



Respiratory burden in obese and young asthmatics: a study of diaphragmatic kinetics

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ABSTRACT

Objective: The aim of this study was to assess the diaphragm kinetics, respiratory function, and serum dosage of leptin and inflammatory cytokines (IL-6 and TNF- α) in three clinical groups: obese, asthmatic, and healthy. **Methods:** This is a clinical exploratory study performed on 73 youths (12-24 years of age, 42.5% male) allocated into three groups: obesity (OG, n=33), body mass index (BMIz-score) $\geq +2$, asthmatic (AG, n=26) controlled mild asthmatics, classified by GINA, and Healthy Control Group (CG, n=14). The participants were subjected to diaphragmatic ultrasound, spirometry, maximal respiratory pressure, serum leptin levels, and IL-6 and TNF- α whole blood cell culture levels. **Results:** Diaphragm thickness was higher in OG in comparison to AG and CG (2.0 ± 0.4 vs 1.7 ± 0.5 and 1.6 ± 0.2 , both with $p < 0.05$). Maximal voluntary ventilation (MVV) was significantly lower in OG and AG in relation to the CG (82.8 ± 21.4 and 72.5 ± 21.2 vs 102.8 ± 27.3 , both with $p < 0.05$). OG has the highest leptin rate among the groups (with the other two groups had $p < 0.05$). All groups had similar TNF- α and IL-6 levels. **Conclusion:** The muscular hypertrophy found in the diaphragm of the obese individuals can be justified by the increase in respiratory work imposed by the chronic condition of the disease. Such increase in thickness did not occur in controlled mild asthmatics. The IL-6 and TNF- α markers detected no evidence of muscle inflammation, even though leptin was expected to be altered in obese individuals. Both obese and asthmatic patients had lower pulmonary resistance than the healthy ones.

Keywords: Obesity; Asthma; Diaphragm excursion; Diaphragm thickness; Adipokines.

INTRODUCTION

Over the past decade, it has been reported that childhood obesity and asthma can lead to immunometabolic mechanisms that cause negative impact on the respiratory system.⁽¹⁾ Although it is well-known that obesity and asthma may alter chest mechanics, reduce lung volumes and capacities and increase airway resistance, changes in the diaphragmatic excursion and thickness in this specific population are yet to be studied^(2,3) Adipokines (serum leptin, IL-6) and other inflammatory cytokines can modify the function of the diaphragm in obese individuals throughout life.⁽⁴⁻⁷⁾

Ultrasound assessment of diaphragmatic kinetics can contribute to the early identification of structural changes induced by higher respiratory demand experienced by

obese individuals throughout life, as well as in inter-crisis periods of asthmatics.^(8,9) Our hypothesis is that obese or asthmatic youngsters may have their diaphragms impaired and adipokines altered, leading to negative impact on pulmonary function and respiratory muscle strength or resistance. This study aimed to assess the diaphragm kinetics, respiratory function, and serum dosage of leptin and inflammatory cytokines in blood cell culture (IL-6 and TNF- α) in the following three clinical groups: obese, asthmatic, and healthy young individuals.

METHODS

This is a clinical-exploratory study performed in the Pulmonary Function Laboratory of the Pulmonology Service at the Federal University of Pernambuco (UFPE)

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Clinical Hospital, in Recife, Pernambuco. Clinical Trial Registration: (Obesity-asthma Endophenotype and Diaphragm Mobility in Adolescence, NCT03029936).⁽¹⁰⁾ All the participants and their guardians were informed on the research procedures and signed terms of free informed consent.

The study includes seventy-three youngsters, both male and female, aged between 12 and 24 years, allocated into three groups: Obesity Group (OG), Asthmatic Group (AG), and Comparative Group (CG). The obesity group includes thirty-three individuals diagnosed with obesity (body mass index – BMIz-score $\geq +2$).⁽¹¹⁾ The asthma group consists of twenty-six individuals diagnosed with persistent mild controlled asthma, according to the criteria of GINA,⁽¹²⁾ with a BMIz-score $< +2$. Finally, the Comparative Group comprises 14 individuals without any respiratory or neurological diseases. Figure 1 shows the study design flowchart.

Considering the spontaneous demand of the service, we selected all patients at the hospital pediatric outpatient clinics, due to. The healthy group was composed of the healthy family members of the patients and the staff. The following exclusion criteria were applied: individuals with congenital, neurological, or genetic

diseases and patients who could not respond to the procedure commands.

Initially, we assessed the anthropometric and clinical data of all participant patients. All measurements were collected from the outpatient clinic in the morning by observing a four-hour fasting at least (to assess adipokine levels). Subsequently, a diaphragmatic ultrasound was performed to assess excursion and thickness, spirometry, and maximal respiratory pressures.

Body weight and height were measured using a digital scale with 0.01 kg precision (Digital scale, Indústrias Fillizola S.A, São Paulo, São Paulo, Brazil) and a 2-m portable 0.1-cm graduation stadiometer (Stadiometer, Sanny®, São Bernardo do Campo, São Paulo, Brazil), respectively. The body mass index (BMI) calculation followed the World Health Organization (WHO) AnthroPlus program (AnthroPlus, WHO, Geneva, Switzerland) and categorized according to the BMI z-score.⁽¹¹⁾

We assessed the body composition by measuring the seven skin folds (subscapular, middle axillary, triceps brachii, thigh, suprailiac, abdomen, and chest) using a digital plicometer (Digital plicometer DGI, Prime Med, Curitiba, Paraná, Brazil). Three measurements were performed and followed by the calculation of the arithmetic mean among them.⁽¹³⁾

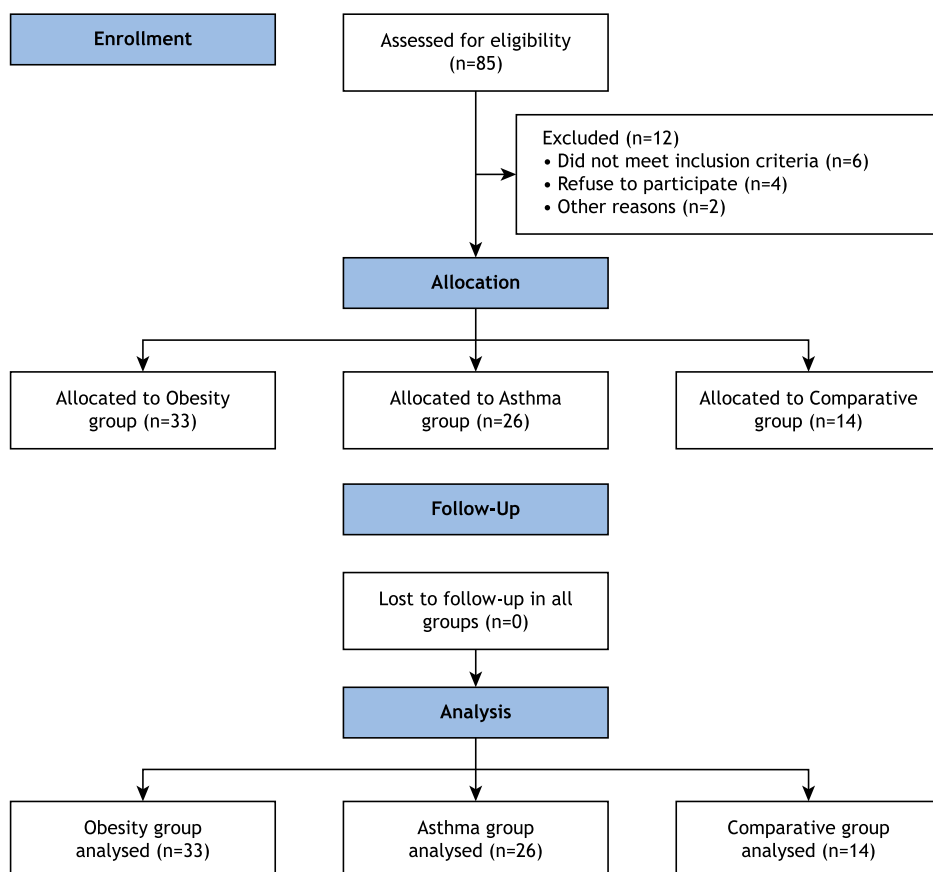


Figure 1. Study design flowchart.

We measured the diaphragmatic excursion with the individuals lying with their thorax supported on a 45° slope by performing an ultrasound (Sono Ace R3 ultrasound system, Samsung Company, Seoul, South Korea) in M mode with a convex transducer (3.5MHz), positioned at the right mid-axillary line.^(14,15) Participants were instructed to breathe deeply and rapidly at the level of total lung capacity (TLC), which was repeated several times. The record of cranio-caudal diaphragmatic excursions during resting breathing and respiration until total lung capacity showed sinusoidal curves.^(14,15) The diaphragmatic excursion (Dexc) appeared in the trajectory obtained between the baseline before the inspiration onset and the plateau in the total lung capacity end. We obtained the average of five measurements with a difference below 10% between them.

We assessed the diaphragmatic thickness through an ultrasound (Sono Ace R3 ultrasound system, Samsung Company, Seoul, South Korea) in B mode. The participant was positioned in the left lateral decubitus position^(9,14,15) and a high-resolution, low-penetration linear transducer (7.5 MHz) was placed perpendicularly to the thoracic cavity between the eighth and ninth intercostal space between the axillary lines.⁽¹⁶⁾ The diaphragm was identified by two parallel bright lines that depict the pleural and peritoneal membrane. The diaphragmatic thickness was measured from the middle of the pleural line to the middle of the peritoneal line. Two thickness measurements were performed between 0.5 and 2 centimeters of the costophrenic sinus visualization in each ultrasound image, and the mean value of these two measurements was used as the final measurement.⁽¹⁵⁾ The mean of three final thickness measurements of the diaphragmatic apposition zone was obtained during the functional residual capacity (relaxed diaphragm thickness - T_{frc}) and in the end of the total lung capacity (contracted diaphragm thickness - T_{tlc}). We also calculated the thickening fraction (TF) as the proportional thickening of the diaphragm from functional residual capacity (FRC) to total lung capacity (TLC). TF represents an index of diaphragmatic thickening as defined by the following Equation 1:

$$TF = \left[\frac{(T_{tlc} - T_{frc})}{T_{frc}} \right] \times 100 \quad (1)$$

where T_{frc} is the diaphragm thickness measured in the end of a quiet expiration (at FRC), and T_{tlc} is the maximum diaphragm thickness measured in the end of a deep breath (at TLC).

We used a multifunctional portable spirometer (Spirobank G USB - MIR; Rome, Italy) to perform the spirometry according to the recommended standards.⁽¹⁷⁾ To assess the maximum voluntary ventilation (MVV), we asked the patient to breathe as fast and deep as possible for 12 to 15 seconds. The volume mobilized in this time period was then extrapolated to the time of 1 minute. All equations used for the calculations were estimated by Pereira et al.⁽¹⁸⁾

We assessed the inspiratory and expiratory muscle strength indirectly by measuring the maximal inspiratory and expiratory pressures (MIP and MEP, respectively) using a digital manovacuometer (MVD-300, GlobalMed, Rio Grande do Sul, Brazil). To obtain the MIP, the patient was required to perform an expiration until residual volume, followed by a maximal inspiration with the airway occluded by a nasal clip. For the MEP, the patient was asked to inhale until total lung capacity, followed by a forced expiration. We performed three maneuvers with the patient sitting and considered the highest value for assessment, based on normal values for the maximal respiratory pressures in young individuals.⁽¹⁹⁾

Ten milliliters of blood were collected in tubes containing sodium heparin for cell culture. Leptin analysis was performed according to the manufacturer's recommendations using the Human Leptin Elisa commercial kit (Leptin ELISA Kit, Millipore Corporation, St Charles, Missouri, USA). Serum sample cytokine levels were quantified through the Cytometric Bead Array (CBA) system, following the methodology suggested by the manufacturer. Firstly, we transferred 50 µl of the capture beads mixture labeled with monoclonal antibodies (anti-IL-2, anti-IL-4, anti-IL-6, anti-IL-10, anti-IFN γ , and anti-TNF- α) with different fluorescence intensities (FL3) to tubes to test the samples and the negative control. Data were acquired using the FACScalibur flow cytometer and analyses were performed on the BD CBA software (BD CBA, Becton, Dickinson and Company, San Jose, USA).

Peripheral blood cells were cultured for a second day standardization performed by Lorena et al.⁽²⁰⁾ We stimulated cultures of *Dermatophagoides pteronyssinus* (DPT), *Phytohemagglutinin* (PHA) (5 µg/mL) and used cultures without stimulus as negative control. Blood was cultured in culture-specific tubes at a ratio of 1mL whole blood to 1mL RPMI 1640 medium supplemented with 10% Fetal Bovine Serum at 37°C at 5% CO₂.

We conducted the statistical testson a statistical software (SPSS, 20.0, Chigado, IL, USA) and built the figures on GraphPad InStat (GraphPad Software, San Diego, CA, USA). A Shapiro-Wilk test verified the assumption of normality and homogeneity of the quantitative variables involved in the study. One-way ANOVA with Tukey's multiple comparison test compared the quantitative and normal variables among the three groups. In case of non-normal quantitative variables, a Kruskal-Wallis test with Dunn's Multiple Comparison was applied. All correlations used Spearman coefficient. Numerical variables were presented as central tendency and dispersion measures. All conclusions resulted from a significance level of 5%.

We calculated the sample on the G*power-3.1.9.4 program through post-hoc power based on the diaphragm thickness data from three analyzed groups. The values considered were $\alpha=0.05$, total sample size=73, number of groups in the one-way ANOVA test=3, and effect size $f=0.4260637$. The effect size was calculated based on the mean, sample size, and

square root of the combined variance for the three analyzed groups. These data generated a Power ($1-\beta$ err prob) of 90%.

RESULTS

Of the total of 73 participating individuals (42.5% men), 14 (42.8% men) were allocated in the health control group (CG), 33 (39.4% men) referred to those with obesity without asthma (OG), and 26 (46.1% men) had persistent mild controlled asthma (AG).

As expected, no difference among the groups was found for either age or height, while the obesity group had higher levels of total weight, BMI z-score, lean body mass, fat mass, fat percentage, and abdominal circumference than both the asthma and comparative groups (Table 1).

Diaphragm Excursion, Thickness, and Thickening Fraction

Figure 2 shows the comparisons of diaphragmatic excursion and thickness among the groups. Regarding diaphragm kinetics, the obesity group had higher thickness at FRC than both the asthma and healthy groups. No difference was indicated between the median (interquartile range) and thickening fraction among obese, asthmatic, and healthy individuals [$158 (79.5)$ vs $157.5 (133)$ vs $161 (109.9)$, $p > 0.05$ - Nonparametric data-Kruskal-Wallis test]. In addition, the thickening fractions were positively correlated with fat mass and body fat percentage ($r = 0.431$, $p = 0.012$ and $r = 0.425$, $p = 0.014$, respectively).

Pulmonary Function Test and Maximal Respiratory Pressures

All three study groups had spirometric variables, including forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), within the normal range (table 2). Both the obese and asthma groups had significantly lower mean MVV% than the healthy individuals (Table 2). All groups had similar maximal respiratory pressures.

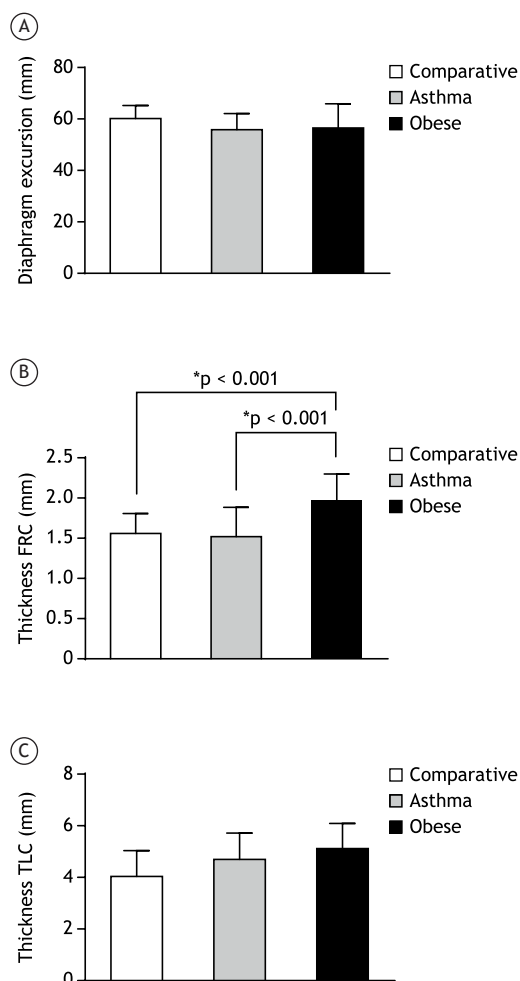


Figure 2. Comparison of diaphragm excursion (A), diaphragm thickness at functional residual capacity - TfrC (B) and diaphragm thickness at total lung capacity - TtC (C), between obese group, asthma group and comparative group (59.6 ± 9.8 vs 54.1 ± 13.9 vs 53.7 ± 16.3 ; $p = 0.4223$, 1.6 ± 0.2 vs 1.7 ± 0.5 vs 2.0 ± 0.4 ; $p = 0.001$ and 4.3 ± 1.2 vs 4.8 ± 1.3 vs 5.2 ± 1.2 ; $p = 0.0735$, respectively). * One-way ANOVA with Tukey's multiple comparison test.

Table 1. Characteristics of the 73 adolescents.

Parameters	Obesity (n = 33)	Asthma (n = 26)	Comparative (n = 14)	p^*
Age (y)	14(3.5)	14(3)	17.5(5.3)	> 0.050
Total weight	83.3 ± 17.8	47.2 ± 10.4^a	54.1 ± 5.6^a	< 0.001
Height	159 ± 7.4	156 ± 10.03	164 ± 5.77	> 0.050
BMIz-score	$2.7(0.8)$	$0.52(0.1)^a$	$-0.6(-0.8)$	< 0.001
Lean Body Mass (Kg)	55.5 ± 9.5	44.1 ± 13.7^a	44.9 ± 4.5^a	< 0.001
Fat Mass (Kg)	25.8 ± 10.3	7.8 ± 5.0^a	8.5 ± 4.3^a	< 0.001
Body Fat (%)	30.8 ± 7	14.6 ± 8^a	15.6 ± 7.1^a	< 0.001
Abdominal Circumference (cm)	99.9 ± 12.8	68.6 ± 13.6^a	73 ± 4.3^a	< 0.001

Data are reported as median and interquartile range or mean $\pm$ standard deviation when applicable. BMIz-score: body mass index. ^adifferences with obesity group. *One-way ANOVA with Tukey's multiple comparison test and Kruskal-Wallis test with Dunn's Multiple Comparison.

Systemic Leptin Levels and Adipokines Cell Culture

The obesity group had higher serum leptin level than both the asthmatic and healthy groups [48.1(35.2) vs

10.1(16.9) vs 8.7(15.5), $p<0.001$]. The whole blood cell culture showed no significant differences of TNF- α and IL-6 responses to DPT and PHA among the three groups (Figure 3).

Table 2. Spirometric parameters of the adolescents.

Parameters	Obesity (n = 33)	Asthma (n = 26)	Comparative (n = 14)	p*
FVC (%)	99.3±15.5	99.6±19.5	96.1±9.8	0,234
FEV1 (%)	96.3±15.2	91.6±18.2	95.1±9.7	0,497
FEV1/FVC	97.0±15,3	92,0±18,8	99,0±9,7	0,3309
MVV (%)	82.8±21.4 ^a	72.5±21.2 ^a	102.8±27.3	<0.001
MIP (cmH ₂ O)	-76.2±25.7	-78.1±21	-72.2±20.5	0,745
MEP (cmH ₂ O)	86.2±24.5	80.3±27.2	79.9±25.2	0,601

FVC: percentage of predicted forced vital capacity, FEV1: percentage of predicted forced expiratory volume in one second, MVV%: percentage of predicted maximal voluntary ventilation, MIP – maximal inspiratory pressure; MEP: maximal expiratory pressure. The equations used to calculate predicted percentages were estimated by Pereira et al.⁽¹⁸⁾. ^adifference with comparative group. *One-way ANOVA with Tukey’s multiple comparison test.

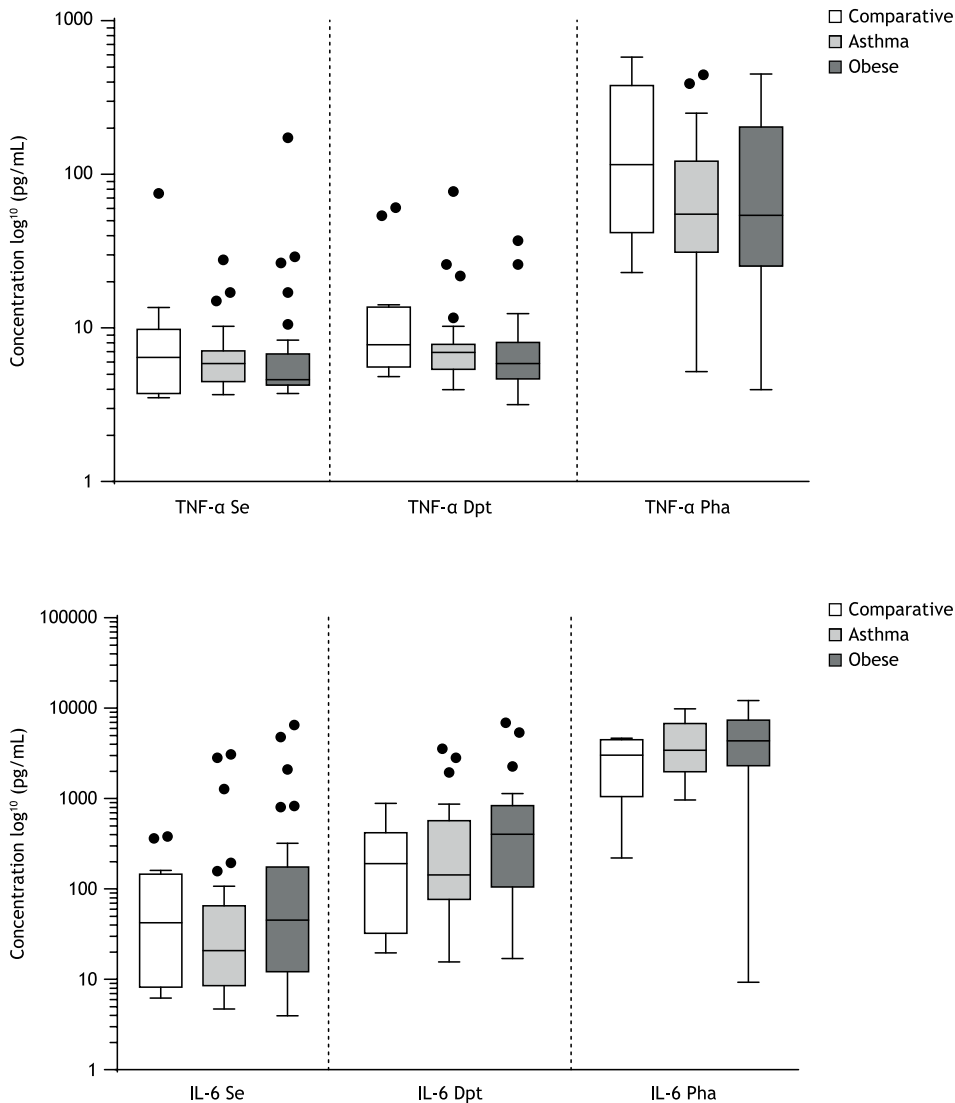


Figure 3. Comparison of (IL-6) and TNF- α levels in obesity, asthma and comparative groups. IL = interleukin, Se = no stimulus, Dpt = Dermatophagoides pteronyssinus, Pha = phytohemagglutinin.

DISCUSSION

This is the first study addressing the assessment of diaphragm excursion and thickness in a sample of obese and asthmatic young individuals with all measurements aimed at investigating the relationships with systemic levels of adipokines (leptin and IL-6) and TNF- α , spirometric variables and maximal respiratory pressures, mainly on diaphragm kinetics. We observed that obesity led to increased diaphragm thickness at functional residual capacity, but diaphragm excursion remained unaltered. Such hypertrophy can be justified by the increase in respiratory work imposed by the chronic condition of the disease. In controlled asthma, occasional asthma attacks did not cause an increase in muscle mass, although both the asthma and obesity groups had lower respiratory resistance than the healthy group. Neither the IL-6 nor TNF- α markers pointed to any evidence of muscle inflammation, altered leptin was expected in obese individuals.

We carried out different assessment of the diaphragm kinetics in youngsters. To our knowledge, only one previous study⁽²¹⁾ had assessed sonographic measurements of diaphragmatic excursion and thickness in healthy infants and children proposing normal values for this age group. However, this study assessed the excursion and thickness of the diaphragm at functional residual capacity (FRC), failing to demonstrate the diaphragm dynamics.

Boussuges et al.⁽⁹⁾ and Ueki et al.⁽¹⁶⁾ clearly state the importance of assessing diaphragm excursion and thickness in total lung capacity (TLC). Until now, no previous studies had assessed sonographic measurements of diaphragmatic excursion and thickness in young obese individuals. Despite the reported difficulty in assessing excursion and diaphragmatic thickness in obese individuals,⁽⁹⁾ our study found no difficulties in assessing diaphragmatic kinetics in obese youngsters.

The thickening of the diaphragm in its apposition zone may boost diaphragmatic excursion when facing an increase in physiological (maximum inspiration) or pathological (dyspnea) load in a well-awake patient.⁽²²⁾ Still, we also found a positive correlation between fat mass and body fat percentage with thickening fractions in the obesity group, allowing to conjecture that individuals with higher degrees of obesity may compromise diaphragm function. As we understand it, much still needs to be clarified about the behavior of diaphragm excursion, thickness, and thickening fraction in the context of obesity, especially regarding their impact on pulmonary function.

Some studies^(23,24) have addressed the assessment of diaphragmatic kinetics with associated pathologies. In intensive care units, thickening fraction (TF) became a new index to predict diaphragm dysfunction.⁽²⁵⁾ It reflects the work of breathing settled by the diaphragm in response to a certain load and can replicate intrinsic diaphragm strength, but it is also influenced by the load degree imposed to the respiratory system.⁽²⁶⁾ A higher TF may reflect increased breathing work in response to higher cardiorespiratory load imposed to the diaphragmatic muscle when assessed under

spontaneous breathing conditions, but no upper limits are known for young individuals.⁽²⁷⁾

Pulmonary function has been widely studied in the scope of obesity and asthma.⁽²⁷⁾ In this study, we found that both obese and asthmatic youngsters have normal pulmonary function, but decreased respiratory resistance (MVV) and showed normal values for maximal respiratory pressures. Recently, the white adipose tissue has been shown to control muscle metabolism and also contribute to the accumulation of intramuscular adipocytes, in addition to increasing insulin resistance.⁽²⁸⁾ This deposit of intramuscular fat may be detrimental to muscle function associated with the release of pro-inflammatory factors, such as leptin, IL-6, and TNF- α , which are harmful to muscle metabolism.⁽⁶⁾

Obesity and asthma have different responses to Th1 and Th2, influenced by the stimulant antigen and presence of cytokines in the environment.⁽⁷⁾ In the context of obesity, TNF- α is among the most studied pro-inflammatory cytokines.⁽²⁹⁾ It may be increased, but not necessarily implicated in inflammatory pathologies or processes. In our study, both the obesity and asthma groups had no significant response to TNF- α levels in relation to the comparative group, even upon DPT stimulation. This finding is consistent with other studies.^(4,30) Serum IL-6 concentrations, known as low-grade chronic inflammatory markers, are associated with obesity and insulin resistance in both adults and children.^(4,29) Smargiassi et al.⁽⁵⁾ reported an association of circulating proinflammatory peptides, including CRP and IL-6, with abdominal adiposity, cardiometabolic risk factors, and insulin resistance in prepubertal children. Our findings the obesity group showed no significant response to IL-6 levels in any of the three stimulations in relation to the other groups. It is possible that the sample size in these variables was limited to identify the difference among the groups.

Thus, we based our hypothesis on the fact that high levels of serum leptin in obese individuals could be linked to changes in the diaphragmatic thickness, as shown in elderly populations in which leptin levels are associated with low physical performance and decreased strength and muscle mass.⁽⁶⁾ We found significant differences in serum leptin levels in the obesity group, while leptin in the asthmatic group was similar to the comparative group. Nevertheless, our results show obese individuals with higher values of diaphragmatic thicknesses in FRC than the asthma and comparative groups. A possible explanation for these findings would be the accumulation of intramuscular adipocytes, although indirect inspiratory muscle training promoted by obesity should not be ruled out.⁽³¹⁾

Our patients were mild asthmatics who had normal pulmonary function in asthma attacks, therefore, no difference in lung function was present. We based our study on a hypothetical model in which a thickening of the diaphragm and alteration in lung function would derive from fat accumulation in the liver resulting from chronic subclinical inflammation. However, our cell culture failed to demonstrate that these cytokines

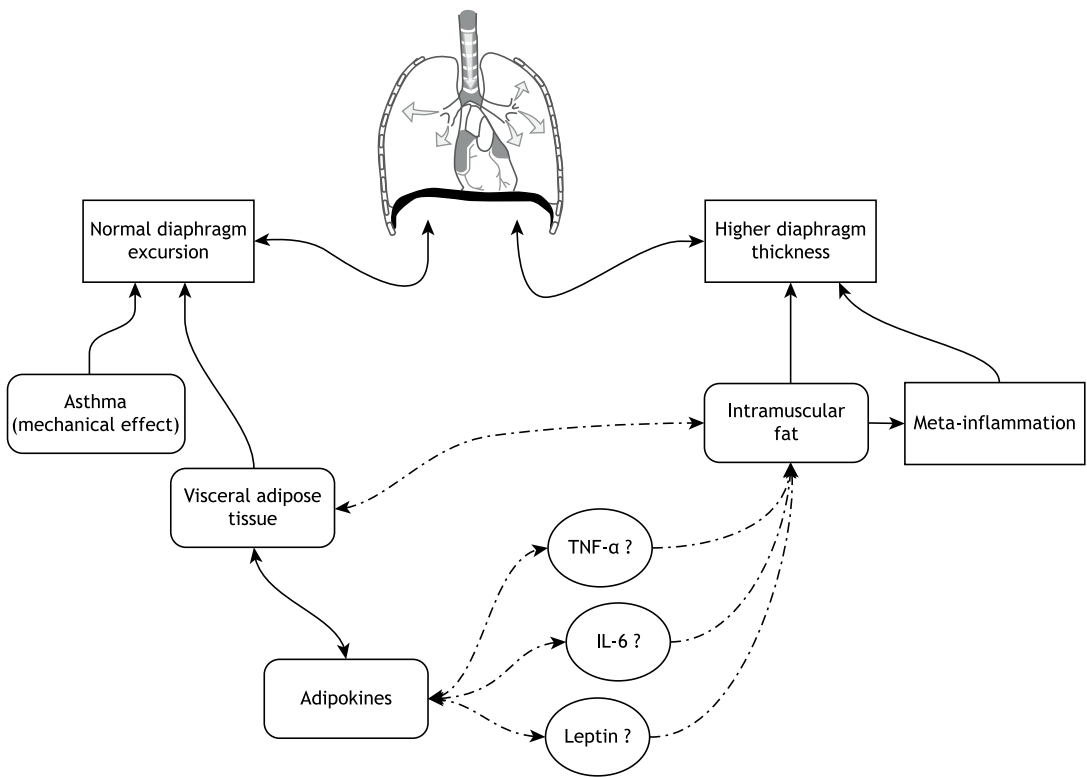


Figure 4. Conceptual model of obesity and asthma influence on diaphragm function. Note – IL – Interleukin. TNF – Tumor necrosis factor. ? – possible influence.

increase in muscle inflammation under allergic stimulus of dermpathophaoides pteronyssinus with significant increase in IL6 and TNF alpha, . The small sample size may have been the resason for such result (Figure 4).

Some limitations were involved in our study. Firstly, we assessed the diaphragm excursion and diaphragm thickness only on the right side, although the literature recommends it as the adequate area for being easily visualized. In addition, it has already been shown to lead to high reproducibility.⁽⁹⁾ Secondly, we acknowledge that obesity has additional multiple risk factors that may affect diaphragm function (systemic inflammation, neuropathies, hypoxia, and drugs potentially involved with myopathy). Thirdly, hormonal influence of the airways, muscle development and lung maturation are confounding factors regarding gender and can affect the results. Seeking to minimize the influence of these variables, we decided that it would be beneficial to reach a balance in the distribution of male and female youngsters in the groups. Furthermore, although it seems that the age in the comparative group was more advanced, all adolescents had surpassed the first pubertal stage, in addition, when comparing the median values among the groups, no significant difference was found.

In conclusion, obese youngsters have greater thickness in FRC, but show no changes in diaphragmatic excursion. Although neither IL-6 nor TNF-a showed an increase, we should not relativize the role of leptin

as an important pro-inflammatory adipokine, able to cause further repercussions in the diaphragm kinetics, differently from asthmatics and healthy individuals. In conclusion, this research introduces new possibilities for researchers to verify the effects of other adipokines and their role in skeletal muscle metabolism in obese or asthmatic young individuals, or both.

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AUTHOR CONTRIBUTIONS

Drs LHST, JAR and ESCS conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. Dr FCV and MSc HCMS designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. Drs AFDA, VMBL, MAVCJ, GVAGL and DM conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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