation (SD).

A: decrease in FEV1 (%) compared to baseline after EVH in individuals with and without EIBA. B, and C: HRV indices that showed significant differences in Table 2 (R-R, and rMSSD intervals) according to groups with and without EIBA. R-R = R-R Intervals; rMSSD - Squared differences between adjacent normal R-R intervals.

Generalized Estimating Equations test.

\* Intra group difference when compared to baseline.

\*\* Difference between groups at all times evaluated