



Preliminary report

High-fat-diet induces airway hyperresponsiveness partly through activating CD38 signaling pathway

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ABSTRACT

CD38 is a plasma membrane bound multifunctional enzyme. It can be activated by inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-13, inducing calcium responses to agonist in airway smooth muscle cells (ASMC). Previous studies have found that high-fat-diet (HFD) induced obesity exhibited innate airway hyperresponsiveness (AHR). This study aimed to detect the effect of CD38 signaling pathway on the AHR of overweight/obese mice. The HFD-fed mice exhibited a significantly higher baseline airway resistance (Rn), and the increasing rates of Rn responded to increasing doses of methacholine compared with the LFD-fed mice. High-fat-diet increased CD38 expressions both in lung tissues and primary cultured ASMCs. Besides, pre-incubation with TNF- α led to a higher expression of CD38 protein and increased intracellular calcium in ASMC of the HFD-fed mice. Furthermore, CD38 gene knockdown through transfection of CD38 siRNA decreased the concentration of intracellular calcium. Additionally, the upregulations of CD38 protein and CD38 mRNA were also found in the lung tissues of HFD-fed mice challenged by ovalbumin (OVA). Collectively, our findings demonstrated a role of CD38 signaling pathway on the AHR of obesity and might be a potential therapeutic target for treating difficult-to-control obese asthma phenotype.

1. Introduction

Asthma in overweight/obese is a unique asthma phenotype that is quite difficult to manage [1]. It is resistant to available corticosteroid treatments, requires higher medication use, and is associated with frequent hospitalizations compared with asthma in normal-weight patients [2–4]. A better understanding of the mechanism underlying the association between overweight/obesity and asthma can guide the development and delivery of effective treatment for difficult-to-control asthma in the overweight/obese patients.

Several studies in the last few years have demonstrated that overweight/obesity exhibits an innate airway hyperresponsiveness (AHR) [5,6], a hallmark of asthma. Nevertheless, the underlying mechanism is still not clear. AHR is caused by the heightened contraction of airway smooth muscle cells (ASMCs), resulting from increased intracellular calcium responses to spasmogens such as acetylcholine, bradykinin, and endothelin-1 (ET-1) [7–9]. CD38 is a plasma membrane bound

multifunctional enzyme induced by cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-13. The upregulation of CD38 can activate its downstream molecule, cyclic adenosine diphosphoribose (cADPR) activity and subsequently intracellular calcium responses to agonists [10]. Recently, some studies have demonstrated a role of CD38 in the regulation of airway smooth muscle (ASM) contraction and AHR induced by allergens [11,12].

The essence of obesity is a systemic inflammation with increased serum concentrations of cytokines, including TNF- α [13]. Therefore, this study proposed the hypothesis that the CD38 signaling pathway might be implicated in overweight/obesity-induced AHR due to the increased concentration of TNF- α , which might aggravate the symptoms of asthma and increase the incidence of asthma. The present study was conducted to examine the effect of CD38 signaling pathway on the AHR of overweight/obesity mice.

Abbreviations: AHR, airway hyperresponsiveness; Rn, airway resistance; BMI, body mass index; cADPR, cyclic adenosine diphosphoribose; BALF, bronchoalveolar lavage fluid; ASMC, airway smooth muscle cell; HFD, high-fat-diet; OVA, ovalbumin

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2. Materials and methods

2.1. Animal models

2.1.1. High-fat-diet induced overweight/obese mice model

Specific pathogen-free C57/B6J mice (3–4 weeks old) obtained from Shanghai Slac Laboratory Animal Center (Shanghai, China) were randomly divided into two groups. One group was fed with low-fat diet (LFD; 10% fat, Mediciene Ltd., Yangzhou, China, ID# MD12031) and the other high-fat diet (HFD; 45% fat, ID# MD12032) for 12 weeks.

2.1.2. Ovalbumin (OVA) sensitized and challenged mice model

Mice were randomly divided into four groups. Two groups were fed with low-fat-diet (LFD) and the other two high-fat-diet (HFD). After 8 weeks feeding, on days 57 and 69, one group of the LFD-fed mice and one group of the HFD-fed mice were sensitized by i.p. injection of 0.01%OVA/Al(OH)₃ suspension. Then they were challenged in an airtight box filled with 1% OVA aerosol for 30 min per day from days 81 to 88. The other groups of LFD-fed mice and HFD-fed mice were sensitized and challenged by normal saline on the same schedule.

All animal procedures were approved by the Laboratory Animal Ethics Committee of Wenzhou Medical University, China, and were consistent with the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.2. Isolation and purification of mice ASMCs

After the last challenge, the mice were sacrificed using anesthesia with chloral hydrate. The bronchial trees were isolated carefully, cut into pieces, and then incubated with collagenase I for 1 h. The cells were then seeded in cell flasks with Dulbecco's modified Eagle medium (high glucose) containing 20% fetal bovine serum and 1% penicillin–streptomycin solution. The cells were purified using differential adhesion and grown under standard conditions (37 °C, 5% CO₂) in a humidified incubator. Passages 3–6 were used for the study.

2.3. Knockdown of CD38 gene by siRNA transfection in vitro

ASMCs isolated from both HFD- and LFD-fed mice were treated with 20 ng/mL TNF- α . Cells were seeded into 6 well plate in advance. When the cells at 30–50% confluence, added 5 nm siRNA oligo-siRNA-Mate compound. The cell groups were as follows: LFD group (cells isolated from LFD-fed mice transfected with vehicle for 12 h), HFD group (cells isolated from HFD-fed mice transfected with vehicle for 12 h), HFD positive group (cells isolated from HFD-fed mice transfected with nicotinamide adenine dinucleotide phosphate (NADPH) siRNA for 12 h), HFD negative group (cells isolated from HFD-fed mice transfected with scramble siRNA for 12 h) and HFD CD38 siRNA group (cells isolated from HFD-fed mice transfected with CD38 siRNA for 12 h).

2.4. Flow cytometry

The expression of cell surface molecular CD38 was detected by flow cytometry using sheep anti-mouse CD38 antibody (1:100; R&D system, Shanghai, China) followed by donkey anti-sheep immunoglobulin H&L (Alexa Fluor® 488) (1:200; Abcam, USA).

2.5. Airway resistance detection

After the last challenge, the mice were given tracheotomy, and then mouse airway resistance (Rn) was evaluated using the flexiVent system (SCIREQ Scientific Respiratory Equipment Inc., Montreal, Canada). The flexiVent uses the indirect measurement technique to assess respiratory mechanics. It uses measurements of position and pressure, both which can be measured with great precision and consistency, to calculate mechanics outcomes.

2.6. Western blot

Lung tissues were homogenized in RIPA lysis containing protease inhibitor. Aliquots of 50 μ g of protein were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes, which were then blocked in 5% dry skim milk for 2 h. Then, the primary antibodies were incubated overnight at 4 °C with 1:1000 dilutions for CD38 (Antigen Affinity-purified Polyclonal Sheep IgG, R&D system, Shanghai, China), 1:4000 for tubulin (Bioworld Technology, Shanghai, China). The secondary antibodies were goat anti-rabbit (Abcam, USA) or a rabbit anti-goat (Abcam, USA) used at 1:5000 or 1:6000 dilution for 1 h at room temperature. Protein concentrations were measured using the ChemiDoc XRS gel imaging system (Bio-Rad, USA).

2.7. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

The total RNA was extracted using a Trizol reagent (Thermo Fisher Scientific, USA) and reverse transcribed into cDNA using the RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific, USA). 3 μ L total RNA were added into the reverse transcription mixture. Then performed for 60 min at 42 °C and 5 min at 70 °C. Polymerase chain reactions (PCRs) were performed for 3 min at 95 °C, followed by 40 cycles of 3 s at 95 °C, 30 s at 59 °C, and 1 s at 72 °C using the FAST PROBE qPCR Master Mix and mCD38 Taqman probes (Gene Pharma, Shanghai, China) for CD38 and glyceraldehyde 3-phosphate dehydrogenase (GADPH). The relative quantity of mRNAs was obtained using the 2^{− $\Delta\Delta C_t$} method. The primers for CD38 were as follows: forward, CAAACACCTGGCCCATCAAT; reverse, CAGGGTGTCTCCAGGGTG; and probe, CTTGGATCCAGGAGAA. The primers for GADPH were as follows: forward, GCACAGTCAAGGCCGAGAA; reverse, CCTCACCCCATTTGATGTTAGTG; and probe, CATCACCATCTTCCAGGAGCGAGACC. Each reaction was run in triplicate.

2.8. Immunofluorescent staining

The intracellular calcium was observed under an inverted fluorescent contrast phase microscope using the Fluo3-AM reagent (DOJINDO, Tokyo, Japan). The inverted fluorescent microscope has three filters (Green, Blue and Ultraviolet filters) and blue filter was used to observe the intracellular calcium. ASMCs were seeded into 6 well plate and then incubated with Fluo3-AM for 45 min away from light after washing. The fluorescence excitation wavelength was 488 nm, and the emission wavelength was 526 nm. The integrated density was calculated for the fluorescence intensity.

2.9. Statistical analysis

All results were expressed as means $\pm$ standard error of the mean (SEM) and analyzed by *t*-test or one-way analysis of variance using SPSS 17.0. For samples with unequal variances, Dunnett's T3 test was used. Statistical significance was determined as *P* < 0.05.

3. Results

3.1. Physiological parameters of mice

At the beginning, the mean body weight of mice from each group had no significant differences. After 12 weeks of feeding, the differences in appearance (Fig. 1a) and body weight between LFD- and HFD-fed mice could be obviously observed. The increase in weight (Fig. 1b) of the HFD-fed mice was significantly higher than that of the LFD-fed mice (*P* < 0.01). The mean liver weight (Sup. Fig. 1c), body length (Fig. 1d), and Lee's index (Fig. 1e) of the HFD-fed mice were also significantly higher than those of the LFD-fed mice.

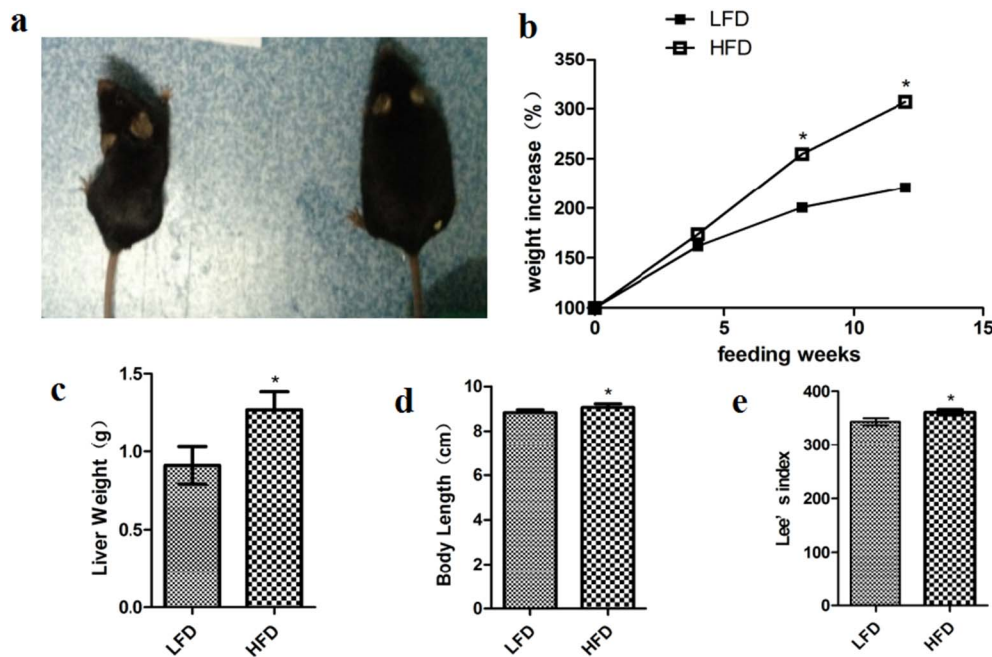


Fig. 1. The appearance (a), weight increasing rate (b), liver weight (c), body length (d), and Lee's index (e) of mice. Body weight was measured every 4 weeks. Values are means $\pm$ SEM; $n = 10$ mice/group. * $P < 0.01$ versus LFD.

3.2. Impact of HFD on airway inflammation

The degree of inflammatory cell infiltration in the airways was evaluated to examine the airway inflammation in HFD-fed mice. The mice had airway inflammation as detected using hematoxylin and eosin (HE) staining (Fig. 2a) and total and differential inflammatory cell counts were increased in bronchoalveolar lavage fluid (BALF) (Fig. 2b). However, the concentrations of Th2-specific cytokines IL-5 and IL-13 did not increase in the HFD-fed mice compared with the LFD-fed mice ($P < 0.01$), whereas the concentration of Th1-specific cytokine interferon (IFN)- γ significantly increased in the BALF of the HFD-fed mice ($P < 0.01$) (Fig. 2c), indicating that obese mice showed non-Th2 response. The concentration of IFN- γ was significantly increased in the BALF of HFD-fed mice ($P < 0.01$) (Fig. 2c).

3.3. Impact of HFD on AHR

The airway resistance (Rn), reflecting small airway function, was detected and different doses of methacholine were used to detect the responses of airway to bronchoconstrictor according to Kim et al. [6] to study the effect of HFD on the lung function of mice. As shown in Fig. 3a, the HFD-fed mice exhibited a significantly higher baseline Rn (0.5334 ± 0.011) compared with that in the LFD-fed mice (0.323 ± 0.007) ($P < 0.01$), indicating a role of HFD on small airway function. At increasing doses of methacholine (0, 10, 20, 40, 60, and 80 mg/mL), the changes in Rn increasing rates were similar to baseline Rn with respect to the responses to bronchoconstrictor (Fig. 3b).

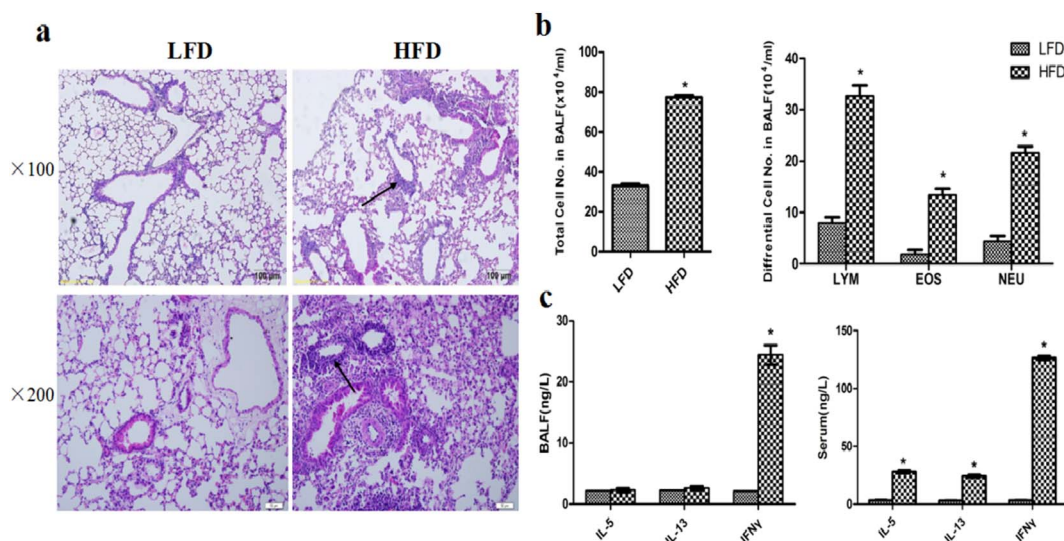


Fig. 2. HFD-fed mice showed non-Th2 airway inflammation. (a) Representative photomicrographs of HE staining in sections of lungs from mice showing some inflammatory cell infiltration around bronchus and many inflammatory cell infiltration around blood vessels. (b) The total cell numbers and differential cell numbers in BALF. (c) The concentrations of IL-5, IL-13, and IFN- γ in BALF and serum. Values are means $\pm$ SE; $n = 10$ mice/group. * $P < 0.01$ versus LFD. Calibration bars = 50 or 100 μ m.

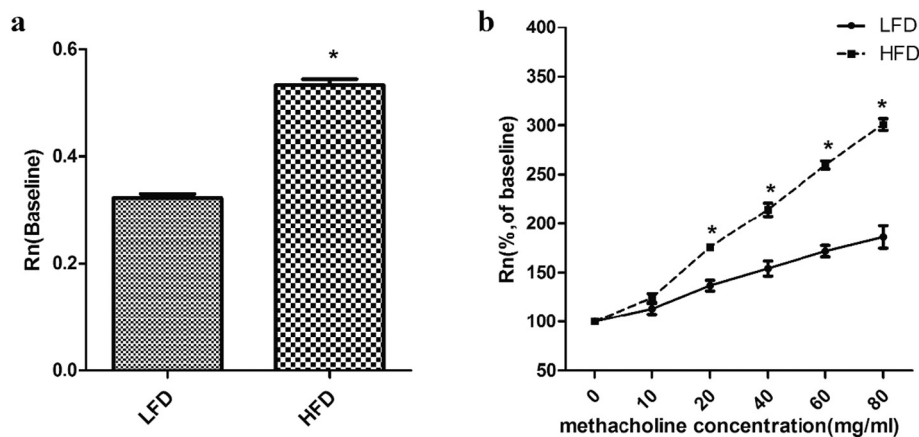


Fig. 3. High-fat-diet induced airway hyperresponsiveness (AHR). (a) The resistance of airway (Rn) without challenge. (b) The changes in airway resistance (Rn) with the increasing doses of the challenge of methacholine. Values are means $\pm$ SE; $n = 7$ mice/group. * $P < 0.01$ versus LFD.

3.4. Upregulation of CD38 signaling pathway in the lungs of HFD-fed mice

The concentrations of TNF- α (Fig. 4b) and cADPR (Fig. 4a) significantly increased in the BALF and serum of HFD-fed mice ($P < 0.01$). Similarly, the expression of CD38 protein (Fig. 4c) and CD38 mRNA (Fig. 4d) was also significantly higher in the HFD-fed mice compared with the LFD-fed mice ($P < 0.01$).

3.5. CD38 signaling pathway induced ASMC constriction in HFD-fed mice

Collagenase I digestion, rather than the tissue-sticking method, was used to isolate ASMCs from mice. The preliminary cultured cells grew well after 6 days of culture, and the purity of the fourth passage was $> 90\%$ (Sup. Fig. 1a and b). Immunofluorescent staining with specific mAb against mouse α -smooth muscle actin (α -SMA) demonstrated a positive cell count of $> 90\%$ (Sup. Fig. 1c). The third- to sixth-passage cells were used for experiments. The expression of CD38 protein in ASMC from LFD- and HFD-fed mice increased significantly with the pretreatment with recombinant mouse TNF- α compared with those without TNF- α pretreatment, indicating a role of CD38 signaling pathway in ASMC function (Sup. Fig. 1d). However, the rate of increase

was obviously higher in the HFD-fed mice compared with the LFD-fed mice, as shown by the positivity for flow cytometry in 10.9%–27.7% of the LFD-fed mice compared with 51.2%–96.2% of the HFD-fed mice (Sup. Fig. 1d).

The results showed that the expression of CD38 protein successfully decreased after transfecting CD38 siRNA, from 98.4% to 30.9% (Fig. 5a and b), in accordance with the expression of CD38 mRNA (Fig. 5c). The concentration of intracellular calcium, which was significantly higher in HFD-fed mice compared with LFD-fed mice, decreased as expected with the transfection of CD38 siRNA (Fig. 5d), demonstrating a regulation of CD38 signaling pathway on the ASMC constriction in HFD-fed mice.

3.6. High expressions of CD38 protein and CD38 mRNA in the lung tissues of HFD-OVA mice

Additionally, to examine the effect of CD38 in the overweight/obese asthma, ovalbumin (OVA) was used to sensitize and challenge mice (Sup. Fig. 2). The mice challenged by OVA showed higher airway inflammation (Fig. 6). Besides, the HFD-fed mice challenged by OVA exhibited the highest airway resistance (Fig. 7). Further, the expressions

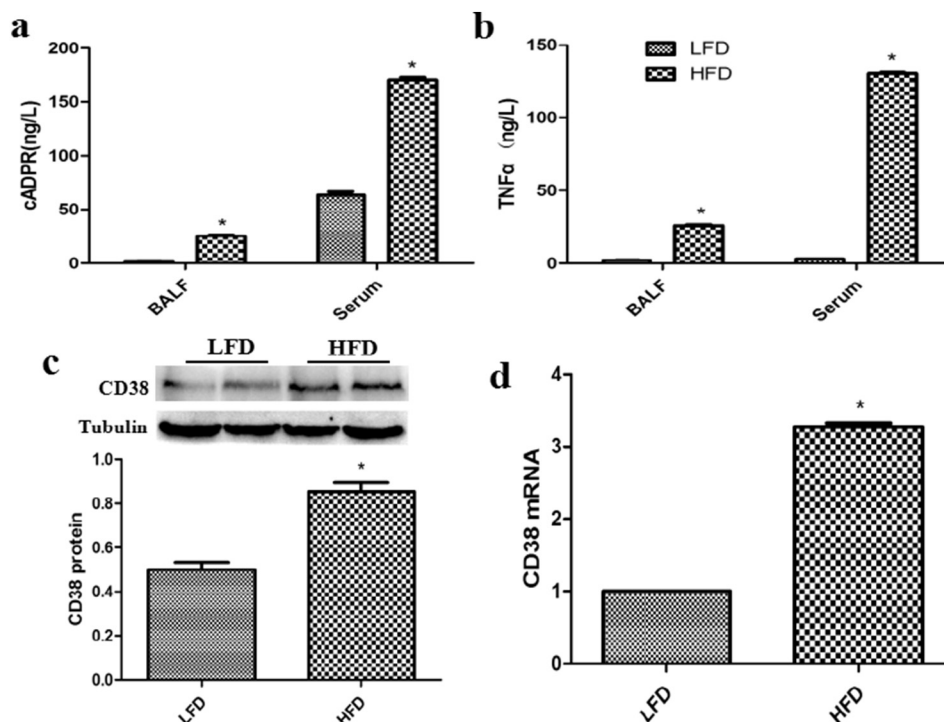


Fig. 4. Expressions of TNF- α , CD38 and cADPR were upregulated in the lungs of HFD-fed mice. (a, b) The concentrations of cADPR protein and TNF- α protein in BALF and serum. The expressions of CD38 protein (c) and CD38 mRNA (d) in lung tissues. Values are means $\pm$ SE; $n = 6$ mice/group. * $P < 0.01$ versus LFD.

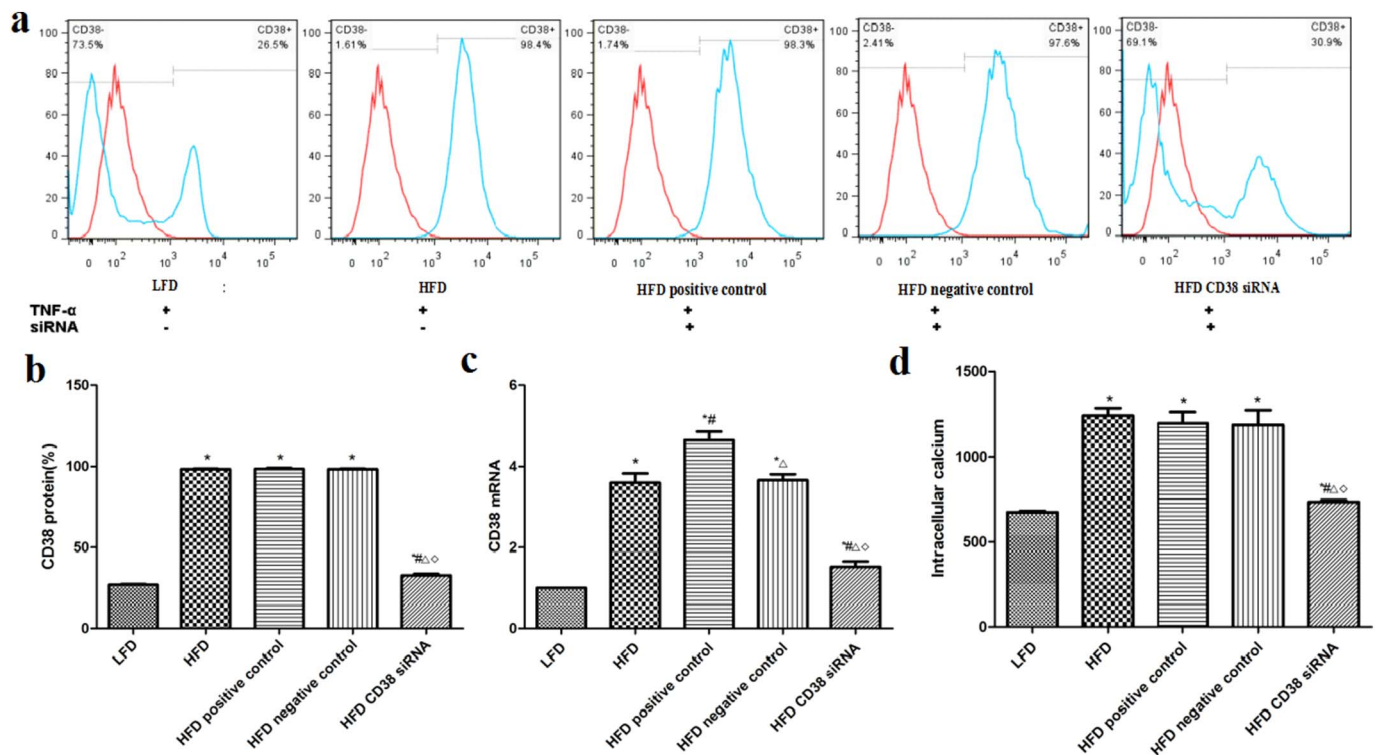


Fig. 5. The CD38 signaling pathway regulated ASMC constriction to methacholine in HFD-fed mice. siRNA transfection reduced TNF- α -mediated expression of CD38 protein (a, b) and mRNA (c), accompanied by reductions of intracellular calcium in ASMCs (d). Values are expressed as mean $\pm$ SE; $n = 3$ independent experiments. * $P < 0.01$ versus LFD, # $P < 0.01$ versus HFD, $\Delta P < 0.01$ versus HFD-positive control group, $\Diamond P < 0.01$ versus HFD-negative control group.

of CD38 protein and CD38 mRNA were detected. As expected, high-fat-diet increased both protein (Fig. 8a and b) and mRNA (Fig. 8c) expressions of CD38 in the lung tissues of mice after OVA challenge.

4. Discussion

Mounting epidemiological data have demonstrated an association between high body weight (at least overweight status) and asthma in children and adults [14,15]. Even small changes in mean population body mass index (BMI) may translate into significant increases or

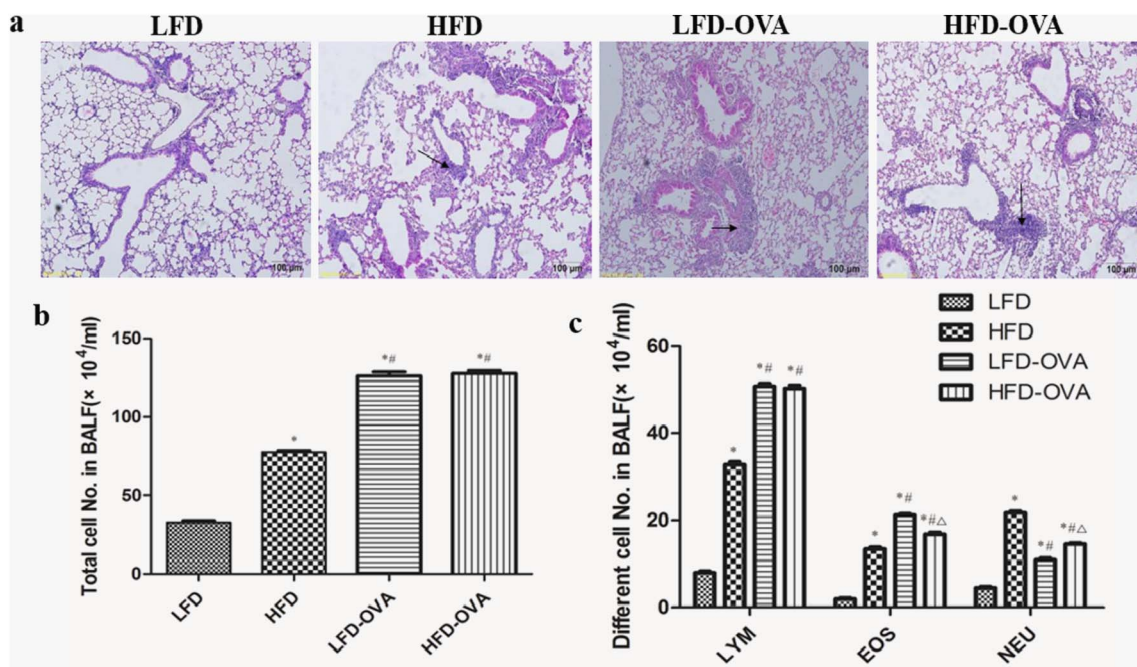


Fig. 6. The airway inflammation was augmented in HFD-fed mice challenged by OVA. (a) Representative photomicrographs of HE staining in sections of lungs from mice ($\times 100$). The total cell numbers (b) and differential cell numbers (c) in BALF. Values are means $\pm$ SE; $n = 10$ mice/group. * $P < 0.01$ versus LFD, # $P < 0.01$ versus HFD, $\Delta P < 0.01$ versus LFD-OVA. Calibration bars = 50 or 100 μ m.

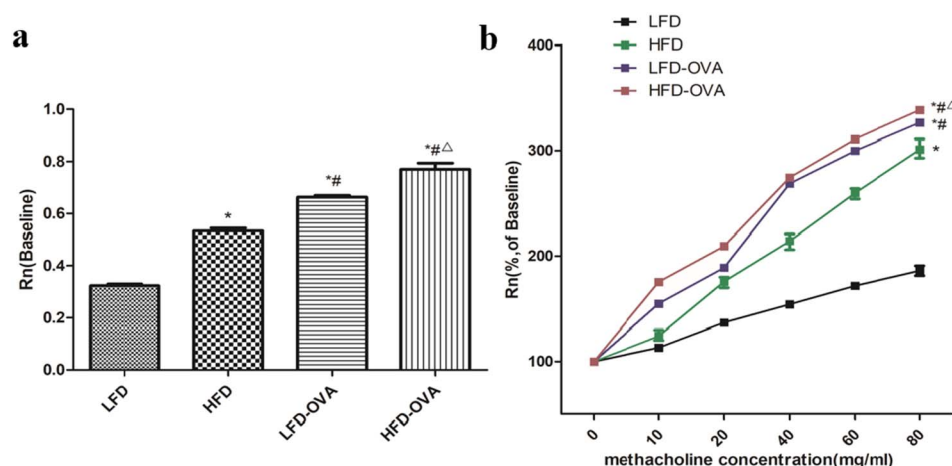


Fig. 7. HFD-fed mice challenged by OVA (HFD-OVA) showed the highest AHR. (a) The resistance of airway (Rn) without challenge. (b) The changes in airway resistance (Rn) with the increasing doses of the challenge of methacholine. Values are means $\pm$ SE; $n = 7$ mice/group. * $P < 0.01$ versus LFD, # $P < 0.01$ versus HFD, $\Delta P < 0.01$ versus LFD-OVA.

decreases in asthma incidence in all ages [16,17]. As a unique asthma phenotype, asthma in the overweight/obese is more difficult to control [18–20] and is associated with worse quality of life and more health care resources compared with nonobese patients with asthma [21].

A proposed hypothesis is that a high body weight, as a state of low-grade inflammation, exacerbates airway inflammation, contributing to the development of asthma [2]. A typical Western diet may act as an inflammatory stimulus [22]. If inflammation is a mechanism linking high body weight and asthma, it is reasonable that diet plays some role. In this study, a 45% HFD or a 10% LFD was used. Compared with the LFD-fed mice, the body weight of HFD-fed mice increased gradually, and the rate of increase was significantly higher compared with the LFD-fed mice at the end of 12 weeks of feeding.

Given the low-grade inflammatory state of a high body weight, the infiltration of airway inflammatory cells was examined. The results of the present study showed that unlike asthma, overweight/obese mice presented increased infiltration of airway neutrophils and exhibited a non-Th2 airway inflammation, consistent with the results of previous studies [23,24]. This indicated that the airway inflammation type of overweight/obesity and asthma was different and might not be the underlying mechanism of asthma in overweight/obese mice.

Several recent studies found that overweight or obesity itself exhibited AHR [25,26] and demonstrated an association between BMI and the onset of AHR [27–29], a common feature of asthma, although the mechanisms underlying this association were not clearly understood. Consistent with the aforementioned results, the airway resistance (Rn) in the HFD-fed mice in this study was significantly higher compared

with the LFD-fed mice and increased more quickly with the increase in doses of methacholine, confirming the role of high body weight on the airway responsiveness.

Speculating that overweight/obesity might influence ASM function and airway responsiveness is logical because ASM is the key end-effector of acute airway narrowing. ASM contraction requires elevation of intracellular calcium [8], and some studies have demonstrated that CD38/cADPR-mediated calcium release is vital in the regulation of calcium homeostasis in ASMCs [30,31].

CD38 is an ectoenzyme expressed on SMCs and immune cells, which catalyzes the conversion of β -nicotinamide adenine dinucleotide (β -NAD) into cADPR, a second messenger molecule capable of releasing calcium from the sarcoplasmic reticulum via activation of the ryanodine receptor (RyR) channels [32]. The calcium response elicited by the addition of acetylcholine or endothelin-1 (ET-1) in airway myocytes obtained from CD38-knockout mice was significantly attenuated compared with the responses elicited in myocytes obtained from wild-type mice. Furthermore, preincubation of myocytes from wild-type mice with ryanodine, which inhibited calcium release from the sarcoplasmic reticulum through ryanodine receptor channels, caused significant attenuation of calcium responses to acetylcholine, while having no significant effect on the calcium response in myocytes obtained from CD38-knockout mice [11].

The expression of CD38 may be modulated by multiple factors including cytokines such as TNF- α [33,34] and IL-13 [35]. Deshpande et al. [36] and Tirumurugan et al. [37] found an increased expression of CD38 and NAD $^{+}$ -catalyzed cADPR generation by exposing human

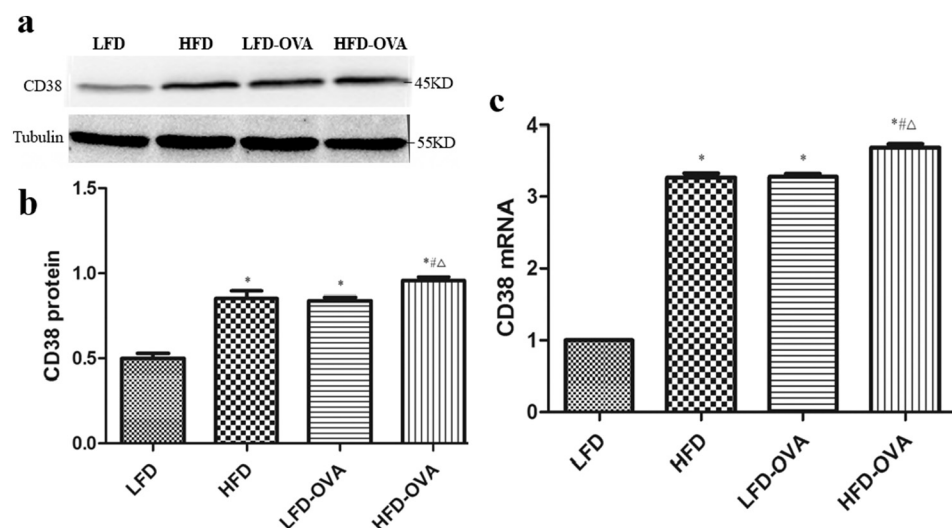


Fig. 8. Expressions of CD38 protein and CD38 mRNA were increased in HFD-fed mice challenged by OVA (HFD-OVA). (a, b) The expression of CD38 protein in the lungs of mice. (c) The expression of CD38 mRNA in the lungs of mice. Values are means $\pm$ SE; $n = 7$ mice/group. * $P < 0.01$ versus LFD, # $P < 0.01$ versus HFD, $\Delta P < 0.01$ versus LFD-OVA.

airway SMCs to inflammatory cytokines such as IL-13 or TNF- α . The intracellular calcium responses to agonists, such as acetylcholine, bradykinin, and ET-1, were significantly augmented in cells treated with TNF- α or IL-13 compared with responses in vehicle-treated cells. Moreover, preincubation of cells with 8-bromo-cADPR, a cell-permeant competitive cADPR antagonist, resulted in a significant reduction in the intracellular calcium responses to agonists, and the responses seen in the cells were comparable to those seen in cells treated with the vehicle. Knockdown of expression of CD38 by siRNA or following transfection with antisense oligonucleotides also caused a reduction in the intracellular responses to agonists in cytokine-treated cells [38]. All the aforementioned results clearly showed that the augmented intracellular calcium responses to agonists following TNF- α or IL-13 exposure could be attributed to the increased expression of CD38 and cADPR-mediated calcium release. Moreover, the expression of CD38, together with cADPR-mediated calcium responses to agonists, was expected to be greater in an inflammatory milieu such as in the lungs of asthmatics.

A systematic study delineating the expression of CD38 in ASM obtained from asthmatics and nonasthmatics is lacking. However, a study comparing the induction of expression of CD38 by TNF- α in ASM cells obtained from asthmatics and nonasthmatics revealed that exposure to TNF- α resulted in a concentration-dependent induction of the expression of CD38 and cADPR activity in ASM cells from both [39]. Nevertheless, the magnitude of CD38 induction was significantly greater in cells from asthmatics than in those from nonasthmatics, suggesting a greater capacity for cADPR-mediated calcium response to agonists in asthmatics [40].

As a systemic inflammatory cytokine, TNF- α was also greatly up-regulated in the overweight/obese patients [41], and the increased concentrations of TNF- α in BALF and serum of HFD-fed mice were observed in the present study. The expression of CD38/cADPR also increased in the lung tissues. Furthermore, the expression of CD38 and the intracellular calcium response to agonist were found to be significantly higher in HFD-fed mice after exposure to TNF- α via isolation and culture of ASMCs from HFD- and LFD-fed mice, indicating a role of CD38 signaling pathway in AHR of overweight/obese mice. Moreover, the knockdown of CD38 gene expression on ASMCs obtained from HFD-fed mice by siRNA transfection led to a reduction of intracellular calcium response to agonist, suggesting that CD38 signaling pathway-induced AHR might be a potential pathogenesis process linking overweight/obesity to asthma.

In this regard, the expressions of CD38 protein and CD38 mRNA in the lung tissues of HFD-fed mice challenged by OVA were also detected. After the last challenge of OVA, the airway resistance of HFD-fed mice was significantly increased compared with saline-challenged HFD-fed mice. Furthermore, OVA sensitization and challenge significantly increased the CD38 expressions in the lungs of HFD-fed mice, indicating a role of CD38 on the AHR of the overweight/obese asthma.

However, there are also some shortcomings in this research. For example, a CD38 gene knockdown animal model was not used. To explore the pathogenesis of a disease, the *in vivo* experiments are more important than *in vitro* experiments. Besides, TNF- α is a nonspecific cytokine upregulated in obesity. Instead, Kim and colleagues [5] found an indispensable role for IL-17 in the high-fat-diet induced AHR. Therefore, future studies are needed to explore the associations between IL-17 and CD38 on the development of high-fat-diet induced AHR. Also, a focus on this potential therapeutic target in overweight/obese mice with asthma is needed.

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Conflict of interest

None.

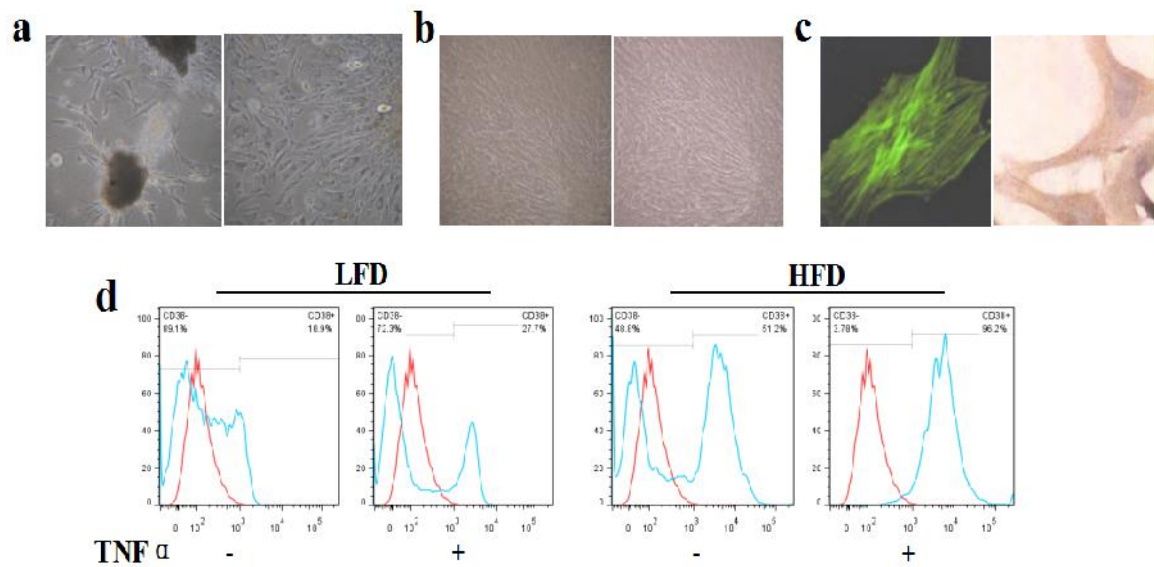
Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2018.01.033>.

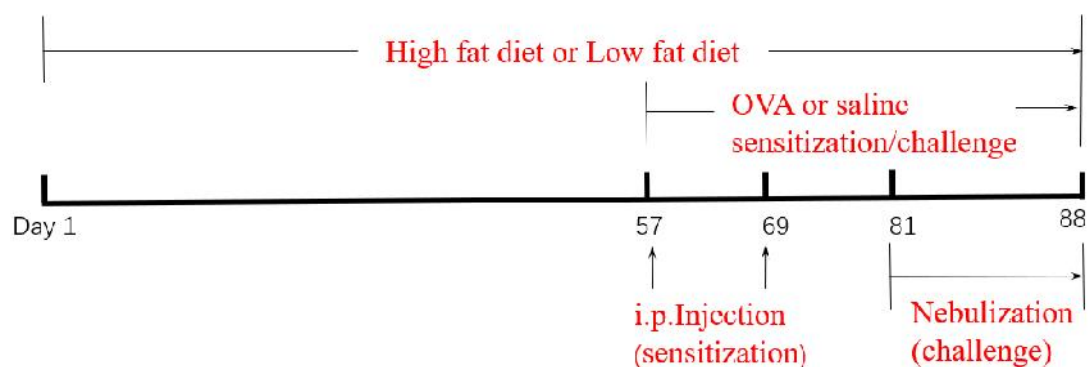
References

- [1] N.G. Papadopoulos, H. Arakawa, K.H. Carlsen, A. Custovic, J. Gern, R. Lemanske, et al., International consensus on (ICON) pediatric asthma, *Allergy* 67 (8) (2012) 976–997, <http://dx.doi.org/10.1111/j.1398-9995.2012.02865.x>.
- [2] D.A. Beuther, Recent insight into obesity and asthma, *Curr. Opin. Pulm. Med.* 16 (1) (2010) 64–70, <http://dx.doi.org/10.1097/MCP.0b013e3283338fa7>.
- [3] C.L. Carroll, P. Stoltz, N. Raykov, S.R. Smith, A.R. Zucker, Childhood overweight increases hospital admission rates for asthma, *Pediatrics* 120 (4) (2007) 734–740, <http://dx.doi.org/10.1542/peds.2007-0409>.
- [4] K.B. Quinto, B.L. Zuraw, K.Y. Poon, W. Chen, M. Schatz, S.C. Christiansen, The association of obesity and asthma severity and control in children, *J. Allergy Clin. Immunol.* 128 (5) (2011) 964–969, <http://dx.doi.org/10.1016/j.jaci.2011.06.031>.
- [5] H.Y. Kim, H.J. Lee, Y. Chang, M. Pichavant, S.A. Shore, K.A. Fitzgerald, et al., Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity, *Nat. Med.* 20 (1) (2013) 54–61, <http://dx.doi.org/10.1038/nm.3423>.
- [6] S.A. Shore, J.J. Fredberg, Obesity, smooth muscle, and airway hyperresponsiveness, *J. Allergy Clin. Immunol.* 115 (5) (2005) 925–927, <http://dx.doi.org/10.1016/j.jaci.2005.01.064>.
- [7] Y. Bai, M.J. Sanderson, The contribution of Ca²⁺ signaling and Ca²⁺ sensitivity to the regulation of airway smooth muscle contraction is different in rats and mice, *Am. J. Phys. Lung Cell. Mol. Phys.* 296 (6) (2009) L947–58, <http://dx.doi.org/10.1152/ajplung.90288.2008>.
- [8] M.J. Sanderson, P. Delmotte, Y. Bai, J.F. Perez-Zoghbi, Regulation of airway smooth muscle cell contractility by Ca²⁺ signaling and sensitivity, *Proc. Am. Thorac. Soc.* 5 (1) (2008) 23–31, <http://dx.doi.org/10.1513/pats.200704-050VS>.
- [9] E. Arteaga-Solis, T. Zee, C.W. Emala, C. Vinson, J. Wess, G. Karsenty, Inhibition of leptin regulation of parasympathetic signaling as a cause of extreme body weight-associated asthma, *Cell Metab.* 17 (1) (2013) 35–48, <http://dx.doi.org/10.1016/j.cmet.2012.12.004>.
- [10] F. Malavasi, S. Deaglio, A. Funaro, E. Ferrero, A.L. Horenstein, E. Ortolan, et al., Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology, *Physiol. Rev.* 88 (3) (2008) 841–886, <http://dx.doi.org/10.1152/physrev.00035.2007>.
- [11] D.A. Deshpande, CD38/cyclic ADP-ribose signaling: role in the regulation of calcium homeostasis in airway smooth muscle, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 288 (5) (2005) L773–88, <http://dx.doi.org/10.1152/ajplung.00217.2004>.
- [12] D.A. Deshpande, A.G. Guedes, F.E. Lund, S. Subramanian, T.F. Walseth, M.S. Kannan, CD38 in the pathogenesis of allergic airway disease: potential therapeutic targets, *Pharmacol. Ther.* 172 (2017) 116–126, <http://dx.doi.org/10.1016/j.pharmthera.2016.12.002>.
- [13] P. Mancuso, Obesity and lung inflammation, *J. Appl. Physiol.* (1985) 108 (3) (2010) 722–728, <http://dx.doi.org/10.1152/jappphysiol.00781.2009>.
- [14] M. Ng, T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, et al., Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013, *Lancet* 384 (9945) (2014) 766–781, [http://dx.doi.org/10.1016/S0140-6736\(14\)60460-8](http://dx.doi.org/10.1016/S0140-6736(14)60460-8).
- [15] J. Patra, Y.I. Maher, S. Mishra, M. Bhatia, D. Alam, D.S. Malini, et al., Effects of body mass index, tobacco smoking, alcohol drinking and solid fuel use on the risk of asthma: Individual Participant Data (IPD) meta-analysis of 175 000 individuals from 51 nationally representative surveys, *BMJ Open Respir. Res.* 3 (1) (2016) e121, <http://dx.doi.org/10.1136/bmjresp.2015-000121>.
- [16] J.L. Ather, M. Chung, L.R. Hoyt, M.J. Randall, A. Georgsdottir, N.A. Daphtary, et al., Weight loss decreases inherent and allergic methacholine hyperresponsiveness in mouse models of diet-induced obese asthma, *Am. J. Respir. Cell Mol. Biol.* (2016), <http://dx.doi.org/10.1165/rcmb.2016-0070OC>.
- [17] D.A. Beuther, E.R. Sutherland, Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies, *Am. J. Respir. Crit. Care Med.* 175 (7) (2007) 661–666, <http://dx.doi.org/10.1164/rccm.200611-1717OC>.
- [18] M. Boudreau, S.L. Bacon, K. Ouellet, A. Jacob, K.L. Lavoie, Mediator effect of depressive symptoms on the association between BMI and asthma control in adults,

- Chest 146 (2) (2014) 348–354, <http://dx.doi.org/10.1378/chest.13-1796>.
- [19] D.M. Mosen, M. Schatz, D.J. Magid, C.J. Camargo, The relationship between obesity and asthma severity and control in adults, *J. Allergy Clin. Immunol.* 122 (3) (2008) 507–511, <http://dx.doi.org/10.1016/j.jaci.2008.06.024>.
 - [20] B. Taylor, D. Mannino, C. Brown, D. Crocker, N. Twum-Baah, F. Holguin, Body mass index and asthma severity in the National Asthma Survey, *Thorax* 63 (1) (2008) 14–20, <http://dx.doi.org/10.1136/thx.2007.082784>.
 - [21] C.S. Farah, C.M. Salome, Asthma and obesity: a known association but unknown mechanism, *Respirology* 17 (3) (2012) 412–421, <http://dx.doi.org/10.1111/j.1440-1843.2011.02080.x>.
 - [22] A. de Vries, L. Hazlewood, P.M. Fitch, J.R. Seckl, P. Foster, S.E. Howie, High-fat feeding redirects cytokine responses and decreases allergic airway eosinophilia, *Clin. Exp. Allergy* 39 (5) (2009) 731–739, <http://dx.doi.org/10.1111/j.1365-2222.2008.03179.x>.
 - [23] L.O. Leiria, M.A. Martins, M.J. Saad, Obesity and asthma: beyond T(H)2 inflammation, *Metabolism* 64 (2) (2015) 172–181, <http://dx.doi.org/10.1016/j.metabol.2014.10.002>.
 - [24] H.A. Scott, P.G. Gibson, M.L. Garg, L.G. Wood, Airway inflammation is augmented by obesity and fatty acids in asthma, *Eur. Respir. J.* 38 (3) (2011) 594–602, <http://dx.doi.org/10.1183/09031936.00139810>.
 - [25] K. Dinger, P. Kasper, E. Hucklenbruch-Rother, C. Vohlen, E. Jobst, R. Janoschek, et al., Early-onset obesity dysregulates pulmonary adipocytokine/insulin signaling and induces asthma-like disease in mice, *Sci. Rep.* 6 (2016) 24168, <http://dx.doi.org/10.1038/srep24168>.
 - [26] L. Everaere, S. Ait-Yahia, O. Molendi-Coste, H. Vorng, S. Quemener, P. LeVu, et al., Innate lymphoid cells contribute to allergic airway disease exacerbation by obesity, *J. Allergy Clin. Immunol.* 138 (5) (2016) 1309–1318, <http://dx.doi.org/10.1016/j.jaci.2016.03.019>.
 - [27] A.A. Litonjua, D. Sparrow, J.C. Celedon, D. DeMolles, S.T. Weiss, Association of body mass index with the development of methacholine airway hyperresponsiveness in men: the Normative Aging Study, *Thorax* 57 (7) (2002) 581–585.
 - [28] S. Chinn, D. Jarvis, P. Burney, Relation of bronchial responsiveness to body mass index in the ECRHS, *Thorax* 57 (12) (2002) 1028–1033.
 - [29] S.H. Kim, E.R. Sutherland, E.W. Gelfand, Is there a link between obesity and asthma? *Allergy, Asthma Immunol. Res.* 6 (3) (2014) 189–195, <http://dx.doi.org/10.4168/aa.2014.6.3.189>.
 - [30] A.G.P. Guedes, D.A. Deshpande, M. Dileepan, T.F. Walseth, R.A. Panettieri, S. Subramanian, et al., CD38 and airway hyper-responsiveness: studies on human airway smooth muscle cells and mouse models, *Can. J. Physiol. Pharmacol.* 93 (2) (2015) 145–153, <http://dx.doi.org/10.1139/cjpp-2014-0410>.
 - [31] A.G.P. Guedes, J.A. Jude, J. Paulin, L. Rivero-Nava, H. Kita, F.E. Lund, et al., Airway responsiveness in CD38-deficient mice in allergic airway disease: studies with bone marrow chimeras, *Am. J. Phys. Lung Cell.* 308 (5) (2015) L485–93, <http://dx.doi.org/10.1152/ajplung.00227.2014>.
 - [32] T.A. White, M.S. Kannan, T.F. Walseth, Intracellular calcium signaling through the cADPR pathway is agonist specific in porcine airway smooth muscle, *FASEB J.* 17 (3) (2003) 482–484, <http://dx.doi.org/10.1096/fj.02-0622fj>.
 - [33] J.A. Jude, M. Dileepan, S. Subramanian, J. Solway, R.A. Panettieri, T.F. Walseth, et al., miR-140-3p regulation of TNF α -induced CD38 expression in human airway smooth muscle cells, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 303 (5) (2012) L460–8, <http://dx.doi.org/10.1152/ajplung.00041.2012>.
 - [34] J.A. Jude, K.G. Tirumurugaan, B.N. Kang, R.A. Panettieri, T.F. Walseth, M.S. Kannan, Regulation of CD38 expression in human airway smooth muscle cells, *Am. J. Respir. Cell Mol. Biol.* 47 (4) (2012) 427–435, <http://dx.doi.org/10.1165/rcmb.2012-0025OC>.
 - [35] A.G.P. Guedes, J. Paulin, L. Rivero-Nava, H. Kita, F.E. Lund, M.S. Kannan, CD38-deficient mice have reduced airway hyperresponsiveness following IL-13 challenge, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 291 (6) (2006) L1286–93, <http://dx.doi.org/10.1152/ajplung.00187.2006>.
 - [36] D.A. Deshpande, S. Dogan, T.F. Walseth, S.M. Miller, Y. Amrani, R.A. Panettieri, et al., Modulation of calcium signaling by interleukin-13 in human airway smooth muscle, *Am. J. Respir. Cell Mol. Biol.* 31 (1) (2004) 36–42, <http://dx.doi.org/10.1165/rcmb.2003-0313OC>.
 - [37] K.G. Tirumurugaan, J.A. Jude, B.N. Kang, R.A. Panettieri, T.F. Walseth, M.S. Kannan, TNF- α induced CD38 expression in human airway smooth muscle cells: role of MAP kinases and transcription factors NF- κ B and AP-1, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 292 (6) (2007) L1385–95, <http://dx.doi.org/10.1152/ajplung.00472.2006>.
 - [38] B.N. Kang, D.A. Deshpande, K.G. Tirumurugaan, R.A. Panettieri, T.F. Walseth, M.S. Kannan, Adenoviral mediated anti-sense CD38 attenuates TNF- α -induced changes in calcium homeostasis of human airway smooth muscle cells, *Can. J. Physiol. Pharmacol.* 83 (8–9) (2005) 799–804, <http://dx.doi.org/10.1139/y05-081>.
 - [39] J.A. Jude, J. Solway, R.A. Panettieri, T.F. Walseth, M.S. Kannan, Differential induction of CD38 expression by TNF- α in asthmatic airway smooth muscle cells, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 299 (6) (2010) L879–90, <http://dx.doi.org/10.1152/ajplung.00021.2010>.
 - [40] J.A. Jude, M. Dileepan, R.A. Panettieri, T.F. Walseth, M.S. Kannan, Altered CD38/cyclic ADP-ribose signaling contributes to the asthmatic phenotype, *J. Allergy* 2012 (2012) 1–8, <http://dx.doi.org/10.1155/2012/289468>.
 - [41] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.J. Ferrante, Obesity is associated with macrophage accumulation in adipose tissue, *J. Clin. Invest.* 112 (12) (2003) 1796–1808, <http://dx.doi.org/10.1172/JCI19246>.



Sup. Fig. 1 TNF- α treatment induced higher expression of CD38 protein in ASMCs obtained from HFD-fed mice. ASMCs were successfully isolated and cultured from mice. (a) Cell primary culture at days 3 and 6. (b) The fourth passage of cells. (c) The identification of cells using anti- α -SMA antibody. (d) The expression of CD38 protein in ASMCs obtained from LFD- or HFD-fed mice with or without TNF- α treatment via flow cytometry. Values are expressed as means $\pm$ SE; $n = 3$ independent experiments.



Sup. Fig. 2 The protocol for establishing OVA sensitized and challenged mice model. Mice were randomly divided into four groups. Two groups were fed with low-fat-diet(LFD) Obesity and the other two high-fat-diet(HFD). After 8 weeks feeding, on days 57 and 69, one group of the LFD-fed mice and one group of the HFD-fed mice were sensitized by i.p. injection of 0.01%OVA/Al(OH)₃ suspension.

Then they were challenged in an airtight box filled with 1% OVA aerosol for 30 min per day from days 81 to 88. The other groups of LFD-fed mice and HFD-fed mice were sensitized and challenged by normal saline on the same schedule.