**Respiratory muscles’s thermographic analysis in asthmatic youth with and without bronchospasm induced by eucapnic voluntary hyperpnea.**

**Abstract**

**Objective:** To compare the thermographic pattern of regions of interest (ROI) of respiratory muscles in young asthmatics with and without bronchospasm induced by eucapnic voluntary hyperpnea (EVH)**. Materials and Methods:** Cross-sectional study carried out with 55 young (55% Male, 45% females) aged 12.5±3.3 years, divided in 9 non-asthmatics, 22 asthmatics without exercise-induced bronchospasm compatible response (EIB-cr) and 24 asthmatics with EIB-cr. The diagnosis of EIB was given to subjects with a fall in forced expiratory volume in the first second (FEV1) >10% compared to baseline. Thermographic recordings of respiratory muscles were delimited in ROI of the sternocleidomastoid (SCM), pectoral, and rectus abdominis intention area. Thermal captures and FEV1 were taken before and 5, 10, 15 and 30 minutes after EVH**. Results:** Twenty-four (52.1%) of asthmatics had EIB-cr**.** There was a decrease in temperature at 10 minutes after EVH test in the SCM, pectoral and rectus abdominis ROIs in all groups (both with p<0.05). There was a decrease in temperature (% basal) in asthmatic with EIB-cr compared to non-asthmatics in the rectus abdominis area (p<0.05). **Conclusion:** There was a decrease in temperature in the ROIs of different muscle groups, especially in asthmatics. The greater drop in FEV1 observed in individuals with EIB-cr was initially associated with a decrease in skin temperature, with a difference between the non-asthmatics in the abdominal muscle area. It is likely that this decrease in temperature occurred due to a temporary displacement of blood flow to the most used muscle groups, with a decrease in the region of the skin evaluated in the thermography.

  **Keywords:** Asthma; Exercise-Induced Bronchospasm; Thermography; Bronchial Hyper-Reactivity, Thermal Imaging.

**Introduction**

 Studies with the application of thermography in specific physical efforts, in the area of sports medicine and in ergonomics, describe acute changes in skin heat according to the execution technique, mode of muscle contraction, requested body area and intensity of effort (Schlader et al. 2011, Bartuzi et al. 2012, Formenti et al. 2013, 2017, Adamczyk et al. 2014, Fernández-Cuevas et al. 2014, Fröhlich et al. 2014, Duruturk et al. 2015, Escamilla-Galindo et al. 2017, Weigert et al. 2018). In this sense, some authors have stated that thermography can provide information about metabolic activity in muscle groups submitted to training and adaptations related to the reestablishment of basal temperature.(Bartuzi et al. 2012, Adamczyk et al. 2014, Fernández-Cuevas et al. 2014, Weigert et al. 2018)

 On the other hand, previous research had reported a fall in temperature when evaluating thermal response after exercise (Adamczyk et al. 2014, Fernández-Cuevas et al. 2014, Fröhlich et al. 2014, Escamilla-Galindo et al. 2017) and a case study had reported a fall in skin temperature in a man after training of respiratory muscles(Ludwig et al. 2012). This drop in skin temperature related to the regions of interest (ROI ́s) of the muscles studied has been described as a physiological response due to a temporary displacement of blood flow to the most used muscle groups in order to provide nutrients for active tissues (Fernández-Cuevas et al. 2014, Fröhlich et al. 2014, Escamilla-Galindo et al. 2017, Formenti et al. 2017).

 Although we did not find any study that evaluated measurements of skin temperature variations in respiratory muscle regions after a bronchial provocation test, changes in local temperature that may be related to changes in the exercise-induced bronchospasm(EIB) represent a challenging topic with innovative characteristics. These responses should stimulate studies, focusing on possible temperature changes in the skin of ROIs related to respiratory muscles and changes in bronchial obstruction objectively evaluated by the forced expiratory volume in one second(FEV1) over time. The present study aimed to describe, analyse, and compare the repercussions of the thermographic pattern of the ROIs of respiratory musculature in youth’s asthmatics with and without bronchospasm induced by eucapnic voluntary hyperpnea.

**Methods**

*Study design, period, and ethics*

 This is an exploratory, cross-sectional analytical study, carried out from April 2019 to February 2020, at the Pulmonary Functional Laboratory of the Hospital das Clínicas at the Federal University of Pernambuco, Recife, Brazil. The study was approved by the institution's ethics and research committee with human beings (No: 2.796.049). All parents or guardians and adolescents signed an informed consent form, as requested by the Brazilian Regulatory Agency. The study flowchart is shows in figure 1.

*Study Population*

 The study included children and adolescents of both sexes, aged between 8 and 20 years, allocated into three groups: 1- non-asthmatic, 2- asthmatic with EIB-cr and 3- asthmatic without EIB-cr. Non-asthmatic individuals were respiratory asymptomatic without a family history of asthma, atopy, or upper respiratory tract infection invited in schools and dissemination in digital media. Asthmatic subjects were diagnosed according to the Global Initiative for Asthma criteria (GINA 2018), confirmed by a specialist physician at the service. Patients were first-time incomers, were in the initial clinical evaluation process and still have not had treatment readjustments to achieve adequate control of symptoms.

 All volunteers were instructed to abstain from exercise (24 hours before test) and caffeine (on test day), from using short- and long-acting bronchodilators for 12 and 48 hours, respectively, and to not use inhaled corticosteroids on the test day (Parsons et al. 2013), in addition to fulfil the necessary requirements for obtaining infrared thermography images(Schwartz et al. 2015).

 Individuals reporting asthma exacerbation or acute respiratory infection in the last six weeks, who regularly used inhaled steroids, or had a baseline FEV1 <60% of predicted value for Brazilians (Pereira and Neder 2002), and those with incapacity to perform the manoeuvres necessary for spirometry or EVH were excluded (Parsons et al. 2013). All those with technical factors that prevent skin exposure to the thermal balance of the room in accordance with internationally recognized protocols(Schwartz et al. 2015) were also excluded.

 Ninety-eight individuals were selected to participate in the research and 43 were excluded [15 participants had symptoms of airway infection in the last six weeks, 11 subjects were unable to perform spirometry or EVH (6 had FEV1 <60% of predicted, 3 failed to perform more than 2 reproducible spirometry exams after 8 attempts, 2 did not understand how to perform HEV), seven wore makeup and/or tight clothes creating one compression area, four used inhaled corticosteroids on the day of collection and 6 withdrew from performing the tests during the initial interview].

*Procedures*

 Information was collected on symptoms during exercise, medication used, and then the patient’s weight(kg) and height(cm) were measured on a calibrated scale and stadiometer, respectively (Welmy W 200, Santa Barbara d´Oeste, SP, Brazil). Participants answered the Asthma Control Test (ACT), version validated for Brazil(Roxo et al. 2010). Air temperature and relative humidity were measured by a Thermo-Hygro-Anemometer (Incoterm, Porto Alegre, Brazil).

*Thermographic Analysis*

 The subjects were instructed to wear comfortable light cotton clothes for the collection(shorts for boys, top and shorts for girls)(Schwartz et al. 2015). Soon after, they stayed in an air-conditioned room (mean±SD ambient temperature of 21.8±0.7°C and 50.5±3.2% relative humidity), where thermographic images were collected by digital, infrared and portable (FLIR E5 Ex Series thermal imager, model E 63900, Flir Systems, USA), with uncooled microbolometer and fixed focus, thermal resolution IR = 120×90 pixels, thermal/NETD sensitivity =<0.10°C/<100 Mk, image frequency = 9 Hz, temperature readout range from –20° to 250°C, 3.0-inch color LCD screen (320 × 240 dots) , MSX (Multi Spectral Dynamic Imaging) of 320×240 and accuracy (precision) of ±2% or 2°C in an environment of 10°C to 35°C.

 The participants remained for 15 minutes in a sitting position, in a plastic chair and with an approximate angle of 90° in the hip, knee and ankle joints, back resting on the chair back, both hands resting on the iliac crests, with the gaze directed at the camera(Schwartz et al. 2015). For the thermographic record, the camera was positioned in front of the participant, stabilized by the tripod (Pro Camera Support, Bonge/Manfrotto, Italy) and at 60 cm for each demarcated region, adjusted according to the body dimensions of each participant, configured for emissivity 0.98 and thermal range 25° to 36°C. The moments of thermal capture occurred immediately before the collection of FEV1.

 All measurements were performed by the same researcher, always in the morning. Thermographic images were taken, which included the anterior region of the body, including the neck, chest, and abdomen, at each moment of the study. From the collected images, regions of interest (ROI ́s) were traced for thermal analysis of the chosen segment (Figure 2).

 The ROI`s corresponding to the area of intent of the right and left sternocleidomastoid (SCM) muscles were demarcated by elliptical forms (Figure 2 [E/1 e E/2]). The ROIs corresponding to the insertion muscles of the right and left pectoralis were also demarcated with ellipses and are represented in Figure 2 (E/3 e E/4). For the ROI representing the anterolateral abdominal musculature (area of intent of the most superficial musculature such as the rectum of the abdomen, the external oblique, and the transverse of the abdomen) a square area of analysis was used (Figure 2 [BX1]).

 These regions were selected in the software FLIR TOOLS® (version 5.6.16078.1002, 2015, FLIR Systems, Inc. 27700 SW, Parkway Avenue Wilsonville, OR 97070, USA), starting from the limitations of the original image made manually. The Rainbow HC (Rainbow High Contrast) colour palette was used, with images treated in thermal amplitude from 25.0 to 36.0°C and settings adjusted for environmental parameters of each participant, considering each ROI as a result of thermography the average temperature value of each capture region. The focus allowed clear distinction of all the details and contours of the image.

*Spirometry and eucapnic voluntary hyperpnea (EVH)*

 Baseline FEV1 was determined by a spirometer (MicroQuark - COSMED, Rome, Italy) in accordance with international protocols(Parsons et al. 2013). The equipment has been calibrated daily and the predicted values and calculations followed the criteria of execution and acceptability determined by international guidelines(Miller 2005).

For eucapnic voluntary hyperpnea, patients breathed a mixture of dry air at room temperature with the addition of 5% carbon dioxide (CO2) (White-Martins, Recife, PE - Brazil) collected in a Douglas balloon, through the mouth with the nose capped using a unilateral device of low resistance valve (Laerdal, Copenhagen-Denmark). The test lasted six minutes and the target of minute ventilation was set at 21 times higher than baseline(Anderson et al. 2001, Parsons et al. 2013, Weiler et al. 2016). The ventilation rate (VR) was monitored using an analogic ventilometer (Wright Mark 8 NSPIRE Health, Colorado-USA) and the subjects were stimulated every 30 seconds to maintain the target ventilation target.

A decrease of ≥10% in the FEV1 as compared to the baseline value during any measurement time point after the challenge (5, 10, 15, or 30 min) was considered a positive response to the EVH challenge and an EIB-compatible diagnosis(Parsons et al. 2013, Hallstrand et al. 2018). Patients who presented > 10% reduction after 30 minutes of evaluation were administered 400 mcg of spray salbutamol (four inhalations) and after 20 minutes, a new spirometry was performed to monitor the return of FEV1 to baseline levels.

*Statistical Analysis*

 Statistical Analysis was performed by GraphPad Prism 6.0 (USA). Continuous data is reported as means ± SD, while absolute data is reported as total value and percentage. Normality was verified by the Shapiro-Wilk test. For the difference between the proportions, the χ2 test was used. The One-Way ANOVA tests with Tukey's post-test and the t tests were used to evaluate the differences between the means.

 To compare the effects between the temperature and maximal post-EVH changes in temperature (expressed as a percentage of the baseline value) according to the EIB-cr, 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 (Scale response = linear when data assume normal distribution and gamma for non-normal distribution). Bilateral p values were calculated, and the significance level adopted was 5%

 We calculated the sample on the G\*power-3.1.9.4 program through post-hoc power based in the temperature on the ROI of the abdominal muscles data from three analysed groups (expressed as a percentage of the baseline value). The values considered were α=0.05, total sample size=55, number of groups =3, and effect size=0.355. The effect size was calculated based on the mean, sample size, and SD for the three analysed groups. These data generated a Power (1-β err prob) of 99%.

**Results**

 The study included 55 individuals (55% Male, 45% females) aged 12.5±3.3 years, grouped into nine non-asthmatics, 22 asthmatics without EIB-cr and 24 asthmatics with EIB-cr. The sample groups were homogeneous in relation to gender, age, BMI, ventilation rate achieved, basal FEV1 and predicted percentage (Table 1). Environmental parameters (ambient temperature and relative humidity of the room air) were not different in the collections of each group (Table 1). There was no difference in asthma disease control (ACT) among asthma patients with and without EIB-cr, but as expected, the fall in FEV1 was higher in individuals with EIB-cr (Table 1). Of the 24 individuals with EIB-cr, 14 (58.3%) were mild, 9 (37.5%) moderate and 01 (4.2%) severe.

In the ROI`s corresponding to the area of intent of all muscle groups (SCM, pectoral and rectum of the abdominal) occurred temperature decrease in 10 minutes after EVH when compared to baseline in all groups (non-asthmatics and asthmatics without and with EIB-cr) (Figure 3).

Figure 4 shows the behaviour in maximal post-EVH changes in temperature (expressed as a percentage of the baseline value) in the different ROIs over the evaluated times. There was a difference between asthmatic with EIB-cr and non-asthmatic in the ROI of anterolateral abdominal musculature, considering all the times evaluated (Figure 4). Figure 5 shows the time course of FEV1 post EVH. This figure shows the known difference in FEV1 between asthmatics with EIB-cr and asthmatics without EIB-cr and non-asthmatics (EIB-cr diagnosis = fall in FEV1 >10% compared to baseline)

**Discussion**

 This research is the first to present the repercussions and behaviour of the FEV1 and the thermographic pattern of the ROIs representing the musculature (right and left sternocleidomastoid muscles, right and left pectoralis major and area of intent of the rectum of the abdomen, external and transverse oblique of the abdomen) in the youth’s asthmatics with and without EVH-induced bronchospasm. In addition, it was shown that asthmatics with EIB had a greater temperature drop than non-asthmatic in the anterolateral abdominal musculature.

 Although the temperature captured with thermography represents the skin temperature, there are studies showing an association between skin temperature and muscle activity, whose stimulation dissipates heat through both direct conduction in the tissues and the thermoregulatory system that involves the transfer of heat via the blood, to be dissipated at the surface (skin) by radiation in addition to evaporation (Bartuzi et al. 2012, Adamczyk et al. 2014, Fernández-Cuevas et al. 2014, Weigert et al. 2018). Our findings suggest that in asthmatic individuals the temperatures decreased up to 10 minutes after EVH in all ROIs, reaching significantly different baseline values in the tenth minute, and then began to increase slightly. Asymptomatic patients also had significantly lower temperatures at 10 minutes, but their first thermal response was temperature increase, although it was not statistically significant. These results are in line with information observed for bronchial obstruction caused by EVH and objectively visualized by FEV1.

 It is likely that this decrease in temperature occurred by shifting blood flow to the most used muscle groups with cooling in the skin region(Ludwig et al. 2012, Adamczyk et al. 2014, Fernández-Cuevas et al. 2014, Fröhlich et al. 2014, Escamilla-Galindo et al. 2017, Formenti et al. 2017, Priego Quesada et al. 2017). These differences in the physical forms of temperature recording are important to understand the complex heat loss systems that are acting during and after exercise(Hildebrandt et al. 2010). Another question is that this answer was observed in different parts of the body, in the initial moments of bronchoconstriction up to 30 minutes where sweat production could not yet be present. Thus, it can be considered that these blood adjustments in the skin use the process of vasoconstriction of blood vessels. Therefore, it offers greater blood flow and, consequently, more oxygen to the muscle region being exercised(Johnson 2010).

However, soon after exercise, the responses may be different depending on several factors such as the duration and intensity of the exercise. Activities performed for longer with the same intensity can cause an increase in skin temperature in the regions of the hands, forearms, and arms and in the thorax regions(Hillen et al. 2020). Clark et al,(Clark et al. 1977) reported that a 75-minute run can increase skin temperature in the active musculature in relation to direct heat transfer from the active muscles to the skin surface. The constant increase in exercise load has also been associated by causing a continuous cutaneous vasoconstrictive response, dependent on the adrenergic system(Charkoudian 2010, Johnson 2010).

Although it was not measured in this research, respiratory muscle overload and mechanical disadvantage caused by hyperinflation and air trapping is already known in acute asthma(Ferrer et al. 2000, Panditi 2003, Miller 2005, Parsons et al. 2013). It is reported that the mechanical disadvantage caused by bronchospasm (measured by FEV1) may cause an increase in accessory muscle activity of respiration(Martin et al. 1980, Hill 1991, Cavalcanti et al. 2022). The difference in results in the percentage variation of temperature between asthmatics with EIB-cr and asymptomatic presenting initially increased surface temperature in the ROI of the abdomen in the latter group, can be justified because, for non-asthmatic, the EVH test does not require as much effort as for asthmatics. Thus, its thermal response does not mirror the peripheral vascular effect required when the body needs to direct blood flow to deeper tissues due to more intense muscle activity.

Due to the limited number of studies found on this theme, the low number of subjects evaluated in some studies(Ludwig et al. 2012, Duruturk et al. 2015, Pereira et al. 2015, Basu et al. 2016) and consequent absence of statistical treatment(Ludwig et al. 2012), do not allow for more in-depth comparisons on the subject and exposes the exploratory nature of the present study. Basu et al. 2016, analysed hyperventilation caused by stress and anxiety in humans using a thermographic tool aimed at the nose, calculating their breathing rates, and found good accuracy compared with a spirometer. Pereira et al. 2015, addressed the representation of an algorithm to remotely monitor the breathing rate using infrared thermography in the nasal region, as a reliable approach to respiratory function by measuring airflow and nasal temperature.

 A possible limitation of this study was the fact that it found few patients with severe EIB-cr and thus did not find a more intense thermal response, in addition to the low adherence of asymptomatic participants. However, a standardized classification for EIB-cr was used(Parsons et al. 2013), and its severity and the results observed here give an idea of the thermographic behaviour during a bronchoprovocation test with EVH. Other questions are about the muscles used for evaluation, the sensitivity of the camera in identifying the proposed changes and the absence of comparison between exercised/trained and untrained. In any case, there is a diversity of new and interesting lines of research on the thermal responses, obtained through infrared thermography, allowing an analysis of both global and local skin temperature. Future studies should investigate the level of influence of infrared thermography in different genders and age groups.

 Another question is about the use of the top on girls (guided to be small) which occurred in all conditions of thermographic measurement, being verified if the areas close to the garment contained peak points/higher temperature (possible due to friction with the skin during thoracic movement), which did not occur. Therefore, the interference of clothing on body surface temperature with the physical exertion performed in this study is unlikely. In addition, it was verified that the groups were homogeneous regarding sex.

In conclusion there was a decrease in temperature in different ROIs of muscle groups after EVH, especially in asthmatic participants. The abdominal area among the other ROIs of the analysed muscles showed the greatest differences of temperature when comparing individuals without asthma.

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**Conflict of Interest:** The authors report no conflicts of interest.

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**Table 1**. Characterization of children and adolescents according to bronchospasm induced by eucapnic voluntary hyperpnea (EVH)**.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | All (N=55) | Non-asthmatic (n=09) | Without EIB-cr (n=22) | EIB-cr (n=24) | p |
| Sex |  |  |  |  |  |
|  Male | 30 (55) | 05 (55,5) | 11 (50,0) | 14 (58,3) | 0,753 |
|  Female | 25 (46) | 04 (44,5) | 11 (50,0) | 10 (41,7) |
| Age (years) | 12,51±3,3 | 14,89±1,8 | 12,00±3,4 | 12,08±3,4 | 0,135 |
| BMI (Kg/m2) | 20,41±4,2 | 19,26±2,4 | 20,69±4,4 | 20,55±4,6 | 0,873 |
| ACT | 19,43±3,7 | - | 18,59±4,7 | 20,00±2,3 | 0,287 |
| Ventilation Rate(ml/min) | 46,26±18,0 | 58,7±4,2 | 41,1±17,4 | 47,3±19,4 | 0,070 |
| Temperature (C°)\*\* | 21,7±0,7 | 22,1±0,5 | 21,5±0,6 | 21,6±0,8 | 0,088 |
| Relative humidity (%)\*\* | 50,4±3,1 | 48,8±3,1 | 50,9±3,3 | 50,4±2,8 | 0,229 |
| Baseline FEV1 (ml) | 2,4±0,9 | 3,0±0,5 | 2,33±1,1 | 2,30±0,7 | 0,076 |
| FEV1 % | 85,7±16,3 | 91,8±10,3 | 85,9±17,3 | 83,2±17,2 | 0,409 |
| FEV1 drop (%) | 13,3±12,9 | -4,8±3,3\* | -5,3±3,3\* | -23,9±2,9 | <0,001 |

Data are expressed as absolute frequency (%) or mean± standard deviation. Chi square test, one-way ANOVA test and t test. \*Statistical difference with the EIB-cr group. \*\* = ambient temperature and relative humidity; EIB-cr = exercise-induced bronchospasm compatible response.

**Figure legends**

**Figure 1**. Study schedule flowchart. Unfilled circle indicates forced expiratory volume in the first second (FEV1). Gray rectangle is general data collection and EVH protocol, black is study acclimatization and unfilled thermography collection. BD= Bronchodilator

**Figure 2.** Thermographic image of the thorax with the respective regions of interest (ROI's).

**Figure 3.** Means and standard deviation of infrared temperatures of the sternocleidomastoid (SCM), pectoral and rectus abdominis muscles at rest and at five, 10, 15 and 30 minutes after EVH in the evaluated groups. Generalized Estimating Equations (GEE) analyses (scale response = Gamma).

\* = Statistical difference compared to baseline in all groups.

‡ = Statistical difference compared with 10 minutes in all groups.

**Figure 4.** Mean and standard deviation of the maximal post-EVH changes in temperature (expressed as a percentage of the baseline value) of sternocleidomastoid (SCM), pectoral and rectus abdominis muscles at five, 10, 15 and 30 minutes after EVH in the evaluated groups. Generalized Estimating Equations (GEE) analyses (scale response = Linear).

T = Temperature

\* = Statistical difference compared to 5 minutes in all groups.

\*\* = Difference between the EIB-cr and asymptomatic group.

‡ = Statistical difference compared with 10 minutes in all groups.

**Figure 5.** Decrease in FEV1 (%) compared to baseline after EVH in the evaluated groups. Generalized Estimating Equations (GEE) analyses (scale response = Linear), interaction p<0.001, time p<0.001 and group p<0.001.

\* = Difference between the EIB-cr and without EIB-cr and asymptomatic group.