Inhibition of resistin signaling can ameliorate obesity-induced asthma in a murine model

Han, Heejae

Department of
Medical Device Engineering and Management
Graduate School
Yonsei University

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Advisor Park, Hye Jung

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Han, Heejae

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This certifies that the Dissertation of Han, Heejae is Approved.

Committee Chair	**Park, Hye Jung**
Committee Member	**Park, Kyung Hee**
Committee Member	**Kim, Youlim**
Committee Member	**Kim, Sung Ryeol**
Committee Member	**Lee, Sang Chul**

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ABSTRACT

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The global coexistence of obesity and asthma has been steadily rising, emerging as a significant public health concern. This study aimed to elucidate the molecular mechanisms underlying obesity-induced asthma, with a particular focus on the role of resistin and its interaction with the NF-κB that is a central regulator of immune and inflammatory responses signaling pathway.

C57BL/6 mice were assigned to either an ovalbumin (OVA)-induced asthma model or an obesity model group. In the OVA model, mice received intraperitoneal injections of OVA twice at 2-week intervals, followed by intranasal administration for 3 consecutive days. Additional interventions included resistin overexpression (resistin recombinant protein; 4 times over 2 weeks), resistin silencing (resistin antibody; 3 times per week), resistin inhibition (CDN1163; 12 times over 3 weeks), and NF-κB inhibitor (MG132; 3 times per week). For in vitro experiments, Beas-2B bronchial epithelial cells were treated with lipopolysaccharide (LPS), insulin, and siRNAs targeting resistin, Retnlα, and Retnlβ.

The results demonstrated that resistin aggravates airway inflammation and tissue remodeling, including goblet cell hyperplasia, fibrosis, increased levels of inflammatory cytokines, and elevated mRNA expression, via activation of the NF-κB pathway. Notably, inhibition of NF-κB significantly alleviated airway inflammation even in the presence of high resistin expression, while NF-κB inhibition did not significantly alter resistin levels. These findings suggest that resistin may act as an upstream regulator of NF-κB.

These results demonstrate that the resistin–NF-κB signaling pathway plays a key role in the inflammatory mechanisms of obesity-induced asthma and suggest that this pathway may serve as a potential therapeutic target. Further research should focus on delineating the molecular basis of this regulatory axis and evaluating the feasibility and efficacy of dual-targeted strategies that concurrently inhibit both resistin and NF-κB.

Key words : **Obesity-induced asthma, Resistin, NF-κB, Airway hyperresponsiveness, Adipokine**

1. Introduction

The global prevalence of obesity and asthma has risen sharply in recent decades, making them major public health concerns (Pawanker, 2014; To et al., 2012). Asthma is a chronic inflammatory airway disease characterized by airway hyperresponsiveness and inflammation, which leads to symptoms such as dyspnea, coughing, and chest tightness (Mims, 2015). Chronic asthma significantly impairs patients' quality of life and imposes substantial economic and social burdens. Obesity, beyond mere weight gain, induces various metabolic abnormalities and systemic inflammatory responses that can affect immune function and airway inflammation (Fasshauer & Bluher, 2015).

Obesity is a well-established risk factor for asthma, contributing to increased disease prevalence, severity, and reduced responsiveness to standard treatments (Gruchala-Niedoszytko et al., 2013, Carpaij & van den Berge, 2018). Chronic low-grade systemic inflammation and adipokine secretion from adipose tissue are thought to underlie this association. Adipokines such as resistin, leptin, and adiponectin can modulate airway inflammation by activating key signaling pathways (Fantuzzi, 2005), including NF-κB. In addition to inflammatory effects (Wellen, 2005), obesity also imposes mechanical constraints on lung function, further exacerbating asthma symptoms (Aghasafari et al., 2019; Skrgat et al., 2024). Despite the growing recognition of obesity-induced asthma as a distinct clinical phenotype, effective targeted therapies remain limited, highlighting the need to elucidate its underlying molecular mechanisms.

Resistin is an adipokine secreted by adipocytes and immune cells, known for its pro-inflammatory role in obesity-related diseases. Elevated resistin levels are associated with increased airway inflammation (Sood & Shore, 2013), poor asthma control, and reduced responsiveness to conventional therapies (Fang et al., 2015). NF-κB, a key transcription factor in immune regulation, induces cytokines such as IL-4, IL-5, and IL-13 and promotes immune cell infiltration into the airways, contributing to asthma pathogenesis (Li et al., 2024). Recent studies suggest that resistin activates NF-κB signaling, amplifying inflammatory responses in obese individuals. However, the molecular mechanisms linking resistin and NF-κB in obesity-induced asthma remain unclear.

This study aimed to investigate the role of resistin in the context of obesity-associated asthma and to examine its relationship with NF-κB signaling. By elucidating their functional interplay, we sought to evaluate their potential as therapeutic targets for the treatment of obesity-induced asthma.

2. Method

2-1. Animal model design

All experimental procedures in these mouse model studies were approved by the Institutional Animal Care and Use Committee, Animal Research Ethics Board of Yonsei University (Seoul, Korea) (IACUC approval number, 2022-0119) and were performed in accordance with the Committee's guidelines and regulations for animal care (Han et al., 2023).

In the obesity model group, the mice were fed a high-fat diet (HFD, Research Diets, Inc., NJ, USA ; fat accounting for 60 % of the calories) for 13 weeks. Lean mice were fed a normal chow diet (NCD, Research Diets, Inc.; fat accounting for 10 % of the calories). C57BL/6 male mice were used, and ≤ 5 animals were raised in one cage. The animal room was maintained at 22 ◦C and a humidity of 50 % ± 10 %, and the light-dark cycle was advanced every 12 h.

To establish a classic asthma model and an obesity-induced asthma model, C57BL/6 mice were sensitized with ovalbumin (OVA). In the OVA model, a mixture of OVA (100 μg per mouse; Sigma-Aldrich, St. Louis, MO, USA) and Inject Alum (1.6 mg per mouse; Invivogen, CA, USA) were intraperitoneally injected twice at a 2 week interval. After 1 week, OVA (20 μg per mouse) was administered intranasally for 3 consecutive days. The resistin inhibitor CDN1163 (CDN; 50mg/kg; Sigma-Aldrich, St. Louis, MO, USA was administered intraperitoneally 12 times over the course of 3 weeks. To silencing resistin antibody (100 μg/kg; R&D Systems, MN, USA) were intravenous injected three times within a week. NF-κB inhibitor (MG132, 5 mg/kg; Merck, NY, USA) were intraperitoneally injected three times within a week. Recombinant resistin protein (10 μg/kg; R&D Systems, MN, USA) was intravenously injected four times within a week. (Figure.1)

2-2. Measurement of airway hyper-responsiveness

Airway hyper-responsiveness (AHR) to inhaled aerosolized methacholine (MCh; Sigma-Aldrich) was measured using a forced oscillation technique (FlexiVent; SCIREQ, Montreal, QC, Canada) on the sacrifice day, as described in a previous study. Aerosolized phosphate-buffered saline or MCh at varying concentrations (0 mg/ml, 6.25 mg/mL, 12.5 mg/mL, 25.0 mg/mL, 50.0 mg/mL, 100mg/ml), was administered to mice for 10 s via a nebulizer connected to a ventilator. Then, AHR was assessed by measurements of airway resistance.

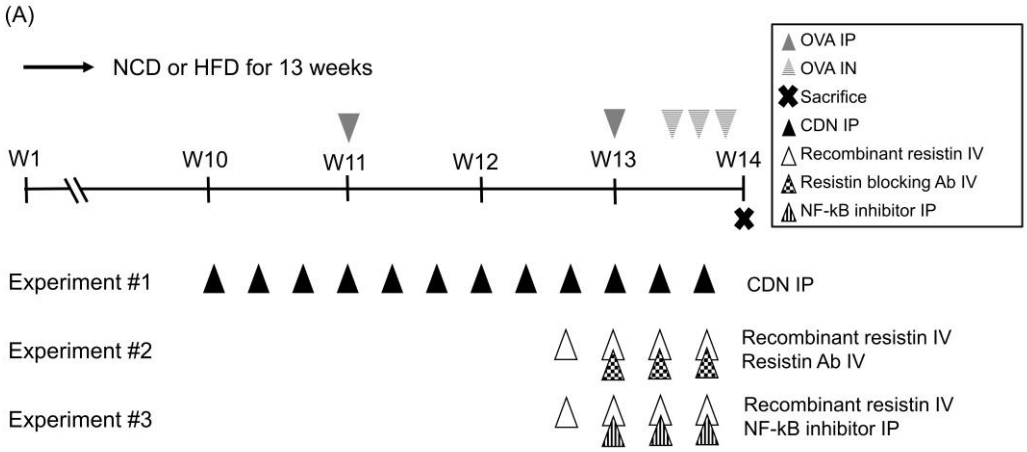

Figure 1. Animal model design

(A) Mice were fed either a normal chow diet (NCD) or a high-fat diet (HFD) for 13 weeks. Ovalbumin (OVA) was administered intraperitoneally (IP) at week 11 and 13, followed by intranasal (IN) administration during weeks 13. Animals were sacrificed at week 14 for analysis.

Experiment 1: Resistin inhibitor (CDN;50mg/kg) administration intraperitoneally (IP).

Experiment 2: Overexpression resistin (10μg/kg) or antibody of resistin (100μg/kg) via intravenous (IV) injection 3-4times in the 13 week.

Experiment 3; Overexpression resistin combined with NF-*κ*B inhibitor (5mg/kg) via intraperitoneal (IP) injection 3-4times in the 13 week.

Each group contained more than five mice in SPF room.

2-3. Inflammatory cell counting in bronchoalveolar lavage fluid

To collect bronchoalveolar lavage fluid (BALF), we performed lung lavage using 1 mL of Hank's Balanced Salt Solution (HBSS) administered through a tracheal tube. Total cell counts were determined using a hemocytometer with trypan blue staining. BALF cells were processed by cytocentrifugation (BioridGE, Shanghai, China) and pelleted onto cytospin slides. The slides were then stained with hematoxylin and eosin (H&E Hemacolor; Merck, Darmstadt, Germany), and a differential count of inflammatory cells was conducted (100 cells per slide).

2-4. Lung homogenate

The lung tissue was resected and homogenized using a tissue homogenizer (MP Biomedicals, CA, USA) in lysis buffer and protease inhibitor solution (Sigma-Aldrich, MO, USA). After incubation and centrifugation, supernatants were harvested and passed through a 0.45 micron filter (SPL, Kyeonggi, Korea). The final preparations were stored at -20 ℃ for cytokine analysis , as described previously.

2-5. Histological analysis

The lung that was not used for BALF collection was fixed in 4% formalin and embedded in paraffin. Lung sections were cut into 2-3μm-thick slices and stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Masson trichrome (M&T) for histological analysis. The slides were observed under a light microscope (×200 magnification). Fibrosis area was measured by estimating at ×400 magnification on M&T staining slides using the Metamorph program (Molecular Devices, Sunnyvale, CA, USA).

2-6. Cell culture and treatment

Beas-2B cell line, epithelial cells isolated from normal human bronchial epithelium, was obtained Yonsei university. Cells were cultured in DMEM/F12 containing 10 % fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific) and 1 % penicillin /streptomycin (Gibco, NJ, USA) in a 37 ℃ in an incubator containing 5 % CO2 at 37 °C. For establishment of *in vitro* model, resistin, Retnlα, Retnlβ siRNA were transfected by using lipofectamine 2000 (Thermo, MA, USA). After then lipopolysaccharide (LPS, 10 μg/mL, Sigma-Aldrich, MO, USA), insulin (50 nM; sigma, CA, USA) was used to treat the cells for 24 h. Recombinant resistin protein (50 ng/ml; R&D, MN,

USA) for resistin overexpression was treated in the cell for 24 h. NF-κB inhibitor (MG132, 5 μM; Merck, NY, USA) was treated in the cell for 24 h.

2-7. Real-time quantitative PCR

RNA was extracted by using TRIzol reagent (Soombio, Seoul, Korea) following the manufacturer's instructions. cDNA synthesis was performed according to Synthesis platinum master mix kit (Genedepot, TX, USA). Real time PCR assays (ThermoFisher Scientific) were used for detecting resistin, Retnl*α*, Retnl*β* and NF-κB gene expression. The primers used in the experiment are listed in Table 1.

2-8. Immunofluorescence (IF) and immunohistochemistry (IHC) staining analysis

Lung tissue was fixed, embedded in paraffin, and sectioned for histological analysis. Slides were deparaffinized, rehydrated, and subjected to antigen retrieval as required. For both immunohistochemistry (IHC) and immunofluorescence (IF), sections were incubated overnight at 4 °C with an anti-resistin antibody (IF 1:200: IHC 1:1000 dilution; R&D Systems, CA, USA), followed by five washes with phosphate-buffered saline (PBS).

For IHC, slides were subsequently incubated for 1 hour at room temperature with an HRP-conjugated goat IgG secondary antibody (1:100 dilution; Santa Cruz Biotechnology, TX, USA), and signals were visualized using a DAB substrate.

For IF, slides were incubated for 1 hour at room temperature with an FITC-conjugated m-IgGκ BP secondary antibody (1:100 dilution; Santa Cruz Biotechnology), and nuclei were counterstained using a PI-containing mounting medium (Invitrogen, CA, USA). Images were captured using an AxioImager M2 microscope (Carl Zeiss, Germany).

2-9. Analysis of cytokines

Concentrations of interleukin (IL)-1*β*, tumor necrosis factor-*α* (TNF-*α*), IL-13, IL-6, IL-5, IL-17, resistin, NF-κB and transforming growth factor-β (TGF-*β*) in lung homogenates were assessed by enzymelinked immunosorbent assay (R&D, CA, USA) (Mybiosource, SD, USA) according to the manufacturer's instructions. All samples were assessed in duplicate.

Table 1. List of primers

Gene	Primer probe	Sequence	Annealing Tm ℃
GAPDH	Forward	GTG AAG GTC GGT GTG AAC GGA TTT	62.6
	Reverse	TGG CAA CAA TCT CCA CTT TGC CAC	
Resistin	Forward	CTG ATG TCG GGG AAG TGA GC	59.5
	Reverse	ACC GGA GGA CAT CAG ACA TC	
Retnlα	Forward	CCC TCC ACT GTA ACG AAG ACT	58.1
	Reverse	AGG AAT TAC TCA CCA GCA GGG	
Retnlβ	Forward	CTG ATA GTC CCA GGG AAC GC	59
	Reverse	GTC TGC CAG AAG TGA CA	
NF-κB	Forward	ACA ACT ATG AGA TGA ACT CCG GG	58.9
	Reverse	CCG TGG GGC ATT TTG TTC AG	

2-11. Statistical analysis

All results are expressed as the mean±standard error. The AHR data were analyzed using repeated-measure analysis of variance (ANOVA), followed by a Tukey test. One-way ANOVA was performed to assess the significance of differences in BALF cell count, cytokine levels, and quantitative fibrosis among groups. All statistical analyses were performed with PASW statistics 18 (SPSS Inc., Chicago, IL, USA). p-values < 0.05 were considered statistically significant.

3. Result

3-1. CDN ameliorated weight changes, metabolic syndrome, airway hyperresponsiveness, and airway inflammation

Mice fed a high-fat diet (HFD) showed a significant increase in body weight compared to the control group ($p < 0.05$; Figure 2A-B). Markers of metabolic syndrome, including fasting glucose and insulin levels, were elevated in the HFD, OVA, and HFD/OVA groups, reflecting metabolic dysfunction induced by the high-fat diet. Treatment with resistin inhibitor, CDN, significantly reduced fasting glucose and insulin levels in the HFD/OVA/CDN group ($p < 0.05$), indicating an improvement in obesity-induced metabolic syndrome (Figure. 2 C-F). Methacholine-induced airway hyperresponsiveness (AHR) was significantly elevated in the HFD/OVA group compared to controls (Figure. 2G, $p < 0.01$). CDN treatment resulted in a significant reduction in AHR in the HFD/OVA/CDN group ($p < 0.05$). BALF analysis revealed increased inflammatory cell infiltration in the HFD/OVA group compared to controls (Figure. 2H). CDN treatment in the HFD/OVA/CDN group significantly reduced the number of inflammatory cells ($p < 0.05$). Histopathological analysis using H&E, PAS, and MT staining demonstrated pronounced airway inflammation, goblet cell hyperplasia, and collagen deposition in the HFD/OVA group (Figure. 2I-L). These pathological changes were significantly mitigated in the HFD/OVA/CDN group, with reduced inflammatory cell infiltration, normalized mucus production, and diminished airway remodeling ($p < 0.01$).

3-2. CDN reduced level of resistin and inflammatory cytokine in HFD, OVA and HFD/OVA group

The immunofluorescence (IF) staining results for resistin reveal that resistin, which was strongly expressed in the HFD and HFD/OVA groups, was notably reduced upon CDN treatment. CDN treatment significantly reduced resistin expression and inflammatory cytokine levels (IL-1β, Il-5, TNF-α, IL-17 and IL-13) in the HFD/OVA/CDN group ($p < 0.05$) (Figure. 3 A-E), indicating an attenuation of systemic and airway-specific inflammation.

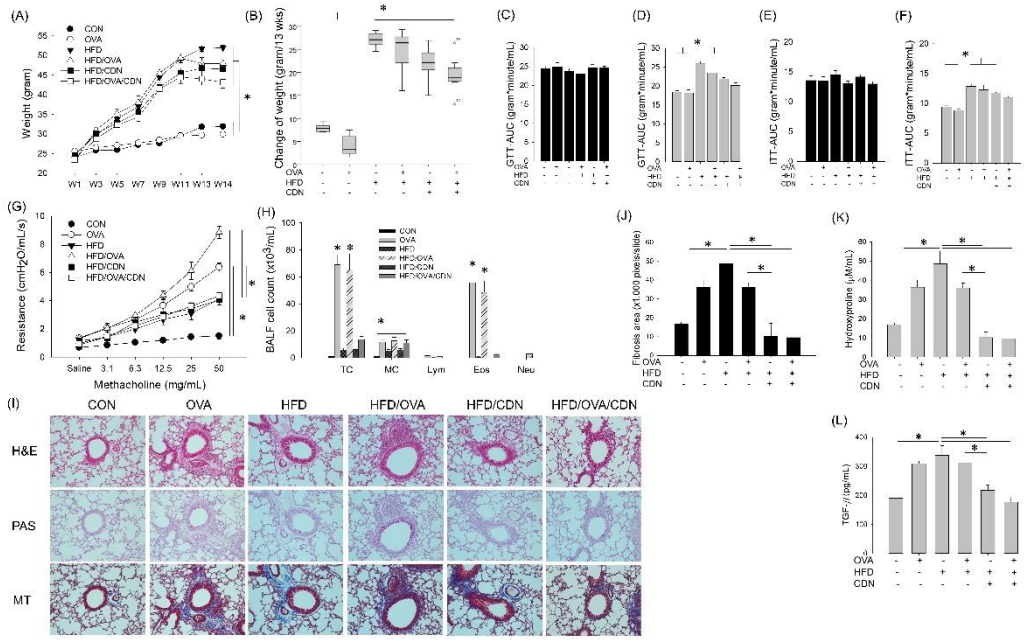

Figure 2. CDN ameliorated weight changes, metabolic syndrome, airway hyperresponsiveness, and airway inflammation

Body weight changes over time in different experimental groups (A). Weight change over 13 weeks (B). Glucose and insulin tolerance tests: glucose tolerance test (GTT) area under the curve (AUC) (C, D), and insulin tolerance test (ITT) AUC (E, F). Airway resistance in response to increasing doses of methacholine (G). Total and differential cell counts in bronchoalveolar lavage fluid (BALF), including total cells (TC), macrophages (MC), lymphocytes (Lym), eosinophils (Eos), and neutrophils (Neu) (H). Representative histological images of lung sections stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Masson's trichrome (MT) to assess inflammation, mucus production, and fibrosis, respectively (I). Fibrosis area quantification (J). Hydroxyproline levels as an indicator of collagen deposition (K). Transforming growth factor-beta (TGF-β) levels (L). Data are presented as mean ± SEM. *$p < 0.05$, indicating statistical significance between groups.

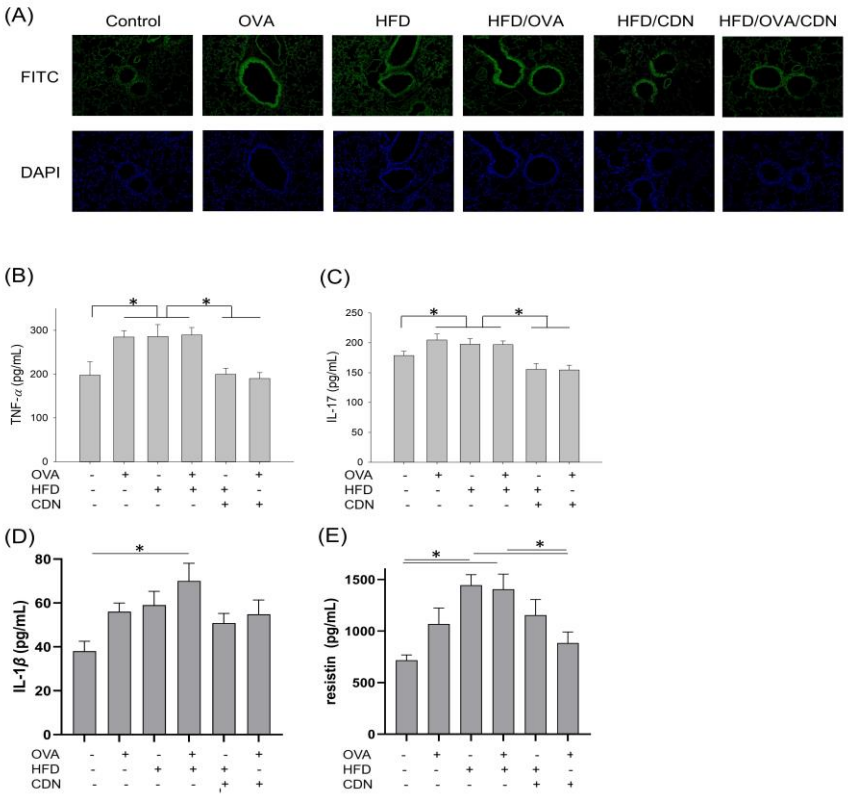

Figure 3. CDN reduced level of resistin and inflammatory cytokine in HFD, OVA and HFD/OVA group

Representative immunofluorescence images of lung sections stained with FITC (green) and DAPI (blue) to assess structural changes and inflammatory infiltration in different experimental groups (A). Levels of inflammatory cytokines measured in lung homogenate, including TNF-α (B), IL-17 (C), IL-1β (D) and resistin (E). Data are presented as mean ± SEM. *$p < 0.05$, indicating statistical significance between groups.

3-3. Effects of resistin overexpression and silencing on airway inflammation and remodeling

Body weights were similar between groups, except for the control group (Figure. 4 A). Compared to the control group, the HFD/OVA and HFD/OVA/resistin groups exhibited significantly higher airway hyperresponsiveness (AHR), and bronchoalveolar lavage fluid (BALF) cell proliferation, with a predominance of eosinophils (Figure. 4 B-C). Resistin silencing significantly reduced in AHR and BALF. Histological analysis using H&E, PAS, and MT staining revealed severe airway inflammation, mucus hypersecretion, and subepithelial collagen deposition in the HFD/OVA group (Figure. 4D). These findings suggest that resistin overexpression and silencing have substantial effects on airway inflammation and remodeling in the HFD/OVA model. Resistin silencing significantly reduced mRNA level of resistin, Retnlα and Retnlβ and NF-κB (Figure. 4 E). Furthermore, inflammatory cytokine concentrations, including resistin, IL-1β IL-5, IL-6 and NF- κB were elevated in the HFD/OVA and HFD/OVA/resistin groups, correlating with increased airway inflammation (Figure.4 F). Resistin silencing significantly inhibited the expression of these cytokines in the HFD/OVA/Ab group, demonstrating its anti-inflammatory effects ($p < 0.05$).

3-4. Effect of resistin overexpressing and silencing on inflammatory cytokine *in vitro*

Compared to the control group, both the LPS and insulin-treated groups exhibited a significant increase in inflammatory cytokine levels, indicating an induced inflammatory response (Figure. 5 A-D). However, in the groups where resistin, Retnlα and Retnlβ were inhibited through treatment with their respective siRNAs, a significant reduction in cytokine expression was observed under both LPS and insulin-stimulated conditions. These results suggest that siRNA effectively suppresses the inflammation triggered by LPS and insulin, highlighting its potential role in modulating inflammatory pathways.

3-5. Effect of resistin overexpression and NF-κB inhibition

Mice fed a high-fat diet (HFD) showed a significant increase in body weight compared to the control group ($p < 0.05$; Figure. 6A). Markers of metabolic syndrome, including fasting glucose and insulin levels, were elevated in HFD, HFD/OVA and HFD/OVA/resistin group (Figure. 6B). Methacholine-induced airway hyperresponsiveness (AHR) was significantly elevated in the HFD/OVA group compared to controls (Figure. 6C). The resistin overexpression group exhibited

levels comparable to those of the HFD/OVA group, while the group treated with the NF-κB inhibitor showed a reduction. BALF analysis revealed increased inflammatory cell infiltration in the HFD/OVA group compared to controls (Figure. 6C). Similarly, the resistin overexpression group exhibited an increased number of inflammatory cells compared to the HFD/OVA group, whereas the group treated with the NF-κB inhibitor showed a reduction. Moreover, the group subjected to both treatments also demonstrated a decrease in the number of inflammatory cells. In the HFD/OVA and HFD/OVA/resistin groups, an increase in the protein levels of IL-6, IL-1β, TGF-β, IL-17 was observed. In contrast, the group treated with the NF-κB inhibitor exhibited a reduction in protein levels (Figure. 6D).

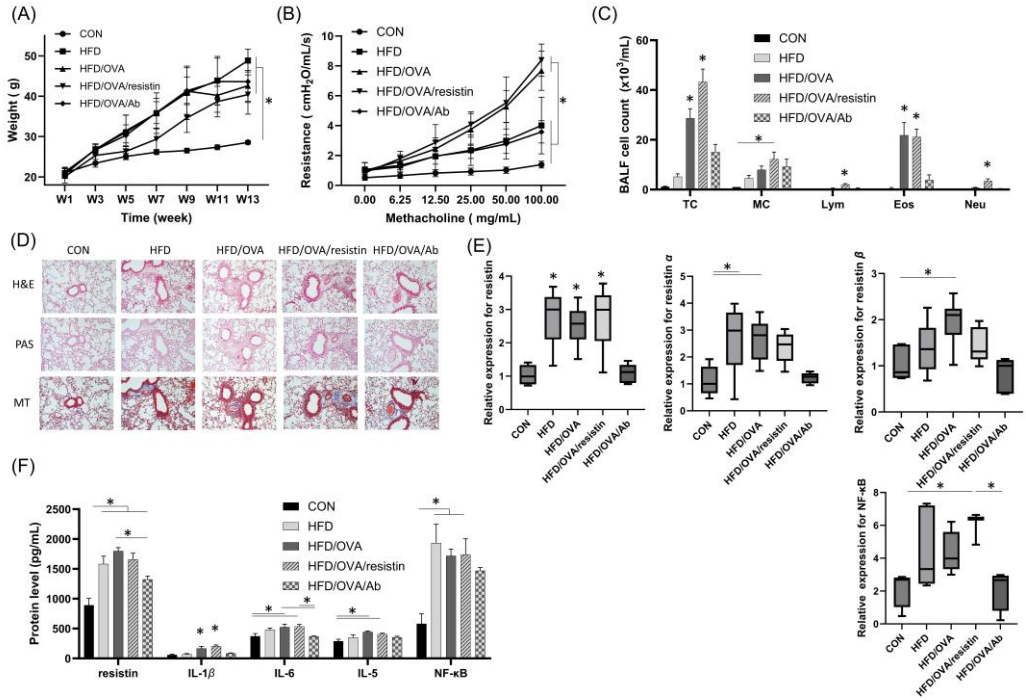

Figure 4. Effects of resistin overexpression and silencing on airway inflammation and remodeling

Body weight changes over time in different experimental groups (A). Airway resistance in response to increasing doses of methacholine (B). Total and differential cell counts in bronchoalveolar lavage fluid (BALF), including total cells (TC), macrophages (MC), lymphocytes (Lym), eosinophils (Eos), and neutrophils (Neu) (C). Representative histological images of lung sections stained with hematoxylin and eosin (H&E), periodic acid–Schiff (PAS), and Masson's trichrome (MT) to assess inflammation, mucus production, and fibrosis, respectively (D). Relative mRNA expression levels of resistin, Retnlα, Retnlβ, and NF-κB in lung tissue (E). Protein levels of resistin, IL-1β, IL-6, IL-5, and NF-κB in lung homogenate(F).
resistin; resistin overexpression by recombinant protein; Ab; silencing resistin by antibody.
Data are presented as mean ± SEM. *p < 0.05, indicating statistical significance between groups.

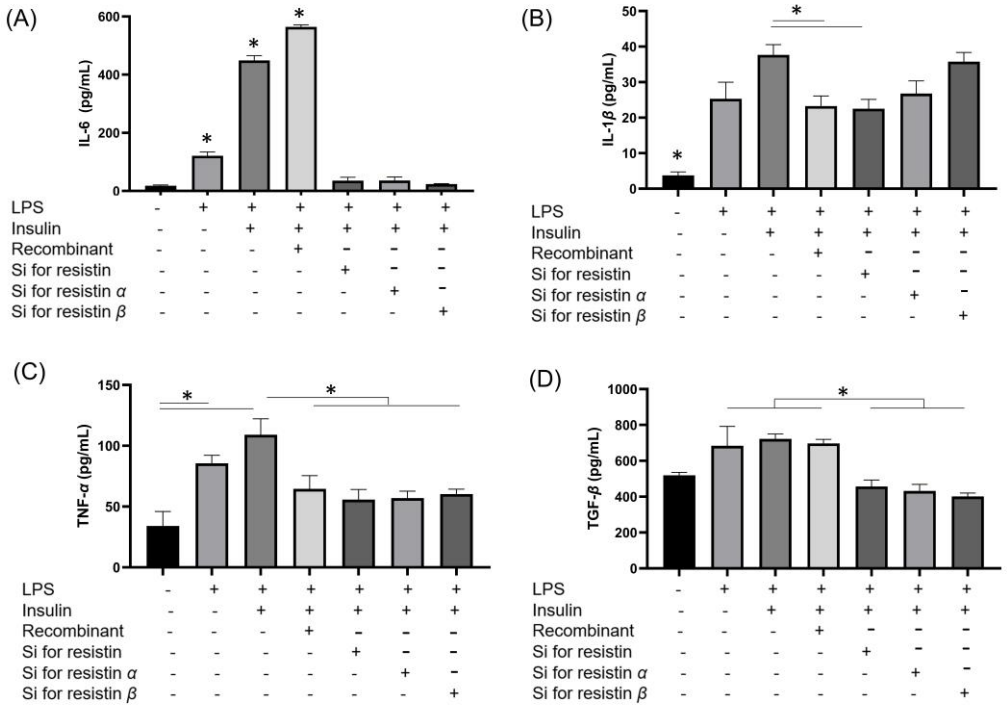

Figure 5. Effect of resistin overexpression and silencing on inflammatory cytokine *in vitro*

Expression level of IL-6 (A), IL-1β (B), TNF-α (C), and TGF-β (D) in cell supernatant. In Beas-2B cell, both LPS, insulin and recombinant resistin protein significantly increased inflammation relevant cytokine level. Inflammatory cytokines were significantly reduced after treatment with resistin siRNA following LPS and insulin stimulation.

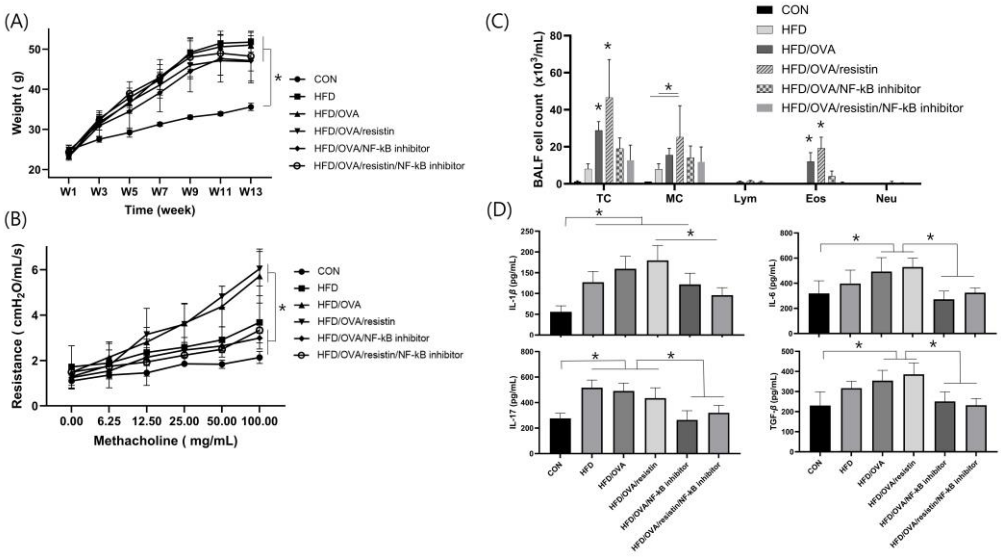

Figure 6. Effect of resistin overexpression and NF-*κ*B inhibition

Body weight changes over time in different experimental groups (A). Airway resistance in response to increasing doses of methacholine (B). Total and differential cell counts in bronchoalveolar lavage fluid (BALF), including total cells (TC), macrophages (MC), lymphocytes (Lym), eosinophils (Eos), and neutrophils (Neu) (C). Level of Inflammatory cytokines IL-1β, IL-6, IL-17 and TGF-β in lung homogenate (D).
resistin; resistin overexpression by recombinant protein; NF-*κ*B inhibitor; MG132

3-6. Expression and localization of NF-κB and resistin in lung and adipose tissues

The expression level of NF-κB was reduced in both the HFD/OVA/NF-κB inhibitor group and the HFD/OVA/resistin/NF-κB inhibitor group (Figure. 7A). However, protein and mRNA levels of resistin remained unchanged in these groups (Figure. 7B-C).

Immunohistochemistry (IHC) using a resistin antibody showed that resistin expression was similar across the HFD/OVA group, the HFD/OVA/resistin group, and the NF-κB inhibitor group in both lung and adipose tissues. Similarly, immunofluorescence (IF) staining with a resistin antibody revealed no significant differences in resistin expression between the HFD/OVA and HFD/OVA/resistin groups and their respective NF-κB inhibitor group counterparts (Figure. 7D).

3-7. Effect of resistin overexpression and NF-κb inhibition cytokine *in vitro*

In Beas-2B cells, the LPS, insulin, LPS/insulin, and LPS/insulin/resistin overexpression groups exhibited an increase in inflammation-related cytokines, including IL-17, IL-6, TGF-β, TNF-α and NF-κB. However, in the LPS/insulin/resistin overexpression group treated with the NF-κB inhibitor, a reduction in these cytokines was observed (Figure. 8A-E). The level of resistin remained unchanged in both the resistin overexpression group and the resistin overexpression with NF-κB inhibition group (Figure. 8F).

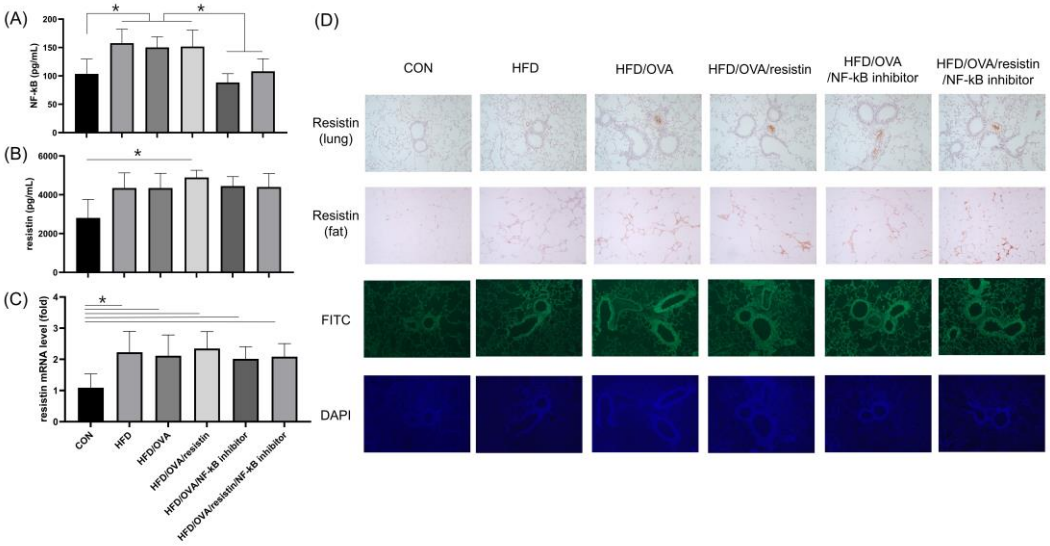

Figure 7. Expression and localization of NF-κB and resistin in lung and adipose tissues

Protein level of NF-κB (A) and protein level of resistin (B). mRNA expression level of resistin (C). Immunohistochemical (IHC) staining using anti-resistin antibody was performed on lung and adipose tissues (×200 magnification) and immunofluorescence (IF) staining with anti-resistin antibody in lung tissue (D).

resistin; resistin overexpression by recombinant protein

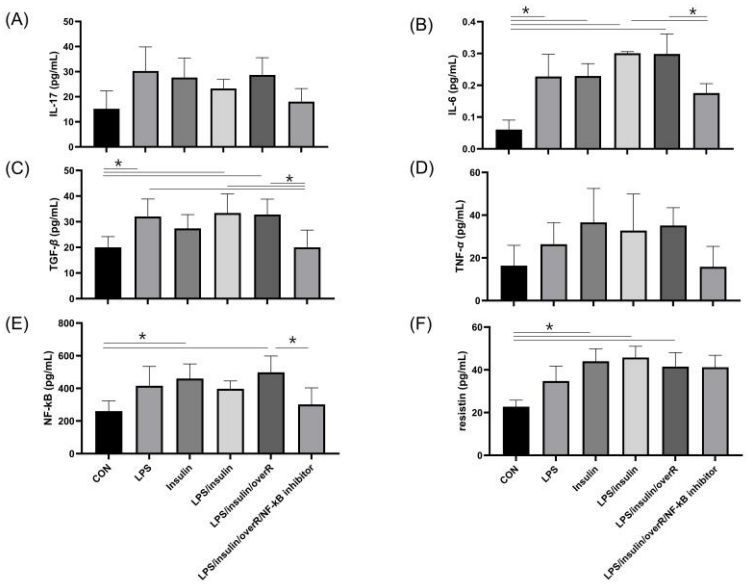

Figure 8. Effect resistin overexpression and NF-κB inhibition on cytokine *in vitro*.

Expression levels of IL-17 (A), IL-6 (B), TGF-β (C), TNF-α (D), NF-κB (E), and resistin (F) were measured in Beas-2B cells treated with lipopolysaccharide (LPS), insulin, or a combination of LPS and insulin, with or without recombinant resistin protein and the NF-κB inhibitor MG132.

resistin; resistin overexpression by recombinant protein

4. Discussion

This study, through both *in vivo* and *in vitro* experiments, demonstrates that resistin plays a pivotal role in the development and exacerbation of obesity-induced asthma, primarily via activation of the NF-κB signaling pathway. In the murine model, treatment with a resistin inhibitor (CDN) or a resistin antibody significantly alleviated obesity-associated airway hyperresponsiveness (AHR), airway cellular proliferation, fibrosis, and elevated pro-inflammatory cytokine levels. Consistent with these findings, *in vitro* experiments using airway epithelial cells revealed that silencing resistin expression markedly reduced LPS- and insulin-induced pro-inflammatory cytokine production. In addition, our results showed that resistin expression was significantly upregulated in the OVA-induced asthma model, accompanied by increased levels of IL-5, IL-6, IL-1β and NF-κB. Resistin overexpression further exacerbated AHR and inflammatory cell infiltration in bronchoalveolar lavage fluid (BALF), whereas administration of a resistin inhibitor significantly reduced airway inflammation in both obesity- and asthma-related models. These data reinforce the role of resistin as a key driver of airway inflammation in obesity-induced asthma. Furthermore, inhibition of NF-κB significantly reduced airway inflammation, mucus production, and the levels of cytokines including IL-6, IL-17, IL-1β, and TGF-β, even in the presence of resistin overexpression. Interestingly, while NF-κB inhibition suppressed downstream inflammatory responses, it did not affect resistin expression, further supporting the notion that resistin functions upstream of NF-κB in the inflammatory pathway.

Obesity and asthma are increasingly prevalent global health issues (Thomson, Clark, Camargo, & Investigators, 2003). Obesity has been shown to aggravate asthma by enhancing both systemic and airway inflammation (Hayley A. Scott et al, 2022).

Beyond our current findings, resistin, an adipokine secreted by adipose tissue, plays a crucial role in regulating systemic inflammatory responses and is associated with various diseases (Silswal et al., 2005). In the present study, we confirmed that resistin exacerbates inflammation in both obesity and asthma. Previous studies have also demonstrated that resistin contributes to the pathogenesis of metabolic disorders, cardiovascular diseases, and chronic inflammatory conditions. Specifically, resistin induces insulin resistance in diabetes and promotes atherosclerosis and vascular inflammation in cardiovascular diseases (H. K. Park, Kwak, Kim, & Ahima, 2017). In autoimmune diseases, resistin overexpression enhances immune cell activation, thereby worsening inflammatory responses (Nirupama Silswal & Sudip Ghosh, 2005; Filkova, Haluzik, Gay, & Senolt, 2009). Particularly in obesity-induced asthma, the role of resistin appears to be more pronounced (Sara Rojas-Dotor, 2013; Sood & Shore, 2013). In obesity, the expansion and functional alteration of adipose tissue lead to increased secretion of various adipokines, including resistin, thereby exacerbating systemic and airway inflammatory responses (Hosny et al., 2021). Elevated levels of resistin have been associated with greater asthma severity and decreased responsiveness to conventional therapies. Thus, in obesity-induced asthma, resistin likely acts as a key mediator of

airway inflammation, which may partly explain the increased severity and treatment resistance observed in obese asthmatic patients. (Sara Rojas-Dotor1, 2013)

NF-κB is a key transcription factor that regulates immune and inflammatory responses and plays a crucial role in various chronic diseases (Peter J. Barnes, 1997; Liu, Zhang, Joo, & Sun, 2017). Research has shown that NF-κB is activated in a wide range of conditions, including asthma, obesity, diabetes, cardiovascular diseases, and autoimmune disorders, functioning as a major regulator of inflammatory pathways (Barnes, 2009; Hayden & Ghosh, 2011). Specifically, dysregulation of the NF-κB signaling pathway contributes to the pathological progression of chronic inflammatory diseases (Firestein, 2001). For instance, NF-κB promotes insulin resistance in diabetes and enhances the expression of pro-inflammatory cytokines in atherosclerosis, leading to vascular damage (Lawrence, 2009; Park, 2016). In autoimmune diseases such as rheumatoid arthritis and Crohn's disease, excessive NF-κB activation exacerbates immune cell-mediated inflammatory responses (M. H. Park & Hong, 2016; Atreya, Atreya, & Neurath, 2008). In asthma, NF-κB upregulates the expression of inflammatory cytokines, contributing to airway inflammation (Lim et al., 2023).

Resistin and NF-κB are closely interrelated in the regulation of inflammatory responses and are known to contribute to the pathogenesis of various chronic diseases (Lee et al., 2014; Bertolani et al., 2006). One of the primary mechanisms by which resistin exerts its pro-inflammatory effects is through the activation of the NF-κB signaling pathway (Tripathi, Kant, Pandey, & Ehtesham, 2020; Li et al., 2021; Rompou et al., 2024). These findings support the hypothesis that resistin acts as an upstream regulator of NF-κB. In this study, we further investigated the impact of resistin activation and NF-κB inhibition on airway inflammation. NF-κB inhibition effectively reduced airway inflammation even under conditions of resistin activation, indicating a strong interconnection between these factors in the inflammatory responses of obesity-induced asthma.

Further investigation is warranted elucidating the molecular interactions between resistin and NF-κB and exploring novel therapeutic strategies targeting these pathways. Furthermore, a dual-targeting strategy inhibiting both NF-κB and resistin may offer a particularly effective approach for the treatment of obesity-induced asthma, warranting further studies to assess its clinical applicability.

5. Conclusion

This study demonstrates that resistin and NF-κB play important roles in obesity-induced asthma and that they function as a key mechanism promoting airway inflammation. Administration of a resistin inhibitor effectively reduced inflammatory responses in obesity-induced asthma models, and the findings also suggest that resistin functions as an upstream regulator of NF-κB. These results indicate that resistin and NF-κB may serve as potential therapeutic targets for asthma treatment, particularly supporting the potential of dual-targeted strategies that inhibit both pathways. Further clinical studies are needed to validate these findings and to evaluate their applicability in asthma therapy.

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Abstract in Korean

레지스틴과 NF-κB가 비만으로 유발된 천식에 미치는 영향

최근 수십 년간 비만과 천식의 유병률은 전 세계적으로 급격히 증가하고 있으며, 이로 인한 공중보건상의 부담 또한 심화되고 있다. 특히, 비만이 천식의 위험도를 높이고 증상을 악화시킨다는 역학적, 생물학적 증거들이 다수 보고되고 있으며, 이에 따라 비만 유발 천식(obesity-induced asthma)의 병태생리를 규명하려는 시도가 활발히 이루어지고 있다. 본 연구는 이러한 흐름에 기반하여, 비만과 천식의 병합 기전에 관여할 것으로 추정되는 아디포카인(adipokine)인 레지스틴(resistin)과 면역·염증 반응 조절의 중심축인 NF-κB(Nuclear Factor kappa-light-chain-enhancer of activated B cells)의 기전에 주목하였다.

레지스틴은 지방조직에서 분비되는 염증 관련 단백질로서, 기존에는 주로 인슐린 저항성과 대사증후군에 관여하는 인자로 인식되었으나, 최근에는 호흡기계 염증과도 밀접한 관련이 있음이 밝혀지고 있다. NF-κB는 다양한 자극에 반응하여 활성화되며, 염증성 사이토카인의 유전자 발현을 촉진하고 면역세포의 활성화를 유도하는 전사인자로서, 천식을 비롯한 다수의 만성 염증질환에 핵심적인 역할을 수행한다.

본 연구는 레지스틴이 NF-κB를 통해 기도 염증을 유발하거나 증폭시키는지를 확인하고자 하였으며, 이를 위해 고지방 식이(high-fat diet, HFD) 및 난백알부민(ovalbumin, OVA)을 이용한 비만 유발 천식 생쥐 모델을 구축하고, 레지스틴의 과발현 및 억제, NF-κB의 억제 실험을 염증관련 단백질, mRNA, 병리학적 분석을 통해 기도 염증 반응을 분석하였다.

실험 결과, HFD/OVA 및 HFD/OVA/Resistin overexpression 군에서 레지스틴과 NF-κB의 발현이 모두 증가함을 확인하였으며, 이는 조직 내 염증세포 침윤, 기도과민반응(AHR), 점액 과다 분비, 섬유화 등 천식의 병리적 특징들과 일치하였다. 특히 NF-κB 억제제 투여군에서는 이러한 염증 반응이 현저히 감소하였고, NF-κB의 발현은 줄어든 반면 레지스틴 발현은 유의미한 변화가 없었다, 이는 레지스틴이 NF-κB의 상위 조절 인자로 작용할 가능성을 뒷받침하는 결과로 해석된다.

또한, 레지스틴의 과발현은 NF-κB 활성화 및 하위 염증관련 사이토카인(IL-6, IL-1β, IL-13, TNF-α 등)의 증가로 이어졌으며, 이는 기도 리모델링과 점액세포 과형성을 촉진하는 기전과 연관된다. 반대로, 레지스틴의 억제를 통해 염증 반응이 효과적으로 억제되었으며, 이는 NF-κB의 활성화를 차단함으로써 기도 염증의 중심 경로를 끊어낼 수 있음을 시사한다. 이와 같은 상호작용은 *In vivo* 모델뿐만 아니라 *In vitro* 모델(Beas-2B)에서도 일관되게 관찰되었으며, 특히 LPS 및 인슐린으로 유도된 염증 환경에서 레지스틴 siRNA 및 NF-κB 억제를 통해 염증관련 사이토카인 발현이 유의하게 감소하였다.

결론적으로, 본 연구는 레지스틴이 NF-κB의 상위 조절 인자로 작용하면서 비만 유발 천식에서 염증 반응을 증폭시키는 핵심 경로임을 실험적으로 입증하였다. 이는 향후 천식 치료 전략 개발에 있어 레지스틴 및 NF-κB를 동시에 표적하는 이중 억제 전략(dual inhibition)이 유망한 접근이 될 수 있음을 시사한다. 실제로 본 연구에서는 이중 억제를 통해 염증 반응이 가장 효과적으로 억제되는 결과를 확인하였다. 이러한 기전적 통찰은 기존의 항염증 치료에 한계를 보이는 비만 유발 천식 환자군에게 새로운 치료 옵션을 제시할 수 있으며, 향후 임상 적용 가능성에 대한 연구가 더욱 활발히 진행될 필요가 있다.

핵심되는 말: 레지스틴, NF-κB, 비만으로 유발된 천식, 기도 과민반응, 지방세포