



Therapy with resveratrol attenuates obesity-associated allergic airway inflammation in mice

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ABSTRACT

Obesity and insulin resistance have been associated with deterioration in asthma outcomes. High oxidative stress and deficient activation of AMP-activated protein kinase (AMPK) have emerged as important regulators linking insulin resistance and inflammation. This study aimed to evaluate the effects of resveratrol on obesity-associated allergic pulmonary inflammation. Male C57/Bl6 mice fed with high-fat diet to induce obesity (obese group) or standard-chow diet (lean group) were treated or not with resveratrol (100 mg/kg/day, two weeks). Mice were sensitized and challenged with ovalbumin (OVA). At 48 h thereafter, bronchoalveolar lavage fluid was performed, and lungs collected for morphological studies and Western blot analysis. Treatment of obese mice with resveratrol significantly reduced hyperglycemia and insulin resistance, as well as the body measures (body mass, fat mass, % fat, and body area). OVA-challenge promoted a higher increase in pulmonary eosinophil infiltration in obese compared with lean mice, which was nearly abrogated by resveratrol treatment. Resveratrol markedly increased the phosphorylated AMPK expression in lung tissues of obese compared with lean mice. Resveratrol reduced the p47phox expression and reactive-oxygen species (ROS) production, and elevated the superoxide dismutase (SOD) levels in lung tissues of obese mice. The increased pulmonary levels of TNF- α and inducible nitric oxide synthase (iNOS) in obese mice were also normalized after resveratrol treatment. In lean mice, resveratrol failed to affect the levels of fasting glucose, p47phox, ROS levels, TNF- α , iNOS and phosphorylated AMPK. Resveratrol exhibits protective effects in obesity-associated lung inflammation that is accompanied by local AMPK activation and antioxidant property.

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1. Introduction

Asthma is a disorder of the conducting airways leading to variable airflow obstruction in association with airway hyperresponsiveness [1]. Airway eosinophilia is commonly associated with increased risk for asthma exacerbation, severity, and poor prognosis [2]. Eosinophils have been classically considered effector cells with pro-inflammatory actions via the synthesis and release of multiple substances such as the highly basic and cytotoxic granule proteins major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EPX) [1]. Eosinophils are also capable of undergoing de novo synthesis of nitric oxide (NO), and measurement of exhaled NO level has been employed as a biomarker of airway inflammation [3]. The enzyme inducible NO synthase (iNOS) is highly expressed in lung tissues and eosinophils of asthmatic patients and

animals, generating high levels of NO in the exhaled air [4]. Activation of NF- κ B by TNF- α increases iNOS transcription [5], leading subsequently to NO overproduction, which in turn modulates the eosinophil recruitment into the pulmonary tissue [6,7]. Moreover, high levels of NO-reacting oxidants such as superoxide anion, hydrogen peroxide, and hydroxyl radicals have been implicated in allergic lung diseases, which may be produced by resident and non-resident cells infiltrating the airways of asthmatic individuals [8]. Increased oxidative stress leads to inactivation of superoxide dismutase (SOD) in asthmatic individuals aggravating airway obstruction [9].

Obese individuals with asthma exhibit worse asthma-related quality of life, worse asthma control, and more asthma-related hospitalizations in comparison with those with asthmatics with normal body mass index [10,11]. Studies indicate a high prevalence of insulin resistance (IR) in obese and asthmatic patients versus obese non-asthmatics, suggesting that insulin resistance may be a causal link between asthma and obesity [12–15]. The exacerbation of the allergic eosinophilic inflammation in obese mice is normalized by suppression of insulin resistance, which is accompanied by reductions of the levels of pro-inflammatory markers such as TNF- α and NO metabolites [16,17].

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Resveratrol (*trans*-resveratrol) is a natural polyphenolic compound found in the skin of red grapes. In rodents and humans, resveratrol promotes cardio-neuroprotective, anti-diabetic and anti-cancer effects [18]. Resveratrol therapy can also prevent the hypertensive response in rats fed a high-fat diet [19]. Recently, resveratrol was shown to alleviate thermal hyperalgesia and mechanical allodynia in a neuropathic mice model [20]. Resveratrol activates AMP-activated protein kinase (AMPK) [21,22], and AMPK improvement in turn improves high-fat diet-induced insulin resistance [17,23]. In a mouse model of asthma, resveratrol significantly reduced the levels of the Th2 cytokines, airway hyperresponsiveness, eosinophilia, and mucus hypersecretion [24]. In the same animal model, the protective effects of resveratrol was associated with restored mitochondrial function and inositol polyphosphate-4-phosphatase A expression in the lungs, as well as with decreased PI3K–Akt signaling [25]. There are growing evidences suggesting that the obesity-related asthma phenotype does not necessarily involve the classical Th2-dependent inflammatory process [26]. In the present study we tested the hypothesis that resveratrol attenuates the pulmonary allergic inflammation in insulin-resistant obese mice. Therefore, we treated high-fat-fed obese mice with resveratrol, and evaluated the pulmonary eosinophilic inflammation in response to ovalbumin (OVA) challenge. The levels of oxidant and antioxidant biomarkers in the lung tissue, as well as the protein expressions of iNOS and phosphorylated AMPK were evaluated in this study.

2. Materials and methods

2.1. Animals and diet

All animal procedures and experimental protocols are in accordance with and were approved by the Ethics Committee in Animal Use, State University of Campinas (CEUA-UNICAMP), protocol 2012/2709-1. Animals were housed on a 12-h light–dark cycle and fed for 12 weeks with either a standard chow diet (70% carbohydrate, 20% protein, 10% fat) or a high-fat diet that induces obesity (29% carbohydrate, 16% protein, 55% fat) [16]. Lean and obese mice were treated with vehicle (water) or resveratrol (100 mg/kg/day) by gavage for two weeks [27].

2.2. Mouse densitometry

Mice were weighed, anesthetized with intraperitoneal injection of a mixture of xylazine (10 mg/kg) and ketamine (90 mg/kg), and then subjected to a dual-energy X-ray absorptiometry scan (DEXA; QDR Series, Hologic Apex Software Inc., Bedford, MA, USA). Body scan measurements allowed the following parameters: total mass, fat mass, % fat, body area and bone mineral composition (BMC). Mouse body scans were obtained for each animal.

2.3. Insulin tolerance test (ITT)

After 6-h fasting, systemic insulin sensitivity was analyzed by the Insulin Tolerance Test (ITT). Briefly, tail blood samples were collected before (0 min) and at 5, 10, 15, 20, 25 and 30 min after an intraperitoneal injection of 1.00 U/kg of regular insulin (Novolin R, NovoNordisk, Bagsvaerd, Denmark). Glucose concentrations were measured using a glucometer (ACCUCHEK Performa; Roche Diagnostics, Indianapolis, IN, USA) and the values were used to calculate the constant rate for blood glucose disappearance (K_{ITT}), which is based on the linear regression of the Neperian logarithm of glucose concentrations obtained from 0 to 30 min of the test. K_{ITT} was calculated using the formula $0.693 / (t_1 / 2) \times -1 \times 100$ [28].

2.4. Sensitization procedure and OVA challenge

Lean and obese mice were actively sensitized with a subcutaneous injection (0.4 ml) of 100 µg of OVA (grade V; Sigma-Aldrich Co., St.

Louis, MO) mixed with 1.6 mg Al(OH)₃ in 0.9% NaCl (day zero). One week later (day 7), mice received a second subcutaneous injection of 100 µg OVA (0.4 ml). On days 14 and 15, mice were intranasally challenged with OVA (10 µg/50 µl) twice a day. At 48 h after the first challenge, the BAL fluid was performed, and lungs were also collected for morphological studies and Western blot. The lungs were washed by flushing phosphate-buffered saline (PBS). The PBS was instilled through the tracheal cannula in 5-aliqouts of 300 µl. The fluid recovered after each instillation was centrifuged (500 × g, 10 min, 4 °C), and BAL fluid supernatant stored at –80 °C. The cell pellet was resuspended in 200 µl of PBS and total (Neubauer) and differential (Diff-Quick stain) cell counts were done. Fig. 1 depicts the experimental protocols for airway sensitization and challenge with ovalbumin (OVA) in mice treated or not with resveratrol.

2.5. Morphometrical lung analysis

Lungs were perfused via the right ventricle with 10 ml PBS to remove residual blood, immersed in 10% phosphate buffered formalin for 24 h and then kept in 70% ethanol until embedding in paraffin. Tissues were sliced (5 µm sections) and stained with hematoxylin/eosin for light microscopy examination. Morphometrical analysis was performed using a Leica DM 5000B digital camera, and Leica Q Win Image Processing and Analysis Software. For each different staining, the area of positivity was measured in mm² for 5 bronchioles per slide.

2.6. Measurements of TNF-α, SOD and glutathione (GSH)

TNF-α in BAL fluid was measured using commercially available DuoSet ELISA kits, following the instructions of the manufacturer (R & D, Minneapolis, USA). SOD and GSH concentrations in lung tissue were determined using commercially available kits (Cayman Chemical, Ann Arbor, MI, USA).

2.7. Western blotting for iNOS, p47phox, PDE4, and phosphorylated AMPK

Lung tissues were homogenized in SDS lysis buffer with a Polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Inc., Westbury, NY) and centrifuged. Protein concentrations in supernatants were determined by the Bradford assay, and an equal amount of protein from each sample (50 µg) was treated with Laemmli buffer containing dithiothreitol (100 mM). Samples were heated in a boiling water bath for 10 min and resolved by SDS-PAGE. Electrotransfer of proteins to a nitrocellulose membrane was performed for 60 min at 15 V (constant) in a semi-dry device (Bio-Rad, Hercules, CA, USA). Nonspecific protein binding to nitrocellulose was reduced by pre-incubating the membrane overnight at 4 °C in blocking buffer (0.5% non-fat dried milk, 10 mM Tris, 100 mM NaCl, and 0.02% Tween 20). Specific antibodies such as anti-

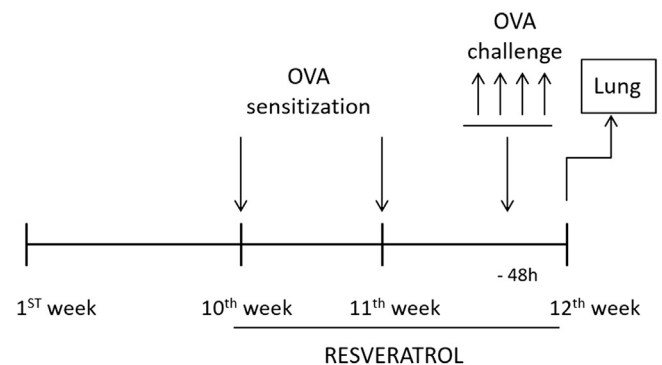


Fig. 1. Protocol for sensitization and challenge with ovalbumin (OVA) in high-fat obese and standard-chow-fed lean mice, treated or not with resveratrol (100 mg/kg, 2 weeks). BAL, bronchoalveolar lavage fluid.

iNOS, anti-phospho-AMPK α 1/ α 2, anti-p47phox, anti-PDE4 or anti β -actin were used for detection. After incubation with primary antibodies, membranes were incubated with secondary antibodies according to the source of the primary antibody. Before detection, membranes were incubated with a solution containing luminol and then exposed for varying times to X-ray films. Densitometry was performed using the Scion Image Software (Scion Corporation, Frederick, MD). Results were represented as the ratio of the density of the primary antibodies' band to the density of the β -actin band.

2.8. Measurement of reactive-oxygen species (ROS)

The lungs were removed, placed in freezing medium for TCA, and frozen in liquid nitrogen. Lung tissues were cut (14 μ m) in a cryostat and placed on glass slides and placed in hot plate (37 °C) for 20 min. The sections were incubated with dihydroethidium (DHE; 2 μ M), diluted in phosphate buffer for 30 min at 37 °C in a humid chamber. The sections were observed with a fluorescence microscope (Eclipse 80i, Nikon, Japan) and camera (DS-U3, Nikon, Japan) with filters for rhodamine,

using a 10 $\times$ objective. The quantification was done by image J Software (National Institutes of Health, Bethesda, MD, USA).

2.9. Drugs and antibodies

Resveratrol, anti-p47phox (sab4502808), and anti β -actin (A3854) were obtained from Sigma (St. Louis, MO, USA). Anti-inducible nitric oxide synthase (iNOS; ab15323-500) and anti-phospho-AMPK α 1/ α 2 (Thr 172; #2531) were obtained from AbCam Technology (Cambridge, England, UK). Anti-AMPK (#2603) and anti-phosphodiesterase 4 (PDE 4; orb6656) were obtained from Cell Signaling (Danvers, MA, USA) and Biorbyt (Cambridge, UK), respectively.

2.10. Statistical analysis

Data were expressed as means $\pm$ SEM. The program GraphPad version 5.0 software was used for statistical analysis. Statistically significant differences were determined using one-way analysis of variance

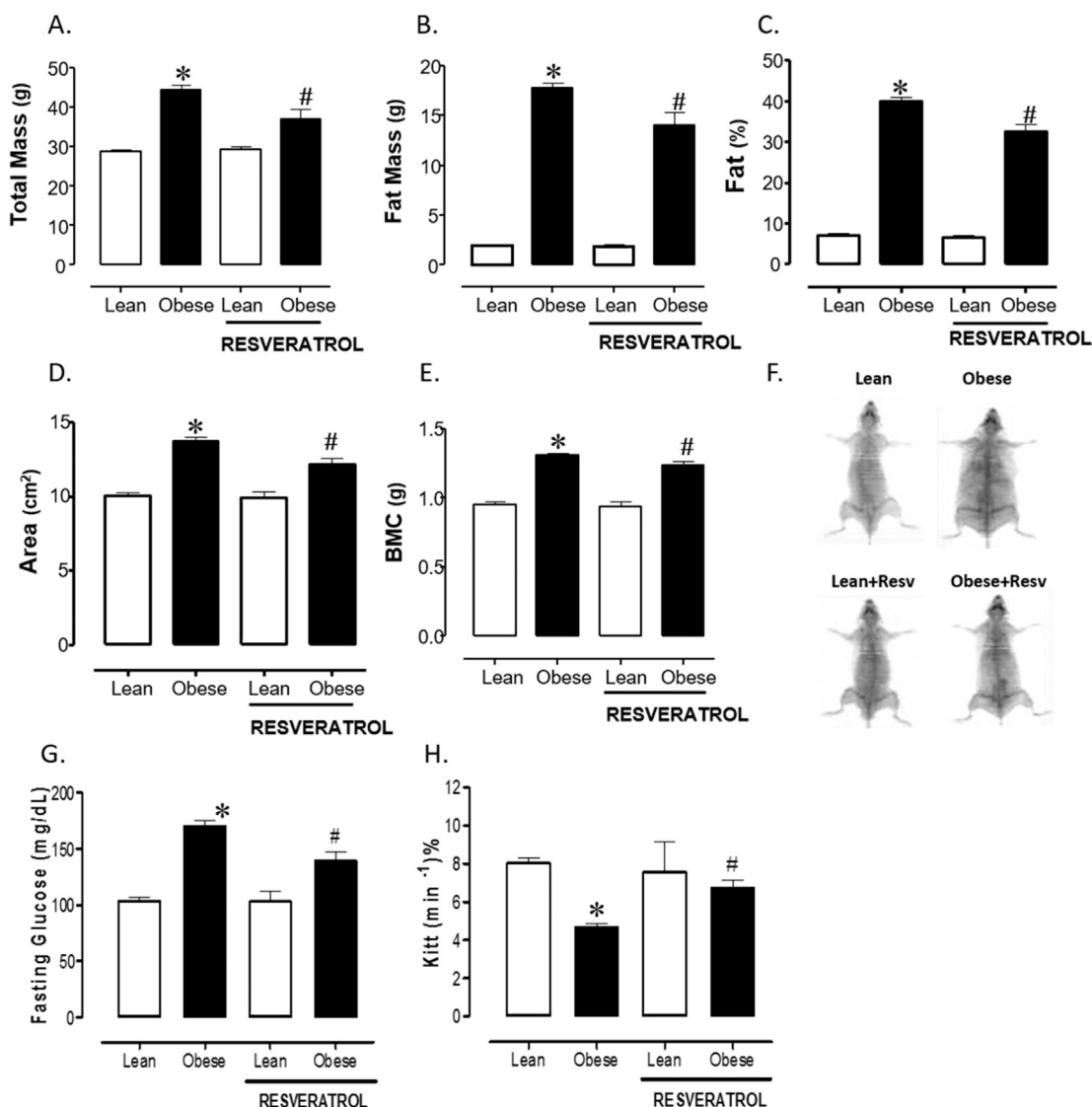


Fig. 2. Effect of resveratrol on total mass (A), fat mass (B), % fat (C), body area (D), bone mineral composition (BMC; E), representative X-ray absorptiometry scan images (F), blood glucose levels (G) and constant rate for blood glucose disappearance (K_{ITT} ; H). Male mice (lean and obese) were treated with resveratrol (100 mg/kg) for 2 weeks. Each column represents the mean $\pm$ SEM for $n = 5$. * $P < 0.05$ compared with lean mice, # $P < 0.05$ compared with untreated obese mice. In panel F, the light gray areas indicate body fat whereas the dark gray areas indicate boney structures.

(ANOVA) for multiple comparisons followed by a Tukey test. A value of $P < 0.05$ was accepted as significant.

3. Results

3.1. Effects of resveratrol on adiposity, glucose levels and insulin resistance

High-fat diet-fed mice (obese group) exhibited significantly higher ($P < 0.05$) body mass, fat mass, % fat, body area and bone mineral composition (BMC) compared with standard diet-fed lean mice ($n = 5$), all of which were significantly reduced by treatment with resveratrol (100 mg/kg/day, two weeks; Fig. 2A–F). Resveratrol treatment did not alter any of these parameters in lean mice. Fasting glucose level was higher in the obese group ($P < 0.05$), which was significantly reduced by resveratrol, with no changes in the lean group ($n = 5$; Fig. 2G). The glucose disappearance rate (as estimated by the K_{ITT} values) was lower in the obese group compared with the lean group, an effect restored by resveratrol treatment ($n = 5$; Fig. 2H). No changes in K_{ITT} were observed in the lean group.

3.2. OVA-induced pulmonary eosinophilic inflammation

To assess the effects of treatment with resveratrol on the allergic pulmonary inflammation, we quantified the total inflammatory cells and eosinophils in the lung tissues. Initially, we carried out the differential cell

counts in BAL fluid in non-sensitized mice (intranasally instilled with saline). In the lean group, leukocytes consisted of 98% mononuclear cells and 2% neutrophils, whereas in obese mice, leukocytes comprised 99% mononuclear cells and 1% of neutrophils ($n = 5$ –6). Eosinophils in both of these non-sensitized control groups were nearly absent.

In the lung tissue of sensitized lean mice, intranasal challenge with OVA markedly increased the infiltration of total inflammatory cells and eosinophils in peribronchiolar regions compared with non-sensitized mice. However, in lung tissue of obese mice, OVA challenge promoted a significantly higher increase ($P < 0.05$) of total inflammatory cells (Fig. 3A,C) and eosinophils (Fig. 3B,C) when compared with lean mice (Fig. 3A,C). Treatment with resveratrol in lean mice significantly reduced the number of total cells and eosinophils in the lung tissue compared with untreated lean group (2.3- and 1.75-fold reductions, respectively; $P < 0.05$). In obese mice, however, resveratrol treatment produced markedly higher reductions of total cells and eosinophils compared with untreated obese group (8.0- and 4.8-fold reductions, respectively; $P < 0.05$).

3.3. Resveratrol possesses antioxidant activity in lung tissues of obese mice

The p47phox expression in lung tissues did not differ between obese and lean groups. However, resveratrol significantly reduced the p47phox expression in obese ($P < 0.05$), without affecting this protein expression in lean mice ($n = 5$ –6; Fig. 4A). Increased ROS levels in lung tissues of obese mice were also observed, an effect

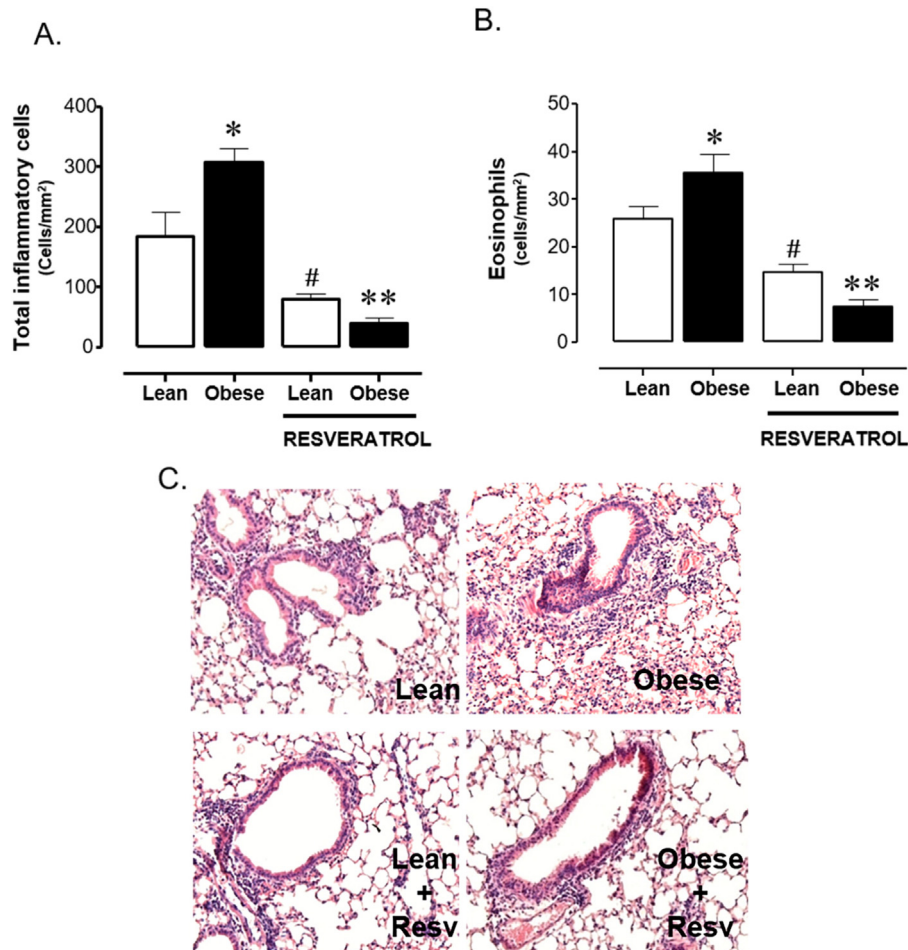


Fig. 3. Resveratrol decreases the number of total inflammatory cells and eosinophils in lung tissue. (A) Number of total inflammatory cells (A) and (B) eosinophils in lung tissue from ovalbumin (48 h)-challenged lean and obese mice. Panel C shows representative photomicrographs (magnification 200×) for each experimental group. Data represent the mean $\pm$ SEM ($n = 6$). * $P < 0.05$, # $P < 0.05$ compared with untreated lean mice. ** $P < 0.05$ compared with untreated obese mice.

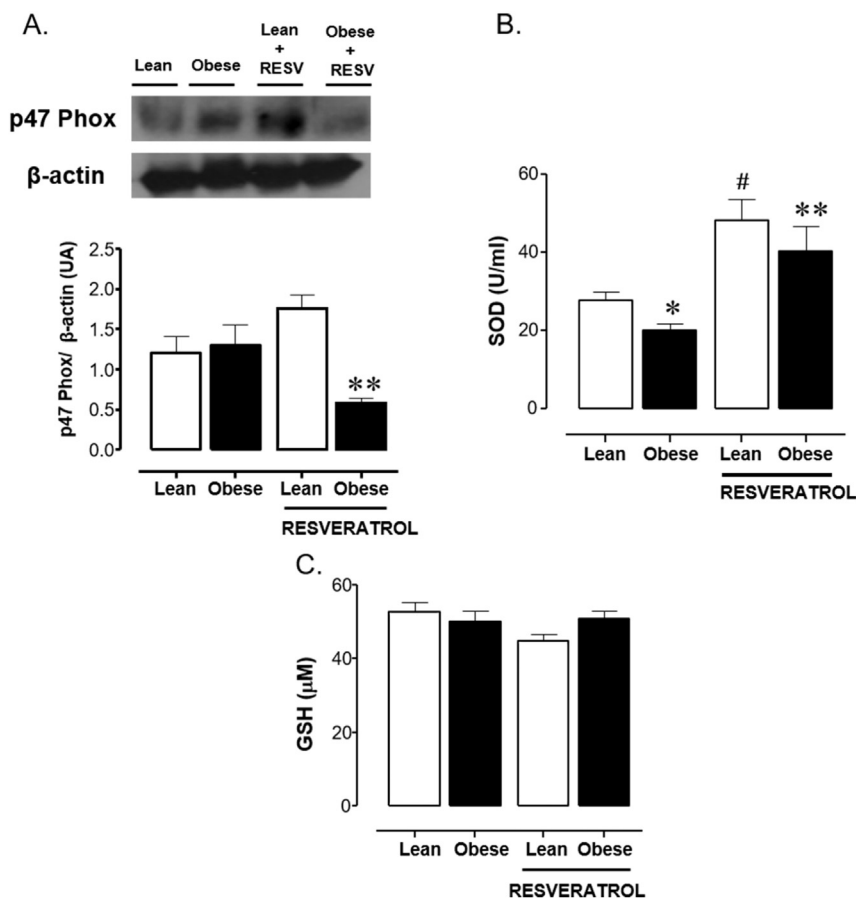


Fig. 4. Effect of resveratrol on p47phox expression, SOD and GSH in lung tissue. (A) Expression of the subunit p47phox, (B) levels of superoxide dismutase (SOD) and (C) glutathione (GSH) in lung tissue of lean and obese mice, treated or not with resveratrol (Resv; 100 mg/kg/day, 2 weeks). Lungs were examined at 48 h following intranasal challenge with ovalbumin in previously sensitized mice. The membranes probed with p47phox antibody were normalized with β -actin. Data are expressed as mean $\pm$ SEM ($n = 5-6$). * $P < 0.05$, # $P < 0.05$ compared with untreated lean mice. ** $P < 0.05$ compared with untreated obese mice.

markedly reduced by resveratrol treatment ($n = 6$; $P < 0.05$; Fig. 5). Resveratrol had no effect in ROS levels in the lean group. The SOD levels in lung tissues of obese mice were significantly lower compared with the lean group. Treatment with resveratrol significantly elevated the SOD levels in obese and lean mice ($n = 6$; Fig. 4B). The levels of GSH in lung tissues did not significantly differ in any experimental group ($n = 6$; Fig. 4C).

3.4. Resveratrol abrogates the increased TNF- α production and iNOS expression in lung tissues of obese mice

TNF- α levels in BAL fluid were 2.0-fold greater in obese compared with lean mice ($P < 0.05$), which was markedly reduced by resveratrol treatment. In the lean group, resveratrol did not significantly affect TNF- α levels ($n = 6$; Fig. 6A). Lung tissue of obese mice

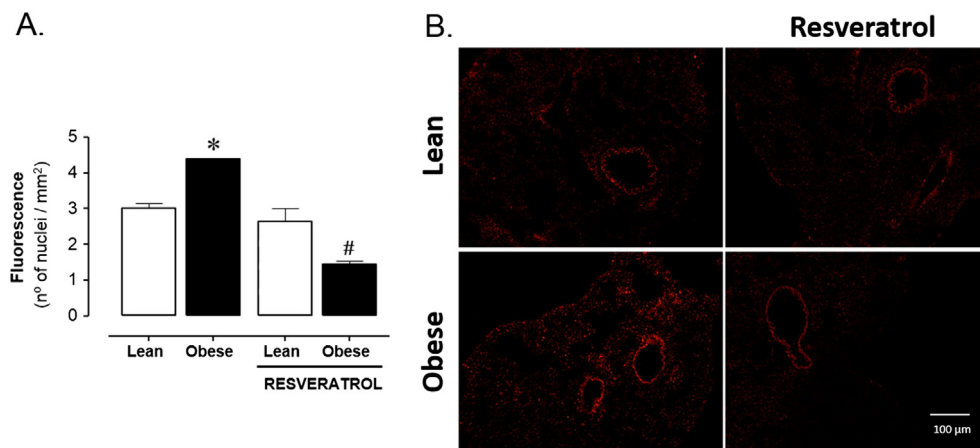


Fig. 5. (A) Levels of reactive-oxygen species (ROS) through dihydroethidium (DHE)-induced fluorescence in lung tissue from lean and obese mice, treated or not with resveratrol (Resv; 100 mg/kg/day, 2 weeks). (B) Representative photomicrographs of lungs in all groups (magnification 200 $\times$). Lungs were examined at 48 h following intranasal challenge with ovalbumin in previously sensitized mice. Data are presented as mean $\pm$ SEM ($n = 6$). * $P < 0.05$ compared with untreated lean mice; # $P < 0.05$ compared with untreated obese mice.

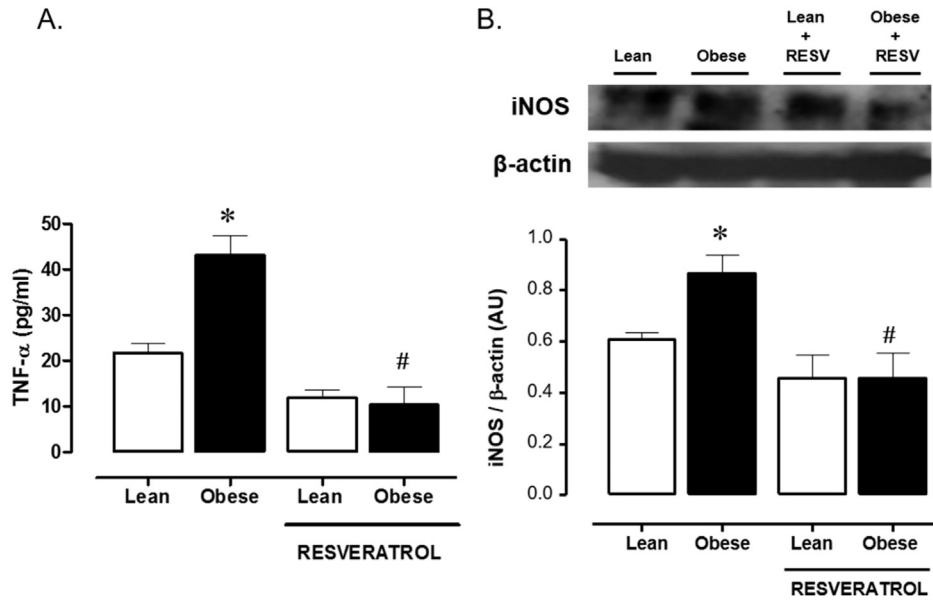


Fig. 6. Effect of resveratrol on TNF- α levels in BAL fluid and inducible nitric oxide synthase (iNOS) expression in lung tissue. (A) TNF- α levels in BAL fluid (B) and iNOS protein expression in lung tissue from lean and obese mice, treated or not with resveratrol (Resv; 100 mg/kg/day, 2 weeks). Lungs were examined at 48 h following intranasal challenge with ovalbumin in previously sensitized mice. The membranes probed with iNOS antibody were normalized with β -actin. The data represent the mean $\pm$ SEM ($n = 6$). * $P < 0.05$ compared with untreated lean mice; # $P < 0.05$ compared with untreated obese mice.

exhibited a significant increase in the expression of iNOS compared with the lean group ($P < 0.05$), which was fully prevented by resveratrol treatment ($n = 6$; Fig. 6B). Resveratrol had no effect in the iNOS expression in lung tissue of lean mice.

3.5. Protein expressions of p-AMPK and PDE4 in lung tissues

The p-AMPK expression in lung tissues did not differ between obese and lean groups. However, resveratrol largely increased the expression of p-AMPK in lung tissue of obese mice without significantly affecting this protein expression in the lean group ($n = 6$; Fig. 7A).

The PDE4 protein expression in lung tissues did not significantly differ between obese and lean groups ($n = 5-7$). Resveratrol treatment

significantly reduced the PDE4 expression in both lean and obese mice with no difference between them ($P < 0.05$; Fig. 7C).

4. Discussion

In the present study, we have used a murine model of asthma associated with obesity and insulin resistance to evaluate the effects of the polyphenol resveratrol. We show that two-week therapy with resveratrol nearly abrogates the allergic pulmonary eosinophilic infiltration in obese mice, which is accompanied by reductions of TNF- α levels, iNOS expression and pulmonary oxidative stress in the lungs of obese mice.

Obesity is a risk factor for developing type 2 diabetes and insulin resistance [14,29]. Mice fed high-fat diet exhibit hyperglycemia and

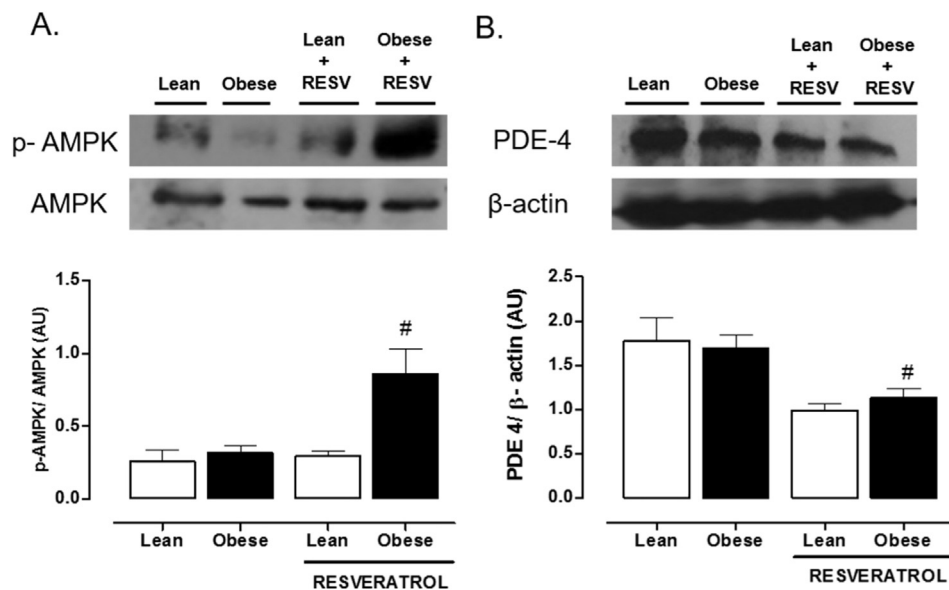


Fig. 7. Expressions of phosphorylated AMPK (A) and phosphodiesterase 4 (PDE4) (B) in lung tissue from lean and obese mice, treated or not with resveratrol (Resv; 100 mg/kg/day, 2 weeks). Lungs were examined at 48 h following intranasal challenge with ovalbumin in previously sensitized mice. Phosphorylated AMPK (p-AMPK) and PDE4 were normalized for β -actin. The data represent the mean $\pm$ SEM ($n = 5-6$). # $P < 0.05$ compared with untreated obese mice.

insulin resistance [17]. Resveratrol treatment significantly attenuated hyperglycemia and increased insulin sensitivity in obese mice, which are consistent with previous studies in obese humans [30] and mice undergoing a high-caloric diet [21]. Additionally, using mouse X-ray absorptiometry to evaluate the anthropometric measures, we showed that resveratrol significantly attenuated all the analyzed parameters, including total mass, fat mass, % fat and body area, which may be due to inhibition of pre-adipocyte proliferation, and inhibition of adipogenic differentiation [31].

Asthma and obesity have in common high levels of oxidative stress biomarkers [32]. Asthmatic patients and OVA-challenged mice show elevated ROS production in lung tissues, and in turn airway inflammation may be triggered by oxidizing agents [8,25,33]. Increased oxidative stress also accounts for the increased adipose tissue and insulin resistance [34]. Reactive-oxygen species include free radicals such as superoxide anion and hydroxyl radicals, as well as non-radicals such as hydrogen peroxide. Several potential ROS-generating systems exist, including the NADPH oxidase complex (NOX), which is a multienzyme oxidant complex composed of membrane-bound and cytosolic subunits [32]. The NADPH oxidase cytosolic subunit p47phox is an essential component of this complex to generate superoxide. Antioxidant enzymes like SOD play a critical role for the management of oxidative stress in different pathophysiological conditions, including asthma [9]. Thus, we studied the expression of p47phox protein and levels of ROS and SOD in the lung tissues to evaluate a potential antioxidant effect of resveratrol. We found that resveratrol treatment nearly abrogated the OVA-induced pulmonary eosinophilic inflammation in obese mice, which was accompanied by marked reductions of p47phox expression and ROS production in the lung tissue, along with greater elevation of SOD levels, all indicating that such antioxidant property of resveratrol contributes to the amelioration of obesity-associated asthma. Previous studies have shown that the antioxidant GSH acts to protect airway inflammation associated with oxidant stress. Treatment of mice with a membrane-permeating GSH precursor reduced the eosinophilic infiltration, whereas GSH depletion increased ROS production, worsening the allergic airway inflammation [33,35]. We firstly hypothesized that GSH levels in lung tissue might contribute to the amelioration by resveratrol of obesity-associated murine asthma. However, the GSH levels did not significantly change in any experimental group, ruling out the reduction of the involvement of glutathione redox in the modulation of p47phox expression and ROS levels by resveratrol in lung of obese mice.

Activation of the TNF- α -iNOS signaling pathway resulting in NO overproduction is reported to play an important role in asthma pathophysiology [36]. Treatment with anti-TNF- α antibody conveys the pulmonary eosinophilic inflammation in insulin-resistant obese mice to the levels of lean mice. Likewise, the iNOS inhibitor aminoguanidine significantly attenuates the eosinophil infiltration in the lung tissue of obese mice [17]. In addition, TNF- α has been clearly implicated in adipocyte fat accumulation and insulin resistance that may occur through NF- κ B-iNOS activation [37]. Our present data shows that resveratrol normalizes the increases of TNF- α levels and iNOS expression in lung tissue and hence the obesity-associated pulmonary eosinophilic inflammation. Of interest, resveratrol was shown to upregulate the expression of NF- κ B in immunosuppressive mice [38].

AMP-activated protein kinase has also emerged as a major regulator linking inflammation and metabolism [18]. Activation of AMPK enhances the *in vitro* phagocytic activity of neutrophils and macrophages [39] and reduces acute lung injury [40,41]. The metabolic effects of resveratrol was associated with AMPK activation, as evaluated in mice deficient of the catalytic subunit of AMPK (AMPK α 1^{-/-}) fed with high-fat diet [22]. In our study, resveratrol treatment markedly elevated the phosphorylation of AMPK in the lung tissue of obese mice, strongly suggesting that amelioration of this asthma phenotype relies on AMPK activation in a similar fashion to that of the classical AMPK activator metformin [17]. Interestingly, activation of AMPK leads to a reduction of TNF- α -induced NF- κ B activation in vascular endothelial cells [42].

Therefore, additional pharmacological or genetic approaches may be necessary to fully implicate and clarify the requirement for the increased activation of AMPK in mediating protection against allergic inflammation in obese mice.

Phosphodiesterase-4 (PDE4) is highly specific for cAMP, which is the dominant enzyme isoform in the airways and inflammatory cells. Resveratrol increases cyclic adenosine monophosphate (cAMP) levels by competitive inhibition of PDEs [43]. Our data showed that resveratrol significantly reduced the PDE4 expression in the lung tissues, but these reductions were of the same extent in lean and obese mice, which is suggestive that beneficial effects of resveratrol in obesity-associated murine asthma take place independently of PDE4 activation.

In asthmatic non-obese mice (lean group), resveratrol, at the dose employed here (100 mg/kg/day, two weeks) [28], promoted a reduction of OVA-induced pulmonary cell recruitment, despite that it was of lower magnitude compared with obese mice. In addition, resveratrol failed to significantly affect any metabolic effect (hyperglycemia and K_{ITT}) or anthropometric parameter in the lean group. Resveratrol elevated significantly the SOD levels in the lean group, but instead failed to affect the levels of p47phox, ROS, TNF- α , iNOS, and p-AMPK. Therefore, it all seems that the protective effects of resveratrol on allergic murine asthma are better visualized in conditions of high oxidative stress such as obesity-associated asthma. In conclusion, the protective action of resveratrol in an obesity-associated asthma phenotype may involve AMPK activation and suppression of iNOS expression in the lung tissues, concomitantly with reductions of TNF- α levels, p47phox expression, and superoxide production.

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