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# Treatment of obese asthma in a mouse model by simvastatin is associated with improving dyslipidemia and decreasing leptin level

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ABSTRACT

Obesity can cause or worsen asthma. Compared with common asthma, obese asthma is difficult to control. Statins are effective serum cholesterol-lowering agents in clinical practice, and they also have anti-inflammatory properties, which in theory are potentially beneficial in asthma. Many studies have shown that simvastatin has good therapeutic effect in animal models of asthma. However, the therapeutic effect and action mechanism of simvastatin for obese asthma remain unclear. Leptin, a satiety hormone, is in positive correlation with total body fat mass and may also play a significant role in the pathogenesis of asthma. In this study, we use the method of high-fat diet and ovalbumin (OVA) sensitization and challenge to establish the mouse model of obesity and asthma, and find that obese asthmatic mice has higher levels of glucose, lipid and leptin in serum, and neutrophil percentage in bronchoalveolar lavage fluid (BALF), and more severe airway inflammation and structural changes in lung tissues than non-obese asthmatic mice, and respond poorly to dexamethasone treatment, which indicates that obese asthma might belong to steroid-resistant (SR) asthma. Simvastatin treatment reduces the levels of glucose, lipid, leptin and neutrophil percentage, and improves airway inflammation and remodeling, which can be as a potential therapeutic target used in the treatment of obese asthma in humans. Correlation analysis shows that there is positive correlation between neutrophil percentage and serum leptin/cholesterol level, which indicates that the therapeutic efficacy of simvastatin on obese asthma might be associated with improving dyslipidemia and decreasing leptin level.



## 1. Introduction

Asthma is a chronic inflammatory disease of the airways characterized by reversible obstruction of airway hyper-responsiveness, inflammatory infiltrates in the bronchial walls, and mucus hypersecretion. Glucocorticoids as potent anti-inflammatory therapy drugs are commonly used in the treatment of acute exacerbations of asthma. However, certain asthma patients usually with severe asthma fail to respond to glucocorticoid treatment despite high dose or/and extended the duration of therapy, and this condition is termed “steroid-resistant (SR) asthma” [1]. These SR asthma patients continue their therapy with glucocorticoids despite the onset of severe side reaction and bad clinical outcome. Recent studies reported that the SR asthma may result from impaired histone deacetylase, cytokine hypersecretion and neutrophilic granulocytosis [2,3].

Obesity is a known risk factor for many human diseases, including asthma, cardiovascular disease, diabetes, and several types of cancer [4,5]. In addition, obesity can worsen the condition of asthma, and alter the efficacy of standard asthma medications, which makes asthma patients respond poorly to the treatment of glucocorticoids [6]. In recent years, mechanisms underlying the relationship between obesity and asthma are widely investigated. Surely obese breathing with a high respiratory rate and a low tidal volume increases the risk of asthma and asthma severity. Furthermore, there is a growing body of evidence that obesity-induced disorders of lipid metabolism and the endocrine function alterations of adipose tissue worsen the condition of asthma [5,7]. For example, we once investigated whether obesity directly leaded to airway inflammation associated with asthma, and found obesity induced the changes of adipokine secretion, such as leptin, leptin receptor, adiponectin, and vascular endothelial growth factor

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(VEGF), and obesity had no significant direct influence on the development of airway hyperreactivity and lung inflammation in the diet-induced mild obese mice without ovalbumin (OVA) challenge. However, compared with non-obese asthmatic mice, obese asthmatic mice had severe condition of asthma, which suggested that obesity worsening the condition of asthma might be associated with the changes of adipokine secretion. These results were consistent with the studies reported by Saraiva et al. [8] and Jung et al. [9].

Leptin, the product of the *ob* gene, is originally identified as a satiety hormone secreted by adipocytes and is in positive correlation with total body fat mass [10]. As an adipose signal, leptin level increase means the excess of lipid storage, which is implicated in the modulation of the energy balance and restriction of excess accumulation of fat [11]. Leptin is a pleiotropic hormone and also has proinflammatory effect involved in the regulation of immune response. For example, Shirshev et al. [12] demonstrated that the modulation of mononuclear phagocytes by leptin is related to activation of the JAK-STAT pathway, which results in stimulation of phagocytosis, production of reactive species of oxygen and nitrogen, and increased secretion of pro-inflammatory cytokines. Raso et al. [13] also confirmed that leptin can target macrophages and is related to the pathophysiology of inflammation. In addition, Mancuso et al. [14] reported that leptin can enhance leukotriene synthesis in alveolar macrophages, and leukotriene plays the central role in asthma pathophysiology, which implies that leptin might be involved in the occurrence and development of asthma. Further, it has been reported that leptin promotes the release of VEGF by human airway smooth muscle cells, and VEGF is implicated in the severity of asthma [15,16]. Cross-sectional studies in children and adults have shown that plasma leptin level is higher in asthma patients [17,18]. These findings indicate that leptin is associated with asthma, and might serve as a link between obesity and asthma.

Statins, inhibitors of 3-hydroxy-3-methyl-3-glutaryl coenzyme A (HMG-CoA) reductase, are widely used in the treatment of coronary artery disease by decreasing cholesterol [19]. Statins also have several other pharmacological actions, such as anti-inflammatory, anti-oxidant, and immunomodulatory effects, which might be beneficial in airway inflammatory diseases. In fact, simvastatin, one kind of statins, has been widely reported to be effective in the treatment of asthma [20,21]. But for the treatment of obese asthma, its role is still unclear.

Thus in this study, we first established the mouse model of obesity and asthma, and investigated the asthma severity of the obese mice. Furthermore, we investigated the curative effect and action mechanism of simvastatin for obese asthma.

## 2. Materials and methods

### 2.1. Experimental animals

Specific pathogen-free (SPF) female C57BL6J mice (body weight 10.91 ± 0.25 g, 3 weeks old) were purchased from the Experimental Animal Center, Anhui Medical University (Hefei, China). These mice were randomly divided into six groups (15 mice every group): control group, asthma group, obese asthma group, dexamethasone group (treated obese asthmatic mice with dexamethasone), simvastatin group (treated obese asthmatic mice with simvastatin), and combination group (treated obese asthmatic mice with dexamethasone and simvastatin). These mice were raised in the controlled environmental conditions (23 ± 2 °C, 50 ± 10% humidity, and 12 h light-dark cycle) and allowed free access to water and regular rodent chow in the animal laboratory of Qingdao University of Medical Science for 1 week before initiating the experiments. All mice used in this study were kept under a protocol approved by the Animal Ethics Community of Qingdao Municipal Hospital.

### 2.2. Establishment of a mouse model of obesity and asthma, and treatment

To establish a mouse model of obesity and asthma, obese asthma group, dexamethasone group, simvastatin group, and combination group were fed with a high-fat diet (60% kcal from fat) for 15 weeks to induce obesity, while control group and asthma group were fed with standard diet. Obesity was defined as a weight more than 20% over the ideal [22]. Mice were weighted once a week. The start of week 9 was considered as day 1. On days 1, 7, and 14, the mice were sensitized by intraperitoneal injection of 50 μg OVA (Grade V; Sigma Chemical Co., St. Louis, MO, USA) adsorbed on a 1 mg aluminum hydroxide gel as an adjuvant. One week after the final injection, mice were exposed to OVA aerosols (1% in saline) for 30 min once daily for 7 consecutive days from day 21–27 using nebulizer (Pari GmbH, Germany), and then were exposed to OVA aerosols every other day for 3 weeks. The control group was intraperitoneally injected and nebulized with saline at the corresponding time points. The dexamethasone group, simvastatin group, and combination group were given 0.5 mg/kg dexamethasone (Chenxin Pharmaceutical Co.,Ltd, Jining, Shandong, China), 40 mg/kg simvastatin (Hangzhou MSD Pharmaceutical Co., Ltd, Hangzhou, Zhejiang, China), and 0.5 mg/kg dexamethasone combined with 40 mg/kg simvastatin via drinking water, respectively. These treatments were performed daily from day 21 for 4 weeks. The control group, asthma group and obese asthma group were given untreated water.

### 2.3. Serum collection

The mice were anesthetized with 0.4 ml of 5% chloral hydrate (V/V) 24 h after the final OVA challenge. Blood samples were collected by removalling eyeball, kept at room temperature for 30 min, and then centrifuged at 2000 × g for 10 min. The serum was collected and stored at −20 °C.

### 2.4. Measurement of serum leptin and biochemical indexes

The serum leptin level was determined by Mouse Leptin ELISA kit (R&D system, Minneapolis, MN, USA) according to the instructions of the manufacturer. The levels of glucose (Glu), triacylglycerol (TG), and total cholesterol (TC) in serum were determined by biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA).

### 2.5. The preparation of the lung tissue and bronchoalveolar lavage fluid (BALF)

Immediately after blood collection, the thoracic cavity was carefully opened. After tying off the right lung at the mainstem bronchus, right lung was removed by dissection, and fixed in 10% neutral-buffered formalin for 24 h. Then BALF was collected by cannulating the trachea and lavaging left lung thrice with 3 × 0.4 ml of ice-cold PBS (over 85% of the volume inputted was recovered).

### 2.6. Lung histology

After fixation, lung tissues were embedded in paraffin, cut into 5-μm sections, and stained with H&E, periodic acid-Schiff and Masson's trichrome. Slides were examined under a light microscope (Olympus, Japan), and the images were captured and

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