Functional and Morphologic Dysfunctions on Airways of Rats Submitted to an Experimental Model of Obesity-exacerbated Asthma

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Abstract

Obesity-exacerbated asthma phenotype is characterized by more severe asthma symptoms and glucocorticoid resistance. The aimed of this study is to standardize an obesity-exacerbated asthma model by high glycemic level index (HGLI) diet and ovalbumin (OVA) sensitization and challenges in Wistar rats. Animals were divided into groups: control (CG), obese (OG), asthmatic (AG), obese asthmatic (OAG) and obese asthmatic treated with dexamethasone (OADEXAG) and *in vivo* and *in vitro* functional and morphological parameters were measured. After HGLI consumption there was an increase in body weight, abdominal circumferences, body mass index, retroperitoneal, epididymal, inguinal adipose tissues and adiposity index. Respiratory function showed reduction in pulmonary tidal and minute-volume. In isolated trachea, the cumulative concentration-response curves for carbachol showed increase in contractile efficacy in OG, AG, OAG and OADEXAG, while OAG showed the greatest contractile efficacy. Histological sections of lungs showed an increased peribronchovascular inflammatory area on OAG, smooth muscle hypertrophy and remodeling area filled by extracellular matrix, and these changes were not reversed by treatment with dexamethasone. Obesity-exacerbated asthma model was successfully established. Therefore, this model allows further molecular investigations and the search for new therapeutic alternatives for the treatment and relief of symptoms of patients of obesity-induced resistant asthma.

1 Introduction

Obesity can be defined as abnormal or excessive accumulation of fat potentially harmful to health. The World Health Organization (WHO) regards obesity as a global epidemic, and reports that the majority of the population lives in countries where overweight and obesity kill more people than malnutrition [1].

Obesity increases the risk of several chronic diseases, including asthma, also acting as a modifying factor. Asthma is characterized by airway hyperresponsiveness, varying degrees of airflow obstruction and pulmonary inflammation, which generate recurrent attacks of shortness of breath and wheezing in patients, which varies in severity and frequency among different subjects [2, 3].

Obese individuals are more likely to develop asthma and those with this association have more frequent and more severe asthma symptoms, in addition to reduced response to various antiasthma drugs, including the resistance to corticosteroids, referred to as an indicator of severe asthma [4].

Induction of obesity in rodents can be made by different strategies, but one of the most used is induction through dietary alteration, which is considered polygenic [5]. To induction of asthma, exposure to allergens, especially ovalbumin, has been one of the most used forms [6], thus presenting itself as a valuable resource.

For the first time, a model of obesity-exacerbated asthma using this pellet-diet and ovalbumin allergen is being studied. Accordingly, the aim of this study was to standardize a model of association between obesity and asthma in Wistar rats and to evaluate changes in the parameters of obesity, tracheal

responsiveness, respiratory function, and bronchial morphology that mimic the aggravation on tissue and functional parameters of asthma caused by obesity.

2 Results

2.1 Diet Centesimal Composition

The centesimal composition of the standard diet and high glycemic level index diet analyzed in this study and were showed in Table 1.

Table 1
– Centesimal composition of the standard diet (Nuvilab®) and HGLI. Data expressed as mean $\pm$ s.e.m. (n = 3). Test t. * p < 0.05 (standard diet vs. HGLI).

Parameters	Standart diet (g/100g)	HGLI (g/100g)
Total moisture and solids	7.80 $\pm$ 0.12	16.0 $\pm$ 0.11*
Ashes	7.7 $\pm$ 0.02	3.6 $\pm$ 0.05*
Protein	20.5 $\pm$ 0.06	12.3 $\pm$ 0.06*
Lipid	8.2 $\pm$ 0.07	4.1 $\pm$ 0.09*
Glucose	2.1 $\pm$ 0.003	19.2 $\pm$ 0.07*
Maltose	3.7 $\pm$ 0.002	1.9 $\pm$ 0.03*
Sucrose	-	5.8 $\pm$ 0.03*
Fructose	1.8 $\pm$ 0.003	-
Mannose	0.8 $\pm$ 0.002	17.6 $\pm$ 0.008*
Total carbohydrates	6.7 $\pm$ 0.003	44.5 $\pm$ 0.1*

2.2 Evaluation of experimental obesity

2.2.1 Animal's weight evolution and food intake

The initial body mass of all experimental groups was similar, but after 16 weeks of consuming the HGLI, both OG and OAG animals a greater weight compared to the CG. The AG, however, did not show any difference compared to CG, but presented lower body mass than OG and OAG. While OADEXAG after treatment did not increased weight when compared to CG (Table 2, n = 4-6).

Table 2

Initial and final body mass values, body mass gain and food intake of rats from CG, OG, AG, OAG and OADEXAG. One-way ANOVA followed by Tukey's post-test (n = 4-6). * $p < 0.05$ (CG vs. OG, OAG), ^b $p < 0.05$ (AG vs. OAG); # $p < 0.05$ (OADEXAG vs. OAG).

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Food consumption (g)
Control	256.0 ± 11.3	369.0 ± 4.4	113.0 ± 13.1	160.8 ± 3.4
Obese	263.8 ± 15.5	500.0 ± 12.2*	236.2 ± 20.2*	156.6 ± 4.0
Asthma	247.8 ± 12.7	382.2 ± 13.0 ^b	134.3 ± 2.4 ^b	167.2 ± 3.4 ^b
Obese asthma	268.8 ± 11.8	466.3 ± 9.9* ^b	197.5 ± 17.1* ^{b#}	150.1 ± 2.9 ^b
Obese asthma dexamethasone	317.5 ± 28.4	415.3 ± 29.1	97.7 ± 19.2	146.9 ± 4.3

The mean weekly body mass gain was also evaluated between the groups during 16 weeks and it was observed that both OG and OAG obtained a greater body mass gain along this period compared to CG group. The AG group showed less weight gain when compared to groups with dietary changes, but did not differ from control. While the group treated with dexamethasone had the lowest weight gain compared to the other groups. Despite these results, the mean weekly food intake was not changed when compared to the control group (Table 2, n = 4-6).

2.2.2 Murinometric parameters

2.2.2.1 Lee index, body mass index, chest and abdominal circumferences

There was no change in the Lee index values between experimental groups (Table 3, n = 6), but the body mass index was increased in OG, OAG and OADEXAG (Table 3, n = 6).

Table 3

Values of Lee and body mass index, thoracic and abdominal circumference of rats from CG, OG, AG, OAG and OADEXAG. One-way ANOVA followed by Tukey's post-test ($n = 6$). * $p < 0.05$ (CG vs. OG, OAG); ^b $p < 0.05$ (AG vs. OAG).

Groups	Lee index (g/cm)	Body mass index (g/cm ²)	Thoracic circumference (cm)	Abdominal circumference (cm)
Control	0.27 ± 0.004	0.5 ± 0.02	17.2 ± 0.5	18.8 ± 0.3
Obese	0.28 ± 0.003	0.6 ± 0.02*	18.3 ± 0.8	21.7 ± 0.4*
Asthma	0.27 ± 0.002	0.5 ± 0.01 ^b	17.1 ± 0.3	18.1 ± 0.1 ^b
Obese asthma	0.28 ± 0.002	0.6 ± 0.01* ^b	18.0 ± 0.5	21.4 ± 0.1* ^b
Obese asthma dexamethasone	0.29 ± 0.005	0.6 ± 0.02*	16.9 ± 0.3	20.6 ± 0.5*

There was no change in thoracic circumference measurements (Table 3, $n = 6$), however the measures of abdominal circumference when compared to the CG were higher in the animals that received HGLI, OG, OAG and OADEXAG, not differing from each other. The AG, otherwise, did not show any difference when compared to control, however they presented lower abdominal circumference when compared to the OAG and group OADEXAG (Table 3, $n = 6$).

2.2.2.2 Mass of adipose tissue deposits and adiposity index

The inguinal, epididymal, retroperitoneal adipose tissue mass and adiposity index was increased in animals from OG, OAG and OADEXAG when compared to CG and AG that showed no difference between them. Furthermore, AG in turn, presented a lower values for epididymal and retroperitoneal adipose tissue mass and adiposity index, when compared to animals obese and asthmatic (Table 4, $n = 6$).

Table 4

Values of inguinal, epididymal, retroperitoneal adipose tissue mass and adiposity index of rats from CG, OG, AG, OAG and OADExAG. One-way ANOVA followed by Tukey's post-test ($n = 4-6$). * $p < 0.05$ (CG vs. OG, OAG), ^b $p < 0.05$ (AG vs. OAG).

Groups	Inguinal fat (g/100 g)	Epididimal fat (g/100 g)	Retroperitoneal fat (g/100 g)	Adiposity index (%)
Control	0.8 ± 0.06	1.5 ± 0.08	1.2 ± 0.1	3.7 ± 0.3
Obese	$2.2 \pm 0.3^*$	$3.3 \pm 0.3^{*a}$	$3.2 \pm 0.3^*$	$8.7 \pm 0.3^*$
Asthma	1.1 ± 0.1	1.0 ± 0.1^b	1.0 ± 0.1^b	3.4 ± 0.3^b
Obese asthma	$2.0 \pm 0.3^*$	$2.5 \pm 0.1^{*ab}$	$3.4 \pm 0.2^{*b}$	$7.7 \pm 0.5^{*b}$
Obese asthma dexamethasone	$1.9 \pm 0.4^*$	$2.4 \pm 0.2^*$	$3.1 \pm 0.1^*$	$7.4 \pm 0.6^*$

2.3 Respiratory function analysis

Breathing recordings was obtained on days 0, 11 and 21 of asthma induction. Tidal volume was reduced only on 22nd day on AG (5.2 ± 0.6 mL/kg), OAG (6.2 ± 0.6 mL/kg) and OADExAG (4.6 ± 0.2 mL/kg), showing no difference between them, when compared to CG (8.8 ± 0.7 mL/kg) (Figure 2A). Respiratory frequency was not changed between groups during asthma induction (Figure 2B). Differently, ventilation was reduced on 22nd in AG, OAG and OADExAG (539.9 ± 78.8 ; 646.0 ± 47.0 ; 523.6 ± 42.0 mL/kg/min) compared to CG (964.3 ± 80.5 mL/kg/min), with no difference to OG (863.8 ± 58.6 mL/kg/min) (Figure 2C) 9 (Supplementary figure S2A).

2.4 Evaluation of contractile reactivity to ovalbumin in rat trachea - Schultz-Dale reaction

The rat trachea of CG and OG did not show contractile reactivity to OVA stimulation ($E_{\max} = 0\%$), differently in AG, OAG and OADExAG, OVA promoted contractile reactivity ($E_{\max} = 100\%$; 220.0 ± 33.7 and $147.6 \pm 42.3\%$), where OAG showed greater contractile reactivity when compared to AG and OADExAG caused a partial reduction of this increase (Figure 3) (Supplementary figure S3).

2.5 Assessment of tracheal contractile reactivity to KCl or CCh in rat trachea

The cumulative concentration-response curves for KCl (10^{-3} - 3×10^{-1} M) did not show any change in contractile efficacy and potency between the experimental groups (Figure 4, Table 5, $n = 5$) (Supplementary figure S4).

Table 5

$E_{\max}$ and EC_{50} values of contractile and relaxant agents in CG, OG, AG, OAG and OADEXAG isolated rat trachea groups. One-way ANOVA followed by Tukey's post-test ($n = 4-6$). * $p < 0.05$ (CG vs. OG, OAG).

Contractile/ relaxant agent	Parameter	CG	OG	AG	OAG	OADEXAG
KCl	$E_{\max}$ (%)	100.0	88.2 ± 10.2	84.7 ± 7.7 ;	93.1 ± 4.9	-
	EC_{50} (M)	3.3 ± 0.08 $\times 10^2$	$3.3 \pm 0.2 \times 10^2$	3.1 ± 0.3 $\times 10^2$	3.5 ± 0.2 $\times 10^2$	-
CCh	$E_{\max}$ (%)	100	$133.2 \pm 10.4^*$	$126.1 \pm 2.4^*$	$152.6 \pm 5.5^*$	$162.2 \pm 7.4^*$
	EC_{50} (M)	9.7 ± 0.8 $\times 10^{-7}$	$1.4 \pm 0.4 \times 10^{-6}$	1.3 ± 0.5 $\times 10^{-6}$	2.6 ± 1.2 $\times 10^{-6}$	7.5 ± 1.7 $\times 10^{-7}$
Nifedipine	$E_{\max}$ (%)	98.5 ± 2.1	96.2 ± 2.4	100.4 ± 3.6	102.2 ± 3.0	-
	EC_{50} (M)	$2.2 \pm 0.4 \times 10^{-6}$	$8.4 \pm 3.5 \times 10^{-6}$	7.7 ± 4.9 $\times 10^{-6}$	3.5 ± 2.1 $\times 10^{-6}$	-
Isoprenaline	$E_{\max}$ (%)	76.7 ± 2.6	$38.4 \pm 2.8^*$	$30.5 \pm 2.4^*$	$41.1 \pm 5.0^*$	-
	EC_{50} (M)	1.1 ± 0.4 $\times 10^{-4}$	$1.9 \pm 0.5 \times 10^{-5^*}$	3.6 ± 2.2 $\times 10^{-5}$	1.2 ± 0.7 $\times 10^{-5^*}$	-
Aminophylline	$E_{\max}$ (%)	98.8 ± 1.1	91.6 ± 1.6	92.6 ± 2.7	96.9 ± 1.2	-
	EC_{50} (M)	5.7 ± 1.1 $\times 10^{-4}$	$8.4 \pm 0.4 \times 10^{-4}$	7.6 ± 1.1 $\times 10^{-4}$	6.4 ± 0.5 $\times 10^{-4}$	-

The cumulative concentration-response curves for CCh (10^{-9} - 3×10^{-4} M) did not show any change in contractile potency between the groups. However, there was an increase in contractile efficacy in OG, AG, OAG and OADEXAG when compared to CG and the animals that presented the two associated diseases (OAG and OADEXAG) showed increased efficacy also compared to AG group (Figure 5, Table 5, $n = 5$) (Supplementary figure S5).

2.6 Assessment of tracheal relaxant reactivity to nifedipine, isoprenaline or aminophylline in rat trachea pre-contracted with CCh

The relaxant tracheal reactivity to nifedipine was equipotent between groups and the efficacy was also unchanged (Figure 6A, Table 5, n = 5) (**Supplementary figure S6A**).

Differently, the relaxant tracheal reactivity to isoprenaline showed a reduction in relaxing efficacy in the OG, AG and OAG when compared to CG. As for potency, OG and AOG shifted the curve to the right with a reduction in relaxing potency about 5.8 and 9.2 times, respectively, when compared to CG, which was equipotent to AG (Figure 6B, Table 5, n = 5) (**Supplementary figure S6B**).

The relaxant tracheal reactivity to aminophylline was equipotent between groups and the efficacy was also unchanged (Figure 6C, Table 5, n = 5) (**Supplementary figure S6C**).

2.7 Morphologic evaluations

2.7.1 Evaluation of the effects of obesity and asthma on the pulmonary

Histological sections of the lung stained with hematoxylin-eosin (n = 6) showed a parenchyma full of alveolar sacs and structures of the respiratory tree, namely terminal bronchioles, which have a low columnar epithelium, ciliated with few goblet cells, in addition to pulmonary vessels (Figure 7A). In OG (Figure 7B), AG (Figure 7C), OAG (Figure 7D) and OADExAG (Figure 7E), it is possible to observe the presence of a mixed and diffuse exudate in peribroncovascular location, composed mostly of macrophages and eosinophils, however the animals of the OAG (Figure 7D) present a more intense infiltrate with greater destruction of the pulmonary parenchyma than the other experimental conditions.

When the special staining Masson's trichrome was employed, it is possible to observe the maintenance of the organ stroma with an apparent increase in the extracellular matrix (Figure 7, G-I) while treatment with dexamethasone (Figure 7I) did not reverse the changes caused by induction of asthma exacerbated by obesity, when compared to the control group (Figure 7F).

The area of peribroncovascular inflammation was also evaluated and it was observed that the CG did not present this type of inflammation, while there was an increase in this parameter in OG (240.7 ± 19.5 area/ μ^2) and in the AG (105.5 ± 3.1 area/ μ^2) and in the OAG (352.5 ± 16.1 area/ μ^2) compared to the CG, with a decrease after treatment with dexamethasone, but not reversal (148.5 ± 5.2 area/ μ^2) when compared to OAG (Figure 8, n = 6).

The smooth muscle area was also quantified in the experimental groups (n = 6) and it was observed that both the OG (75.3 ± 5.3 area/ μ^2), AG (71.5 ± 7.7 area/ μ^2) and OAG (77.5 ± 6.8 area/ μ^2) showed an increase in this muscle layer, which was not altered by treatment with dexamethasone (67.2 ± 3.9 area/ μ^2) when compared to the CG (13.2 ± 2.1 area/ μ^2) and not differing from each other (Figure 8B, n = 6).

Assessing the remodeling area filled by the extracellular matrix (n = 6), when compared to CG (6.0 ± 0.5 area/ μ^2), there is an increase in OG (27.5 ± 3.4 area/ μ^2), AG (61.3 ± 4.2 area/ μ^2) and OAG (65.0 ± 2.7

area/ μ^2), which showed a reduction of only about 30% after treatment with dexamethasone (45.7 ± 2.5 area/ μ^2). AG and OAG did not differ from each other and induced greater remodeling when compared to obesity alone (Figure 8C n = 6).

3 Discussion

Obesity and asthma affect a large portion of the world population and are characterized by increasing predisposition and severity of asthma, making difficult treatment [7]. In order to understand the main changes caused by this association, in the present work it has been standardized a model of obesity-exacerbated asthma induced by high glycemic index diet (HGLI) and ovalbumin in Wistar rats.

The dietary change is a process resembles current human obesity [8]; thus, the HGLI diet chosen for the study presents condensed milk as one of its main components, and was offered to the animals for 16 weeks. [9] reported in a comparative study between a diet rich in fat and the same diet with a condensed milk, greater increase in body weight gain in mice combined with other changes, such increased inflammation in animals, indicating that this ingredient is more inflammatory than fat. Corroborating these data, [10] used HGLI for the first time and also observed increased inflammation in Wistar rats.

In the present study, after 16 weeks OG and OAG had a greater final weight, and weight gain, compared to the animals that received the control diet (Table 2), being therefore an important factor in the implantation of obesity. The induction of asthma without changing the diet (AG) did not cause changes in weight or body mass of these animals when compared to CG (Table 2), indicating that asthma alone does not affect the weight of these animals. These dates are similar to the observed in other studies [11–13].

One of the main managements of asthma is through treatment with corticosteroids, which are steroid hormones that exert potent anti-inflammatory activity [14], however it has already been observed that obese asthmatics are less likely to achieve asthma control using corticosteroids [15].

Thus, the treatment of obese-asthmatic animal with dexamethasone (OADEXAG) promoted no difference in weight from the standard diet groups at the end of 16 weeks (Table 2), however there was a marked weight loss during the 5 days of corticosteroid administration, a fact also observed during the induction of asthma (data not shown), indicating that the successive intraperitoneal administrations might cause a temporary loss of appetite, which may be responsible for the weight loss, similar to observed by [16] in a 20-days continuous intraperitoneal infusion of hydrocortisone sodium succinate in obese and non-obese rats.

Changes in HGLI consumption were not observed between the experimental groups (Table 2), indicating that the increase in body weight and mass gain observed occurred due to the increase in the amount of energy ingested and not by the amount of dietary consumption.

Lee index and BMI were evaluated, and it has been observed no change in LEE index between groups (Table 3), while there was an increase in BMI in animals that consumed the HGLI diet (OG, OAG and OADEXAG), similarly to demonstrated by [17], in a high carbohydrate diet offered for rats.

Since these parameters does not discriminate body composition, thoracic and abdominal circumferences were evaluated. The chest circumference was not altered by the change in diet or by the induction of asthma, however, the abdominal circumference, in turn, was higher in OG, OAG and OADEXAG, with no difference between them, indicating that asthma does not hinder increase in circumference caused by HGLI, possibly due to an increase in fat deposition in adipose tissue (Table 3).

Determining the weight of the main fat deposits (inguinal, epididymal and retroperitoneal), as well as the adiposity index can be a measure of great value for the assessment of obesity. Thus, it was observed that the OG, OAG and OADEXAG animals showed an increase in the three fat deposits, when compared to the control, leading us to infer that HGLI increased adipose tissue and it can probably configured as a risk factor for worsening asthma. In addition, despite the weight loss caused by treatment with dexamethasone, the main fat deposits were not altered, being resistant to treatment with corticosteroids (Table 4).

The adiposity index was also increased in OG, OAG and OADEXAG when compared to the CG and AG, which did not differ from each other (Table 4). Contrary results by [18], who compared some types of obesogenic diets, demonstrated that only the westernized diet would cause an increase in the adiposity rate of these animals.

In the present study, the lung function was evaluated, as indicated by the tidal volume, respiratory frequency and minute volume, and it was observed that although the respiratory rate was not altered but there was a reduction in tidal and minute volume, indicative of reduced airflow characteristic of increased airway resistance, which implies the bronchoconstriction promoted by asthma induction.

In a study by [19], a reduction of TV and MV was also observed in a model of asthmatic rats, however, differently, there was an increase in respiratory rate. Additionally, the tidal volume decreases were related in other models to lung fibrosis and extensive inflammation [20], which may explain some observed changes. Differently, rats fed a hypercaloric diet demonstrated an exacerbated increase in respiratory frequency and a decreased expiratory time [21].

In functional ex vivo measurement, animals previously sensitized (AG, OAG and OADEXAG) showed a tracheal contractile response to OVA, whereas the animals of the CG and OG did not respond to the same stimulus (Figure 3), confirming functionally asthma implantation in these animals. It is important to point out that OAG showed a greater contractile effect to OVA when compared to AG, leading us to suggest an exacerbation of diseases, and the treatment with dexamethasone showed no difference in the OAG. These data also corroborate the previous data related to the respiratory function, which showed a reduction on pulmonary ventilation.

Tracheal contractile reactivity assessment using a mainly electromechanical agent (KCl) did not change neither efficacy nor potency between the experimental groups (Table 5, figure 4), indicating that the diseases probably do not present a majority participation of the electromechanical component in hyperreactivity, similarly to demonstrated by [22] in the guinea pig trachea with chronic allergic pulmonary inflammation. Besides, the investigation of the effects of treatment with dexamethasone was carried out only in the pathways ways that were altered by the induction of obesityexacerbated asthma.

Knowing that one of the main contractile mechanisms of the airways is promoted by acetylcholine (ACh) via cholinergic innervation [23], it was observed an increase in contractile efficacy in all experimental groups, when compared with CG. However OG and AG did not differ from each other, but and OAG showed an increase in contractile efficacy when compared to AG as well as the OADExAG (Figure 5), characterizing that both obesity and asthma alone cause tracheal hyperresponsiveness and the association of the two diseases causes an exacerbation and this is resistant to treatment with dexamethasone.

The increase in contractile reactivity of the trachea in groups of asthmatic rats, being even greater on simultaneously obese and asthmatic animals, has already been observed in other studies using another muscarinic agonist, methacholine [24].

To evaluate whether asthma, obesity and the association between the two disorders would alter the electromechanical relaxation pathway, the relaxing effect of the cumulative concentration-response curves to nifedipine, a Ca_v channel blocker [25, 26] was evaluated over the tonic contraction induced by CCh. After that, it was observed that there was no change in efficacy or potency between the groups (Figure 6A, Table 5), indicating that the diseases do not change the electromechanical component of relaxation.

The pharmacomechanical component of relaxation was evaluated by employing isoprenaline, a β_2 -adrenergic agonist, [27]. A reduction in the relaxing efficacy in OG, AG and OAG was observed, however the CG did not induce 100% relaxation (Figure 6B), this event can be explained, because despite the expression of β_2 -adrenergic receptors in the airways, they occur mostly in the lower airways, such as bronchioles, when compared to the upper ones, such as the trachea [28].

Another relaxing mechanism can be evaluated getting a relaxation curve to aminophylline, a non-selective PDE inhibitor [29] and there was no change in the relaxing efficacy or potency between the groups (Figure 6C). Altogether, these data indicate that the main changes induced by obesity, asthma and obesity-exacerbated asthma are not due to changes in the tracheal relaxant mechanism.

Through histological analyzes of lungs, there was also an increase in the thickness of the smooth muscle layer in all groups, an increase in peribroncovascular inflammation in asthma group and in even greater levels in obese asthma animals when compared to CG, as well as greater tissue remodelling by the matrix extracellular also in these groups, characterizing asthma and demonstrating that the association of obesity and asthma worsens the inflammatory condition. The increase in these parameters was not

reversed by treatment with dexamethasone (Figures 7 and 8), characterizing the glucocorticoid resistance reported in severe asthma, confirming once again the implantation of the model. Interestingly, the obese group also showed an increase in the inflammatory area, in the thickness of the smooth muscles and also in the filling by extracellular matrix, characterizing the inflammatory condition that obesity alone is able to generate.

In view of these results, the implementation of an obesity-exacerbated asthma model is confirmed, characterized by functional and morphologic respiratory changes, becoming a valid methodology for a better understanding of this association, as well as a search for new therapeutic strategies for the treatment of this condition.

4 Materials And Methods

4.1 Animals

In this research were used male Wistar rats (*Rattus norvegicus*), 50 days of age, weighing between 220-270 g, obtained from the animal production unit (UPA) of Instituto de Pesquisa em Fármacos e Medicamentos (IPeFarM) of Universidade Federal da Paraíba, maintained under temperature control conditions ($22 \pm 1^\circ\text{C}$) and a 12-hour light-dark cycle, with free access to food and water.

4.1.1 Ethical statement

The experimental procedures were performed following the principles of guidelines for the ethical use of animals in applied etiology studies [30] and from the Brazilian Guide for the Production, Maintenance or Use of Animals in Teaching or Scientific Research Activities, from Conselho Nacional de Controle de Experimentação Animal (CONCEA) [31]. Experimental procedures were approved by the Ethics Committee on Animal Use of UFPB (protocol nº 1162100918). **In addition, we confirm that all methods are reported in accordance with ARRIVE guidelines.**

4.2 Chemicals

Sodium chloride (NaCl), potassium chloride (KCl), magnesium sulfate (MgSO_4), potassium phosphate (KH_2PO_4), calcium chloride (CaCl_2), glucose, sodium bicarbonate (NaHCO_3), monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate NaH_2PO_4 were obtained from Êxodo Científica (Sumaré, São Paulo, Brazil). Carbamylcholine hydrochloride (CCh), aluminum hydroxide $[\text{Al}(\text{OH})_3]$, ovalbumin (OVA) (grade II and V), nifedipine, aminophylline and dibasic sodium phosphate (Na_2HPO_4) were obtained from Sigma-Aldrich (São Paulo, São Paulo, Brazil). Arachidonic acid (AA) and isoprenaline were purchased from Cayman Chemical (Ann Arbor, Michigan, USA). Dexamethasone disodium phosphate injection was purchased from Aché Laboratórios Farmacêuticos (Guarulhos, São Paulo, Brazil).

Absolute alcohol was purchased from Neon (Suzano, São Paulo, Brazil), xylol and paraffin were purchased from Dinâmica Química Contemporânea LTDA (Indaiatuba, São Paulo, Brazil). Formaldehyde, hematoxylin, eosin and Masson's trichrome were obtained from different brands.

4.3 Experimental Groups

The animals were randomly divided into 5 experimental groups, with 8 animals each: control (CG) - fed with standard diet and not sensitized; obese (OG) - fed with high glycemic index pellet-diet (HGLI) and not sensitized; asthmatic (AG) - fed a standard diet and sensitized; obese asthma (OAG) - fed with HGLI and sensitized; and obese asthma dexamethasone (OADEXAG) - fed with HGLI, sensitized and treated with dexamethasone.

4.4 Diets

The animals from CG and AG received a standard diet (Nuvilab®) and from OG, OAG and OADEXAG received a HGLI, composed by standard diet (Nuvilab®), refined sugar (Alegre®) and condensed milk (Camponesa®). This diet was characterized as having a high glycemic index, with a value of 77.6, and a high glycemic load, with values of 38.8 [10, 32].

4.4.1 Diet Centesimal Composition

The centesimal composition of the standard diet (Nuvilab®) and HGLI were determined by moisture analysis at 102 °C, fixed mineral residue obtained after carbonization and incineration in a muffle furnace at 550 °C, proteins by the Kjeldahl method, lipids by the method Soxhlet, while the carbohydrate content was obtained using the high performance liquid chromatography technique [33].

4.5 Sensitization and challenges with ovalbumin for asthma induction and dexamethasone treatment

For induction of asthma on rats, the sensitization started at the end of the 13th week after the start of the diets and occurred during the last 22 days (Figure 1). The protocol followed the methodology described by [34, 35]. Animals in the OADEXA group during the last 5 days of disease induction received 5 mg/kg per day of dexamethasone intraperitoneally [36].

4.6 Obtaining of tracheal rings

The rats were euthanized by anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) *ip*, followed by exsanguination, had their trachea divided into rings and isometric contractions were evaluated. The integrity of the epithelium was verify and only rings with intact epithelium were used [37].

4.7 Nutritional Solutions

For the tracheal reactivity protocols, a Krebs nutrient solution was used on organ bath, adjusted to pH 7.4 (with a solution of HCl or NaOH, 1 N), aerated with carbogen mixture (95% O₂ and 5% CO₂) and

maintained at 37°C. It had the following composition, in mM: NaCl (118.0), KCl (4.5), MgSO₄ (5.7), KH₂PO₄ (1.1), CaCl₂ (2.5), glucose (11.0) and NaHCO₃ (25.0).

The samples for histological procedures were kept in a buffered formaldehyde solution (10%) with the following composition: formaldehyde (1:10 v/v), NaH₂PO₄ (0.04:10 m/v), Na₂HPO₄ (0.005:10 m/v) and H₂O (9:10 v/v).

4.8 Evaluation of experimental obesity

4.8.1 Animal's weight evolution and food intake

The body mass (g) of the rats was recorded weekly, always on the same days, with the mass gain being calculated by the difference between final and initial body mass.

Food consumption was also calculated weekly, always on the same days, represented by the difference between the food offered and the residual [38].

4.8.3 Murinometric parameters

4.8.3.1 Lee index, body mass index, chest and abdominal circumferences

At the day of euthanasia, the animals were weighed, and the naso-anal length (cm) was used to calculate the Lee index, which is the ratio between the cube root of body mass and the naso-anal length of the animal [39], and the body mass index (BMI) characterized by the ratio between the weight and the naso-anal length of the animal squared. The thoracic circumference, located in the posterior portion of the front leg and the waist circumference, located in the anterior part of the animal's hind leg were also measured using an anthropometric body measuring tape [17].

4.8.3.2 Mass of adipose tissue deposits and adiposity index

The inguinal, epididymal and retroperitoneal adipose tissues were weighed, which represent the main components of central adiposity in rats [40].

The adiposity index was calculated from the sum of the individual masses of the epididymal, inguinal and retroperitoneal fat layers, using the formula: inguinal fat + epididymal fat + retroperitoneal fat x 100/final body weight [41].

4.9 Respiratory rate analysis

On days 0, 12 and 21 of the asthma induction protocol, after nebulization (OVA or saline), the animals were submitted to a measurement of tidal volume, respiratory frequency and minute volume using the plethysmography technique in a full body chamber, adapted from the literature [42, 43].

4.10 Evaluation of contractile and relaxant reactivity

4.10.1 Evaluation of contractile reactivity to ovalbumin in rat trachea - Schultz-Dale reaction

The trachea rings were stimulated with 100 µg/mL OVA and the contraction amplitude was compared between the CG, OG, AG, OAG and OADEXAG [44, 45].

4.10.2 Assessment of tracheal contractile reactivity to KCl and CCh in rat trachea

Two cumulative concentration-response curves were induced to eletromechanical agent, KCl [46] or CCh, a pharmacomechanical agent [47]. Contractile reactivity was evaluated on the basis of the maximum effect (E_{max}) and concentration of a substance that produced 50% of its maximal effect (EC_{50}) of the contractile agent, calculated from the concentration-response curves obtained, calculated by non-linear regression [48].

4.10.3 Assessment of tracheal relaxant reactivity to nifedipine, isoprenaline and aminophylline in rat trachea pre-contracted with CCh

A contraction with CCh 10^{-5} M was induced and the tonic component was added in cumulative concentrations to nifedipine, calcium channel blocker [49], isoprenaline, an β adrenoceptor agonist receptor [50] or aminophylline, an non-selective phosphodiesterase inhibitor [29] until they reach their E_{max} . Relaxant reactivity was expressed as the reverse percentage of the initial contraction force elicited by CCh and evaluated on the basis of EC_{50} and E_{max} , calculated from concentration-response curves, by non-linear regression [48].

4.11 Morphologic evaluations

4.11.1 Evaluation of the effects of obesity and asthma on the pulmonary morphology of rats

Samples of lung tissue fragments were collected and fixed in 10% buffered formalin solution. After that, dehydration in growing alcohol solutions from 70 °GL to absolute, then the samples were added in a xylol bath. The tissues were embedded in paraffin and cut into a microtome with a thickness of 4 µm, mounted on histological slides and dewaxed in xylol, hydrated in alcohols in decreasing concentrations. The samples were then treated with Harris' hematoxylin and eosin or with Masson's Trichrome solution. The slides were dehydrated in increasing concentrations of alcohols, diaphanized in xylol, and assembled with Entellan® [30].

4.12 Statistical analysis

The results were expressed as the percentage of the mean and the standard error of the mean (e.p.m.), and the E_{max} and EC_{50} were compared and analyzed statistically using analysis of variance (ANOVA) "one-way" followed by the Tukey post-test. The null hypothesis was rejected when $p < 0.05$. All data were analyzed using the GraphPad Prism® version 5.01.

Declarations

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Author Contributions

SF have made substantial contributions to acquisition, analysis and interpretation of data and wrote the first draft of the manuscript. RP, IF, JL, TM, CB and AM have made substantial contributions to acquisition of data. LC, MM, HC, JS, JA and AA have made substantial contributions acquisition of data, analysis and interpretation of data. LV and FC have made substantial contributions to conception, design, analysis, interpretation of data, been involved in drafting the manuscript and revising it critically.

Competing interest

The authors declare no competing interest.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due be in a patenting process, but are available from the corresponding author on reasonable request.

References

1. World Health Organization. Obesity and overweight. <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (2021).
2. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. <https://ginasthma.org/wp-content/uploads/2021/05/GINA-Main-Report-2021-V2-WMS.pdf> (2021).
3. World Health Organization. Asthma. <https://www.who.int/news-room/fact-sheets/detail/asthma> (2021).
4. Peters, U., Dixon, A. E., Forno, E. Obesity and asthma. *J Allergy Clin Immunol.* **141**, 1169–1179 (2018).

5. Lutz, T. A., Woods, S. C. Overview of animal models of obesity. *Curr. Protoc. Pharmacol.* **58**, 5–61 (2012).
6. Ghorani, V. *et al.* The Effects of Allium Cepa Extract on Tracheal Responsiveness, Lung Inflammatory Cells and Phospholipase A2 Level in Asthmatic Rats. *Iran J Allergy Asthma Immunol.* **17**, 221–231 (2018).
7. Miethe, S., Karsonova, A., Karaulov, A., Renz, H. Obesity and asthma. *J Allergy Clin Immunol.* **146**, 685–693 (2020).
8. Meguid, M. M. *et al.* A surgical rat model of human Roux-en-Y gastric bypass. *J Gastrointest Surg.* **8**, 621–630 (2004).
9. Masi, L. N, *et al.* Combination of a high-fat diet with sweetened condensed milk exacerbates inflammation and insulin resistance induced by each separately in mice. *Sci Rep.* **7**, 1–10 (2017).
10. Luz, A. B. S. *et al.* Adipocytes and intestinal epithelium dysfunctions linking obesity to inflammation induced by high glycemic index pellet-diet in Wistar rats. *Biosci. Rep.* **38**, 1–15 (2018).
11. Aslani, M. R. *et al.* Tracheal overexpression of IL-1 β , IRAK-1 and TRAF-6 mRNA in obese-asthmatic male Wistar rats. *Iran J Basic Med Sci.* **19**, 350 (2016).
12. Aslani, M. R. *et al.* Lung altered expression of IL-1 β mRNA and its signaling pathway molecules in obese-asthmatic male Wistar rats. *Iran J Allergy Asthma Immunol.* **15**, 183–197 (2016).
13. Ghobadi, H., Alipour, M. R., Keyhanmanesh, R., Boskabady, M. H., Aslani, M. R. Effect of High-fat Diet on Tracheal Responsiveness to Methacholine and Insulin Resistance Index in Ovalbumin-sensitized Male and Female Rats. *Iran J Allergy Asthma Immunol.* **18**, 48–61 (2019).
14. Barnes, P. J. Glucocorticoids. *Chem Immunol Allergy.* **100**, 311–316 (2014).
15. Boulet, L. P, Frannsen, E. Influence of obesity on response to fluticasone with or without salmeterol in moderate asthma. *Respir Med.* **101**, 2240–2247 (2007).
16. Liu, X. Y. *et al.* Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats. *Exp Ther Med.* **2**, 977–984 (2011).
17. Novelli, E. L. B. *et al.* Anthropometrical parameters and markers of obesity in rats. *Lab. Anim.* **41**, 111–119 (2007).
18. Bortolin, R. C. *et al.* A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. *Int J Obes.* **42**, 525–534 (2018).
19. Arora, P, Ansari, S. H., Anjum, V., Mathur, R., Ahmad, S. Investigation of anti-asthmatic potential of Kanakasava in ovalbumin-induced bronchial asthma and airway inflammation in rats. *J. Ethnopharmacol.* **197**, 242–249 (2017).
20. Sung, J. H. *et al.* Changes of pulmonary function during 60 days of welding fume exposure period. *J. Toxicol Public Health.* **20**, 55–61 (2004).
21. Speretta, G. F. *et al.* High-fat diet increases respiratory frequency and abdominal expiratory motor activity during hypercapnia. *Respir Physiol Neurobiol.* **258**, 32–39 (2018).

22. Vasconcelos, L. H. C. *et al.* Virgin coconut oil supplementation prevents airway hyperreactivity of guinea pigs with chronic allergic lung inflammation by antioxidant mechanism. *Oxidative medicine and cellular longevity*. *Oxid Med Cell Longev*. 2020, 1-16 (2020).
23. Santing, R. E. *et al.* A novel method to assess airway function parameters in chronically instrumented, unrestrained guinea pigs. *Pulm Pharmacol*. **5**, 265–272 (1992).
24. Aslani, M. R., Keyhanmanesh, R., Alipour, M. R. Increased visfatin expression is associated with nuclear factor- κ B in obese ovalbumin-sensitized male Wistar rat tracheae. *Med Princ Pract*. **26**, 351–358 (2017).
25. Fanta, C. H., Venugopalan, C. S., Lacouture, P. G., Drazen, J. M. Inhibition of bronchoconstriction in the guinea pig by a calcium channel blocker, nifedipine. *Am Rev Respir Dis*. **125**, 61–66 (1982).
26. Moura, C. T. M., Bezerra, F. C., Moraes, I. M., Magalhães, P. J. C., Capaz, F. R. Increased responsiveness to 5-hydroxytryptamine after antigenic challenge is inhibited by nifedipine and niflumic acid in rat trachea in vitro. *Clin Exp Pharmacol Physiol*. **32**, 1119–1123 (2005).
27. Carswell, H., Nahorski, S. R. β -Adrenoceptor heterogeneity in guinea-pig airways: comparison of functional and receptor labelling studies. *Br J Pharmacol*. **79**, 965–971 (1983).
28. Yousif, M. H., Thulesius, O. The effect of forskolin on isoproterenol-induced relaxation in rat and guinea-pig tracheal preparations. *Med Princ Pract*. **8**:266–271 (1999).
29. Matsuda, F. *et al.* Comparative effect of amrinone, aminophylline and diltiazem on rat airway smooth muscle. *Acta Anaesthesiol Scand*. **44**, 763–766 (2000).
30. Sherwin, C. M. *et al.* Guidelines for the ethical use of animals in applied ethology studies. *Appl Anim Behav Sci*. **81**, 291–305 (2003).
31. Brazil Ministério da Ciência, Tecnologia e Inovação, Conselho Nacional de Controle de Experimentação Animal. Guia Brasileiro de Produção, Manutenção ou Utilização de Animais em Atividades de Ensino ou Pesquisa Científica. <https://antigo.mctic.gov.br/mctic/opencms/institucional/concea/paginas/guia.html> (2016).
32. Aguiar, A. J. F. C. *et al.* A high glycemic index pellet-diet induces metabolic disorders and increased adipose tissue PPAR γ expression in experimental model. *Ann. Nutr. Metab*. **71**, 1229–1230 (2017).
33. Chemists, A. O. A., Horwitz, W. Association Official Analytical Chemists: Official Methods of Analysis of the Association Chemists. (ed. Gaithersburg) (2002).
34. Salmon, M. *et al.* Involvement of cysteinyl leukotrienes in airway smooth muscle cell DNA synthesis after repeated allergen exposure in sensitized Brown Norway rats. *Br. J. Pharmacol*. **127**, 1151–1158 (1999).
35. Galvão, J. G. F. *et al.* Ouabain attenuates ovalbumin-induced airway inflammation. *J. Inflamm. Res*. **66**, 1117–1130 (2017).
36. Dong, F. *et al.* Puerarin attenuates ovalbumin-induced lung inflammation and hemostatic unbalance in rat asthma model. *Evid Based Complement Alternat Med*. **214**, 1–9 (2014).

37. Tschirhart, E., Frossard, N., Bertrand, C., Landry, Y. Arachidonic acid metabolites and airway epithelium dependent relaxant factor. *J. Pharmacol. Exp. Ther.* **243**, 310–316 (1987).
38. Vadivel, V., Pugalenti, M. Studies on the incorporation of velvet bean (*Mucuna pruriens var. utilis*) as an alternative protein source in poultry feed and its effect on growth performance of broiler chickens. *Trop Anim Health Prod.* **42**, 1367–1376 (2010).
39. Lee, M. O. Determination of the surface area of the white rat with its application to the expression of metabolic results. *Am. J. Physiol.* **89**, 24–33 (1929).
40. Mauer, M. M., Harris, R. B., Bartness, T. J. The regulation of total body fat: lessons learned from lipectomy studies. *Neurosci Biobehav Rev.* **25**, 15–28 (2001).
41. Macrini, D. J. Obesidade e reprodução: efeitos da privação alimentar materna na prole de ratos machos. Thesis, Universidade Paulista <https://repositorio.unip.br/dissertacoes-teses-programa-de-pos-graduacao-stricto-sensu-em-patologia-ambiental-e-experimental/obesidade-e-reproducao-efeitos-da-privacao-alimentar-materna-na-prole-de-ratos-machos/> (2014).
42. Oartlett, D. J., Tenney, S. M. Control of breathing in experimental anemia. *Respir. Physiol.* **10**, 384–395 (1970).
43. Malan, A. Ventilation measured by body plethysmography in hibernating mammals and in poikilotherms. *Respir Physiol.* **17**, 32–44 (1973).
44. Chand, N., Eyre, P. The Schultz-Dale reaction: a review. *Agents actions.* **8**, 171–184 (1978).
45. Lima, W. T., Silva, Z. L. Contractile responses of proximal and distal trachea segments isolated from rats subjected to immunological stimulation: role of connective tissue mast cells. *Gen Pharmacol.* **30**, 689–69 (1998).
46. Du, W. *et al.* Excitation-contraction coupling in airway smooth muscle. *J. Biol. Chem.* **281**, 30143–30151 (2006).
47. Ouedraogo, N, Roux E. Physiology of airway smooth muscle contraction: an overview. *J. Pulm. Respir. Med.* **4**, 1–6 (2014).
48. Neubig, R. R., Spedding, M., Kenakin, T., Christopoulos, A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. *Pharmacol. Rev.* **55**, 597–606 (2003).
49. Cheng, J. B., Townley, R. G. Pharmacological characterization of effects of nifedipine on isolated guinea-pig and rat tracheal smooth muscle. *Arch Int Pharmacodyn Ther.* **263**, 228–244 (1983).
50. Naline, E., *et al.* Relaxant effects and durations of action of formoterol and salmeterol on the isolated human bronchus. *Eur. Respir. J.* **7**, 914–920 (1994).
51. Alves, A. F., Pereira, R. A., Andrade, H. M., Mosser, D. M., Tafuri, W. L. Immunohistochemical study of renal fibropoiesis associated with dogs naturally and experimentally infected with two different strains of *Leishmania (L.) infantum*. *Int J Exp Pathol.* **100**, 222–233 (2019).

Figures

Figure 1

Flowchart and timeline of the study design for asthma.

Figure 2

Evaluation of (A) tidal volume (V_T), (B) respiratory frequency (RF), and (C) VE on days 0, 12 and 21 of asthma induction. Symbols and vertical bars represent the mean and the e.p.m., respectively. One-way ANOVA followed by Tukey's post-test ($n = 6$). $*p < 0.05$ (CG vs. AG, OAG and OADEXAG).

Figure 3

Effect of stimulation of rat trachea with 100 µg/ml of OVA. Symbols and vertical bars represent the mean and the e.p.m., respectively. One-way ANOVA followed by Tukey's post-test ($n = 3-5$). * $p < 0.05$ (CG vs. AG, OAG and OADEXAG); ^a $p < 0.05$ (OG vs. OAG); ^b $p < 0.05$ (AG vs. OAG).

Figure 4

Cumulative concentration-response curves to KCl, in the presence of functional epithelium, in rat trachea. Symbols and vertical bars represent the mean and the e.p.m., respectively. One-way ANOVA followed by Tukey's post-test (n = 5).

Figure 5

Cumulative concentration-response curves to CCh, in the presence of functional epithelium, in rat trachea. Symbols and vertical bars represent the mean and the e.p.m., respectively. One-way ANOVA followed by Tukey's post-test (n = 5). * $p < 0.05$ (CG vs. AG, OG, OAG and OADExAG); ^b $p < 0.05$ (AG vs. OAG).

Figure 6

Cumulative concentration-response curves to nifedipine (A), isoprenaline (B) and aminophylline (C) in pre-contracted rat trachea with CCh 10^{-5} M in the presence of functional epithelium. The symbols and vertical bars represent the mean and the e.p.m., respectively (n = 5).

Figure 7

Photomicrography of the lungs of rats from CG (A and F), OG (B and G), AG (C and H), OAG (D and I) and OADEXAG (E and J) stained in HE and Masson's trichrome, respectively. Cell infiltrate (black arrows).

Figure 8

Measurements of inflammatory area (A), smooth muscle thickness (B) and remodeling through the extracellular matrix (C) in the lungs of animals in the GC, OG, AG, OAG and OADEXA. Symbols and vertical

bars represent the mean and the e.p.m., respectively (n = 6). One-way ANOVA followed by Tukey's post-test. * $p < 0.05$ (CG vs. OG, AG and OAG), ^a $p < 0.05$ (OG vs. OAG), ^b $p < 0.05$ (AG vs. OAG), [#] $p < 0.05$ (OAG vs. OADEXAG).

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