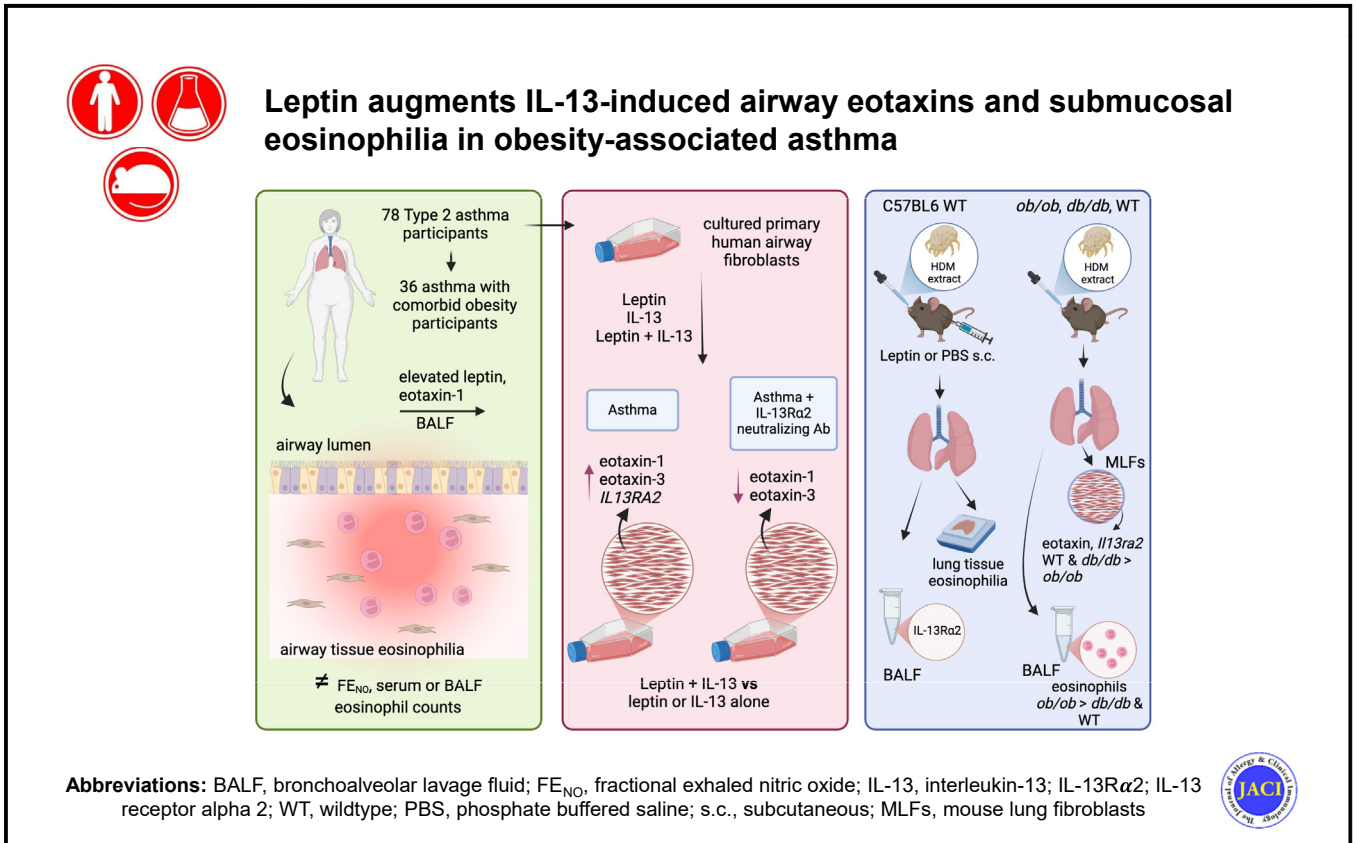
Leptin augments IL-13–induced airway eotaxins and submucosal eosinophilia in obesity-associated asthma

Jennifer L. Ingram, PhD, Victoria L. McQuade, MRes, Jasmine Weiss, MD, MHS, Jack T. Womble, BS, Mark D. Ihrie, PhD, Karen Zhao, BS, et al

GRAPHICAL ABSTRACT



Abbreviations: BALF, bronchoalveolar lavage fluid; FE_{NO}, fractional exhaled nitric oxide; IL-13, interleukin-13; IL-13Rα2; IL-13 receptor alpha 2; WT, wildtype; PBS, phosphate buffered saline; s.c., subcutaneous; MLFs, mouse lung fibroblasts

Capsule summary: Leptin, an adipokine found in high circulating levels in obesity, enhances IL-13–induced eotaxin production in airway fibroblasts in asthma. Inhibition of IL-13 receptor alpha 2 reduces the ability of leptin to promote eotaxin secretion in airway fibroblasts.

J Allergy Clin Immunol 2024;■■■:■■■-■■■.

https://doi.org/10.1016/j.jaci.2024.10.039

Leptin augments IL-13–induced airway eotaxins and submucosal eosinophilia in obesity-associated asthma

Jennifer L. Ingram, PhD,[a] Victoria L. McQuade, MRes,[a] Jasmine Weiss, MD, MHS,[b] Jack T. Womble, BS,[a] Mark D. Ihrie, PhD,[a] Karen Zhao, BS,[a] Dave Francisco, BS,[c] Barbara Theriot, MS,[d] Katelynn May, BSN,[a] Haein Kim, BS,[a] Matthew McCravy, MD,[a] Maor Sauler, MD,[e] Njira L. Lugogo, MD,[f] Mary E. Sunday, MD, PhD,[g] Jeffrey Everitt, DVM,[g] Julia K. L. Walker, PhD,[h] Robert M. Tighe, MD,[a] Monica Kraft, MD,[c]* and Loretta G. Que, MD[a]* *Durham and Chapel Hill, NC; New York, NY; New Haven, Conn; and Ann Arbor, Mich*

Background: Airway tissue eosinophilia can be an observed feature of obesity-associated type 2 (T2) asthma, but the processes mediating this inflammation are unknown.
Objective: To investigate a process whereby leptin, an adipokine elevated in obesity, potentiates pulmonary eosinophilia and eotaxin production by airway fibroblasts in T2 asthma.
Methods: We assessed associations between body mass index and airway eosinophilia as well as leptin and eotaxin production in 78 participants with asthma, 36 of whom exhibited obesity. Cultured human airway fibroblasts and mouse models of chronic allergic airway disease were used to evaluate leptin's effect on eotaxin production and lung eosinophilia. The role of IL-13 receptor alpha 2 (IL-13Rα2) in mediating these processes was examined using specific neutralizing antibodies *in vitro*.
Results: In participants with T2 asthma and obesity, we observed that airway tissue eosinophilia did not associate with traditional T2 inflammation metrics such as peripheral and/or bronchoalveolar lavage fluid eosinophil counts or with fractional exhaled nitric oxide. Alternatively, we observed elevated bronchoalveolar lavage fluid leptin and eotaxin-1 levels. In airway fibroblasts from participants with asthma, leptin augmented IL-13–induced eotaxin-1 and eotaxin-3 production and *IL13RA2* expression. In mice, elevated leptin promoted airway IL-13Rα2 and eotaxin production by lung fibroblasts and lung tissue eosinophilia following chronic house dust mite allergen exposure. Inhibition of IL-13Rα2 reduced combined leptin and IL-13–stimulated eotaxin secretion by human airway fibroblasts.
Conclusions: We identified a potential association explaining airway tissue eosinophil retention in obesity-associated T2 asthma through leptin-mediated enhancement of IL-13–induced eosinophil chemokine production by airway fibroblasts, a process requiring IL-13Rα2. (J Allergy Clin Immunol 2024;■■■:■■■-■■■.)

Abbreviations used

BALF:	Bronchoalveolar lavage fluid
BMI:	Body mass index
CCL11:	Eotaxin-1
CCL26:	Eotaxin-3
FENO:	Fractional exhaled nitric oxide
FVC:	Forced vital capacity
GAPDH/*Gapdh*:	Glyceraldehyde-3-phosphate dehydrogenase
HDM:	House dust mite
ICS:	Inhaled corticosteroid
IL-4Rα (*IL4RA*):	IL-4 receptor alpha
IL-13Rα1 (*IL13RA1*):	IL-13 receptor alpha 1
IL-13Rα2 (*IL13RA2, Il13ra2*):	IL-13 receptor alpha 2
MBP:	Major basic protein
PC_{20}:	Provocative concentration of methacholine resulting in a 20% fall in the FEV_1
PGE2:	Prostaglandin E2
T2:	Type 2
WT:	Wild-type

Key words: *T2 asthma, obesity, eosinophils, eotaxin, IL-13Rα2, leptin, airway fibroblast*

Projections suggest that by 2030, 50% of the US population will be living with obesity.[1] Currently, nearly 40% of patients with asthma in the United States experience comorbid obesity.[2] Obesity is a disease modifier that complicates the diagnosis, pathobiology, and treatment of asthma.[3-7] Large multicenter analyses of participants with asthma have identified specific asthma phenotypes comprising late-onset type 2 (T2) and non-T2 asthma participants with obesity.[8-10] These asthma phenotypes featuring obesity may exhibit eosinophilic, noneosinophilic, or mixed populations of eosinophils and neutrophils in sputum,[8-10] which suggests that biologic processes driving asthma pathology are heterogeneous among patients with obesity.

T2 asthma persists in adulthood and comprises approximately 50% of adult asthma.[11] Indeed, individuals with asthma and comorbid obesity exhibit elevated airway levels of the important T2 cytokines and eosinophil regulators IL-5 and IL-13.[12-14]

From [a]the Department of Medicine, Duke University Medical Center, Durham; [b]the Department of Pediatrics, University of North Carolina, Chapel Hill; [c]the Department of Medicine, Icahn School of Medicine at Mount Sinai, New York; [d]Duke Human Vaccine Institute, Duke University, Durham; [e]the Department of Internal Medicine, Yale University, New Haven; [f]the Department of Medicine, University of Michigan, Ann Arbor; and [g]the Department of Pathology, Duke University Medical Center, and [h]the School of Nursing, Duke University, Durham.

*These authors contributed equally to this work.

Received for publication May 16, 2024; revised October 18, 2024; accepted for publication October 22, 2024.

Corresponding author: Jennifer L. Ingram, PhD, Department of Medicine, Duke University Medical Center, Box 2641 DUMC, 203 Research Dr, Durham, NC 27710. E-mail: jennifer.ingram@duke.edu.

0091-6749/$36.00

https://doi.org/10.1016/j.jaci.2024.10.039

Recent reports suggest that submucosal eosinophils are elevated in airways of both atopic and nonatopic individuals with asthma and comorbid obesity (despite low levels of sputum eosinophils)[12,15,16] and that eosinophil recruitment to the lung tissue of participants with asthma and obesity is increased compared with lean participants with asthma.[17] Similar reports show that tissue eosinophilia is increased in obese mice.[18-20]

Eotaxins (eotaxin-1 [*CCL11*], eotaxin-2 [*CCL24*], and eotaxin-3 [*CCL26*]) are eosinophil-specific C-C chemokines that serve as key chemotactic agents for eosinophil-mediated airway inflammation in T2 asthma.[21,22] Eotaxin-1 and eotaxin-3 are secreted by various airway structural cells, including airway fibroblasts, and are factors attracting eosinophils to sites of allergen-induced airway injury.[22] The importance of eotaxin-1 in asthma pathobiology is supported by evidence from animal modeling (eotaxin-3 is a pseudogene in mice[23,24]), wherein allergen-induced eosinophil recruitment to the airway was inhibited in eotaxin-1–deficient mice.[25]

The T2 cytokine IL-13 is a critical mediator of airway inflammation in asthma.[26,27] IL-13 stimulates eotaxin-1 production in mouse and human lung fibroblasts, and this paracrine signaling may be sufficient to initiate eosinophilic airway infiltration.[28,29] IL-13 elicits responses via a receptor complex comprising a dimer of the IL-13 receptor alpha 1 (IL-13Rα1) and the IL-4 receptor alpha (IL-4Rα), present on the cell surface of various airway cell types.[30-32] Distinct from these IL-13R subunits, IL-13 receptor alpha 2 (IL-13Rα2) was first considered a possible IL-13 decoy receptor, because it has no known signaling motif, and IL-13 binds IL-13Rα2 with high affinity.[33] Thus, IL-13Rα2 was thought to serve as a negative regulator of IL-13 signaling; however, subsequent reports demonstrated that IL-13Rα2 participates in cellular signaling in immune, lung, and intestinal cells.[34-36]

Another important signaling mediator involved in both obesity and asthma is leptin. Leptin is a proinflammatory adipokine.[37] Although leptin is elevated in obesity, it also appears to be independently elevated in asthma because individuals with asthma have higher bronchoalveolar lavage fluid (BALF) leptin levels regardless of obesity.[38,39] Furthermore, leptin-producing cells are found in higher numbers in the airway submucosa of participants with asthma compared with participants without asthma.[40] Leptin has direct effects on eosinophils; it amplifies the chemotactic response of eosinophils to eotaxin and delays eosinophil apoptosis.[41,42]

On the basis of these data, we hypothesized that localized leptin signaling potentiates airway tissue eosinophilia in T2 asthma and comorbid obesity by augmenting IL-13–induced eotaxin production by airway fibroblasts. We also hypothesized that IL-13Rα2, rather than acting as a decoy, participates in this cell signaling pathway. We report here that primary airway fibroblasts from participants with T2 asthma and obesity have elevated responses to combined IL-13 and leptin compared with cells from lean participants with T2 asthma and that inhibition of IL-13Rα2 downregulates the effects of leptin on these cells.

METHODS

Detailed methods are provided in this article's Methods section in the Online Repository at www.jacionline.org.

Human study population

A total of 161 participants (82 with asthma and 79 without asthma; Table I) were recruited from the Durham, NC, community. The study was approved by the Duke University Institutional Review Board (no. Pro00115889), and all participants provided consent before initiation of study procedures. Participants with asthma fulfilled the criteria as defined by the Global INitiative for Asthma guidelines.[43] They exhibited a provocative concentration of methacholine resulting in a 20% fall in FEV_1 (PC_{20} FEV_1) of less than 8 mg/mL and reversibility, or as demonstrated by at least a 12% and a 200-mL increase in FEV_1 or forced vital capacity (FVC) with inhaled albuterol. All participants with asthma were atopic, evidenced by at least 1 positive skin test result to common North Carolina allergens associated with a correlative clinical history. No participants with asthma were on biologic therapies. All nonasthma participants had no clinical history of atopy and showed negative skin test results. Participants with obesity exhibited a body mass index (BMI) of 30.0 kg/m^2 or higher; overweight participants exhibited BMIs greater than or equal to 25.0 kg/m^2 and less than or equal to 29.9 kg/m^2; lean participants exhibited BMIs less than or equal to 24.9 kg/m^2.

At the baseline visit, pre- and postalbuterol spirometry, blood sampling, Asthma Control Questionnaire,[44] and fractional concentration of exhaled nitric oxide (FENO) testing (Niox Vero, Morrisville, NC) were performed and participants' medical history, anthropometric data, including height, weight, and waist circumference measurements, were collected. Complete blood cell counts with manual differentials were performed. Participants underwent bronchoscopy with endobronchial biopsy and bronchoalveolar lavage. BALF was centrifuged to pellet cells that were transferred to slides, and manual cell differentials were performed.

Mouse models

Animal care and experimental protocols were approved by the Duke University Institutional Animal Care and Use Committee and carried out in accordance with the Association for the Accreditation of Laboratory Animal Care international guidelines. All mice were housed in microisolator cages (5 mice/cage) on corn cob bedding in pathogen-free facilities at Duke University and fed a standard chow diet, *ad libitum*. Five-week-old male wild-type (WT) C57BL/6J, *db/db*, and *ob/ob* mice (Jackson Laboratory, Bar Harbor, Me) were intranasally administered house dust mite (HDM; *Dermatophagoides pteronyssinus*) allergen (1.25 μg/μL in 40 μL saline;XPB82D3A2.5, lot no. 198713, Greer Laboratories, Lenior, NC) or saline as control 5 days per week for 6 weeks under light isoflurane anesthesia. In a separate cohort, 5-week-old male and female C57BL/6J mice (Jackson Laboratory) were intranasally administered HDM allergen or saline along with subcutaneous injections of leptin or saline 5 days per week for 6 weeks as described.[45] Mice were weighed at weeks 0 and 6 of the protocol. At 6 weeks and 24 hours after the final dose of allergen/saline, the mice were sacrificed by lethal injection of urethane and tissues (blood, BALF, and left and right lungs) were harvested immediately.

Pulmonary fibroblast culture

Samples from subsets of the participant cohort were analyzed *ex vivo*. These subsets were determined by the availability of

TABLE I. Demographic characteristics and clinical data of the study cohort

Characteristics	All	Participants with asthma (n = 82)			
		Lean	Overweight	Obesity	*P* value
N	82	27	18	37	*.04*
Age (y), median (range)	28 (18-59)	22 (19-48)	27 (18-39)	38 (19-59)	*<.01*
Sex, female/male (% female)	60/22 (73)	18/9 (67)	8/10 (44)	34/3 (92)	*<.01*
Race, White/Black/Asian/Other (% Black)	48/28/4/2 (34)	18/4/3/2 (15)	11/6/1/0 (33)	19/18/0/0 (49)	*.03*
Ethnicity, Hispanic/Non-Hispanic/unknown (% Hispanic)	3/77/2 (4)	1/24/0 (4)	0/18/0 (0)	2/35/0 (5)	.27
Age (y) of asthma onset,* median (range)	11 (0-45)	11 (0-30)	6 (0-28)	11 (0-45)	.10
BMI (kg/m^2), median (range)	27.0 (18.0-59.0)	22.0 (18.0-24.0)	26.0 (25.0-29.0)	39.0 (30.0-59.0)	*<.01*
Mean ACQ score ± SEM	1.45 ± 0.10	1.42 ± 0.22	1.26 ± 0.14	1.54 ± 0.15	.57
Prebronchodilator FEV_1 (L), mean ± SEM	2.95 ± 0.08	3.24 ± 0.11	3.35 ± 0.17	2.55 ± 0.10	*<.01*
Prebronchodilator FVC (L), mean ± SEM	3.94 ± 0.11	4.32 ± 0.13	4.80 ± 0.23	3.26 ± 0.12	*<.01*
Prebronchodilator FEV_1/FVC, mean ± SEM	0.76 ± 0.01	0.75 ± 0.02	0.70 ± 0.02	0.78 ± 0.02	*.02*
Prebronchodilator FEV_1% predicted, mean ± SEM	86.7 ± 1.6	90.1 ± 3.0	88.2 ± 3.2	83.5 ± 2.2	.17
Methacholine PC_{20} (mg/mL), median (range)	0.54 (0.0014-13.45)	0.78 (0.0014-10.56)	0.30 (0.09-2.10)	0.70 (0.04-13.45)	.23
FENO (ppm), median (range)	23.5 (0.7-193.4)	29.8 (6.2-87.0)	40.6 (9.0-193.4)	17.7 (0.7-106.0)	.18
IgE (IU/mL), median (range)	94.0 (29.0-1100.0)	195.0 (42.0-1100.0)	ND	90.5 (29.0-210.0)	.38
Absolute blood eosinophil counts (cells/μL), mean ± SEM	242.4 ± 28.1	239.3 ± 59.5	285.8 ± 62.1	221.7 ± 36.0	.82
Absolute blood neutrophil counts (cells/μL), mean ± SEM	3831.7 ± 340.4	3300.0 ± 198.3	3277.8 ± 312.6	4300.0 ± 604.9	.54
Asthma medication use, yes/no (% yes)					
ICS	7/75 (8.5)	0/27 (0)	1/17 (5.6)	6/31 (16.2)	.06
LABA	5/77 (6.1)	0/27 (0)	1/17 (5.6)	4/33 (10.8)	.20
ICS/LABA	19/63 (23.2)	5/22 (18.5)	1/17 (5.6)	13/24 (35.1)	*.04*
LTRA	6/76 (7.3)	0/27 (0)	2/16 (11)	4/33 (10.8)	.20
Comorbidities, yes/no (% yes)					
Hypertension	5/77 (6.1)	0/27 (0.0)	0/18 (0.0)	5/32 (15.6)	*.04*
Diabetes mellitus	3/79 (3.7)	0/27 (0.0)	0/18 (0.0)	3/34 (8.1)	.15
GERD	19/63 (23.2)	4/23 (14.8)	0/18 (0.0)	15/22 (40.5)	*<.01*
Obstructive sleep apnea	4/78 (4.9)	0/27 (0.0)	0/18 (0.0)	4/33 (10.8)	.08
Allergic rhinitis	9/73 (11.0)	2/25 (7.4)	1/17 (5.6)	6/31 (16.2)	.38
Not reported	0	0	0	0	

ACQ, Asthma Control Questionnaire; *GERD*, gastroesophageal reflux disease; *LABA*, long-acting β-agonist; *LTRA*, leukotriene receptor antagonist; ND, not determined.
*Physician diagnosis or self-reported onset of symptoms.

viable cultured airway fibroblasts for experiments. Human airway fibroblasts and mouse lung fibroblasts were cultured in 12-well plates as described.[31,45] At 80% to 90% confluency, cultures were placed in serum-free media for 24 hours. Each sample was then incubated for 24 hours with the following in triplicate: 10, 20, and 100 ng/mL leptin, 50 ng/mL IL-13, combined IL-13 and leptin, and untreated control (serum-free media). Some cultures were pretreated with 25 ng/mL IL-13Rα2 neutralizing antibody or control goat IgG antibody (R&D Systems, Minneapolis, Minn) for 2 hours before treatments. Cell culture supernatants (1 mL/well) and RNA were collected from each well. RNA was isolated using TRIzol reagent (Gibco, Waltham, Mass) according to the manufacturer's protocol.

Pulmonary tissue eosinophil quantification

Images of major basic protein (MBP)-stained human airway tissue sections (1-2 sections per participant) were captured at 10× magnification and imported into ImageJ software. Nonoverlapping images of each section (4-5 per section) were obtained. Before analysis, all images were edited using ImageJ software to have uniform white backgrounds and to exclude cartilage and glands. Relative quantities of MBP staining were determined by manual counting of immunopositive cells within the submucosal tissue area of airway biopsy section.[46] For mouse lung, eosinophils were enumerated by assessing MBP-immunopositive cells in 10 selected fields at 400× magnification. Random areas of lung were selected and fields to be assessed were chosen by centering terminal bronchioles and associated blood vessels in the randomly chosen field. All slides were evaluated by trained pulmonary pathologists in a blinded fashion.

Statistical analysis

Data were analyzed using the JMP (SAS, Cary, NC) and Prism (GraphPad, Boston, Mass) software. Data are expressed as means ± SEM, unless otherwise noted. Outliers were tested using the Robust Regression and Outlier Removal method[47] and were removed where appropriate. Parametric or nonparametric tests were used accordingly (1- or 2-way ANOVA, Wilcoxon rank-sum test, or *t* test), with appropriate posttest, to evaluate significance. A *P* value less than .05 determined significance and is shown in italics in Table I.

RESULTS

Study cohort characteristics

In our study cohort (Table I), participants with asthma exhibited reduced prebronchodilator FEV_1, FEV_1/FVC, and FEV_1% predicted and had positive methacholine PC_{20} testing result compared with nonasthma participants, as expected. Elevated FENO levels, serum IgE levels, and blood eosinophil counts were observed in participants with asthma and were higher relative to nonasthma participants. Obesity was more prevalent in participants with asthma, with those with comorbid obesity exhibiting a greater percentage of the self-identified female sex and inhaled corticosteroid (ICS) use, with reduced prebronchodilator FEV_1, FVC, and FEV_1/FVC, than lean and overweight participants with asthma. Participants who self-identified as Black were more frequently categorized with obesity, and participants with obesity were older than overweight or lean participants regardless of asthma status. Comorbidities were similar between asthma and nonasthma participants. Participants with obesity exhibited greater frequencies of hypertension and

TABLE I. *(Continued)*

All	Nonasthma participants (n = 79)				*P* value, asthma vs nonasthma
	Lean	Overweight	Obesity	*P* value	
79	33	17	29	.07	.81
29 (19-61)	26 (19-48)	27 (21-61)	34 (19-57)	*<.01*	.61
56/23 (71)	22/11 (67)	11/6 (65)	23/6 (79)	.45	.75
47/27/3/1 (34)	25/5/3/0 (15)	10/7/0/0 (41)	12/15/0/2 (52)	*.03*	.84
5/74/0 (6)	2/31/0 (6)	1/16/0 (6)	2/27/0 (7)	.99	.29
25.8 (18.0-46.5)	23.0 (18.0-24.5)	26.0 (25.0-29.0)	38.0 (30.0-46.5)	*<.01*	.26
3.40 ± 0.08	3.56 ± 0.14	3.56 ± 0.18	3.12 ± 0.12	.07	*<.01*
4.22 ± 0.11	4.33 ± 0.17	4.58 ± 0.26	3.88 ± 0.15	.06	.08
0.81 ± 0.01	0.83 ± 0.01	0.78 ± 0.01	0.81 ± 0.01	.08	*<.01*
98.0 ± 1.2	99.2 ± 2.0	97.1 ± 2.1	97.1 ± 1.9	.70	*<.01*
>16.00	>16.00	>16.00	>16.00	1.00	*<.01*
13.0 (6.0-60.5)	13.0 (6.0-60.5)	19.5 (6.0-33.0)	14.7 (6.0-48.0)	.40	*<.01*
44.5 (9.0-810.0)	63.5 (11.0-810.0)	69.5 (48.0-114.0)	15.5 (9.0-104.0)	.16	*.02*
128.5 ± 15.5	100.0 ± 15.2	172.2 ± 52.5	130.6 ± 20.1	.59	*<.01*
3715.8 ± 258.9	3557.1 ± 453.6	2611.1 ± 293.2	4526.7 ± 374.2	*<.01*	.79
5/48 (9.4)	0/21 (0.0)	0/10 (0.0)	5/17 (22.7)	*.02*	.47
2/51 (3.8)	1/20 (4.8)	0/10 (0.0)	1/21 (4.5)	.78	.97
8/45 (15.1)	1/20 (4.8)	0/10 (0.0)	7/15 (31.8)	*.02*	.25
5/48 (9.4)	0/21 (0.0)	0/10 (0.0)	5/17 (22.7)	*.02*	.30
2/51 (3.8)	0/21 (0.0)	1/9 (10.0)	1/21 (4.5)	.38	.14
26	12	7	7		

gastroesophageal reflux disease, regardless of asthma status. Interestingly, nonasthma participants with obesity exhibited elevated blood neutrophil counts and greater frequency of obstructive sleep apnea compared with overweight and lean participants, but this relationship was not observed in participants with asthma.

Airway eosinophilia in participants with asthma and comorbid obesity

To confirm that participants with asthma and comorbid obesity exhibit increased airway tissue eosinophilia, as reported,[12,15,16] we immunostained airway biopsy tissue sections derived from individuals across the body mass spectrum with an antibody specific for MBP, an eosinophil marker. We observed that, in asthma, participants with obesity have increased MBP-positive cells in airway tissue compared with lean participants with asthma ($P = .007$) (Fig 1, *A*), with the number of airway tissue MBP-positive cells positively associated with BMI in asthma (Fig 1, *B*). Participants with asthma and obesity had significantly elevated numbers of MBP-positive cells in airway tissue compared with those with lean body mass regardless of ICS use ($P = .01$ for no ICS use in obesity compared with lean; $P = .006$ for reported ICS use in obesity compared with lean); however, no difference was found in participants with asthma and obesity between those with or without ICS use ($P = .10$) (see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast, associations between the number of airway tissue MBP-positive cells and percent BALF eosinophils, blood eosinophil counts, or FENO levels in participants with asthma were not observed (Fig 1, *C-E*). Percent BALF eosinophils or blood eosinophil counts were not different between body mass groups in asthma ($P = .57$ in asthma and $P = .59$ in nonasthma for BALF; $P = .82$ in asthma and $P = .67$ in nonasthma for blood) (Fig 1, *F*; see also Fig E2, *A*, in this article's Online Repository at www.jacionline.org), and no associations were observed between percent BALF eosinophils or blood eosinophil counts and BMI in either asthma or nonasthma participants (Fig 1, *G*; see also Fig E2, *B*). Blood eosinophil counts were elevated in participants with asthma compared with nonasthma participants in the respective body mass groups ($P = .03$ for lean; $P = .05$ for overweight; and $P = .03$ for obesity) (Fig E2, *A*). Waist circumference measurements were recorded in a subset of participants, and these measurements were strongly and negatively correlated with percent BALF eosinophils in participants with asthma but not in nonasthma participants (Fig 1, *H*). Blood eosinophil counts were not associated with waist circumference in either asthma or nonasthma participants (Fig E2, *C*). In the same subset of participants in which waist circumference was measured, no relationship was observed between percent BALF eosinophils or blood eosinophil counts and BMI in either asthma or nonasthma participants (Fig 1, *H*; see also Fig E2, *C*). Similar to previous reports,[12-14] we observed that BALF IL-5 concentrations were associated with BMI in participants with asthma, but no relationship between airway IL-5 and body mass was observed in nonasthma participants (Fig E2, *D*).

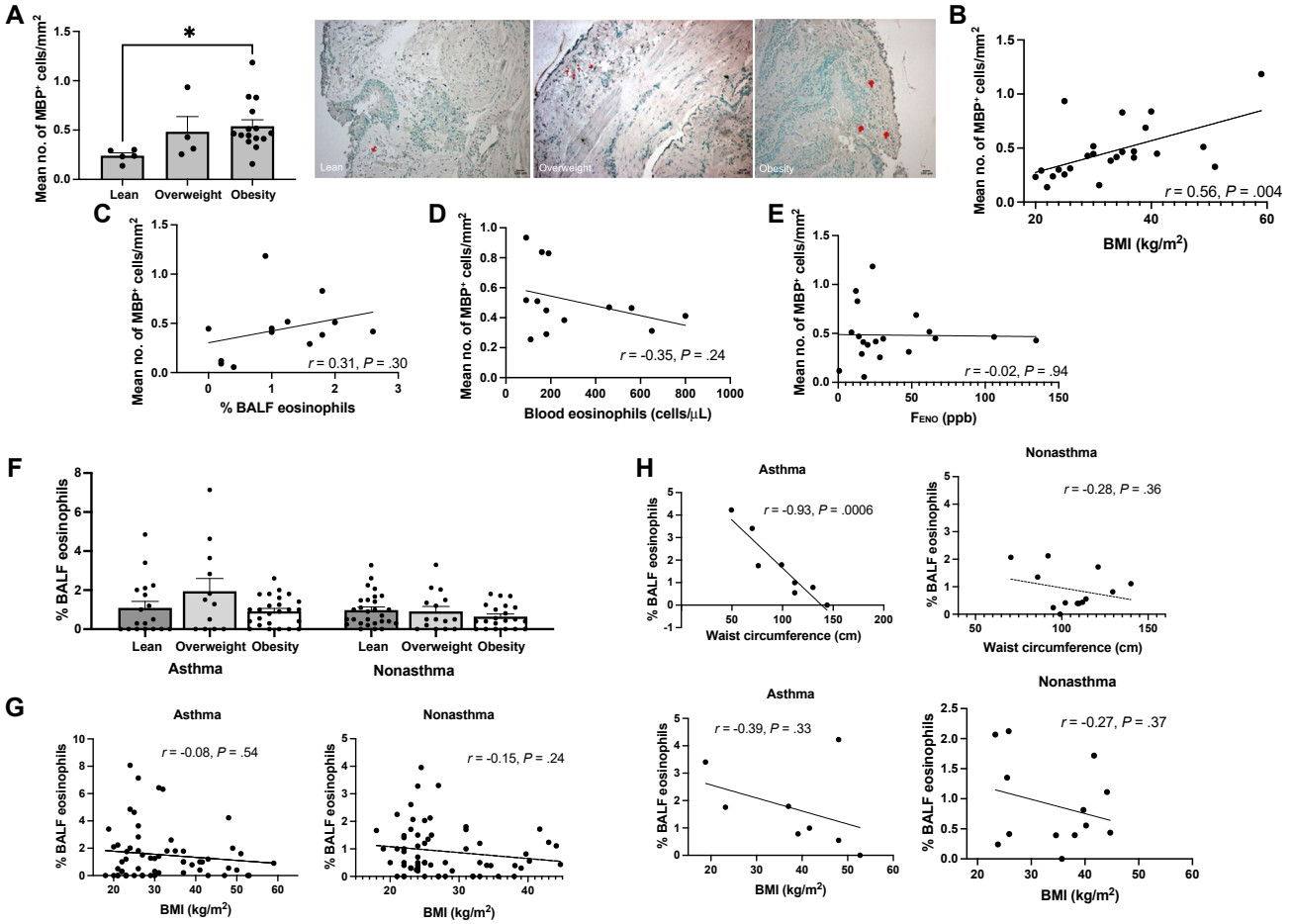


FIG 1. Airway tissue and lumen eosinophilia in participants with asthma and comorbid obesity. **A,** Mean number of MBP-positive (MBP^+) cells/mm^2 ± SEM in airway biopsy tissue of participants with asthma by body mass group (n = 4-16 per group). *$P < .05$. Representative images of MBP staining in airway biopsy tissue obtained from participants with asthma: lean (*left*), overweight (*middle*), and obese (*right*). Arrows indicate MBP^+ cells. **B,** Mean number of MBP^+ cells/mm^2 in association with BMI in participants with asthma. **C,** Mean number of MBP^+ cells/mm^2 in association with percent BALF eosinophils in participants with asthma and comorbid obesity. **D,** Mean number of MBP^+ cells/mm^2 in association with blood eosinophil counts in participants with asthma and comorbid obesity. **E,** Mean number of MBP^+ cells/mm^2 in association with FENO in participants with asthma and comorbid obesity. **F,** Mean percent eosinophils ± SEM in BALF by body mass group in asthma (n = 12-25 per group) and nonasthma (n = 15-26 per group) participants. **G,** Relationship of percent BALF eosinophils with BMI in asthma and nonasthma participants. **H,** Relationship of percent BALF eosinophils with measures of waist circumference (*top*) or BMI (*bottom*) in a subset of asthma (*left*) and nonasthma (*right*) participants.

Leptin levels in overweight or obese participants with asthma

Next, we investigated the association of leptin with eosinophil levels in BALF in participants with asthma. As expected, serum leptin levels were elevated in both asthma and nonasthma participants with overweight and obesity compared with lean participants ($P < .002$ for each comparison), with no difference between asthma and nonasthma in overweight and obese participants ($P = .34$) (Fig 2, *A*). Also, in a subset of participants, BALF leptin levels were greater in overweight and obese participants compared with lean participants, and both serum and BALF leptin levels were associated with waist circumference in this subset of participants with asthma for which both measures were available (Fig 2, *B*; see also Fig E3, *A* and *C*, in this article's Online Repository at www.jacionline.org). In agreement with previous reports,[38,39] we found that participants with asthma and comorbid overweight and obesity exhibited elevated leptin levels in BALF compared with nonasthma participants with obesity ($P = .04$) (Fig 2, *B*). Furthermore, BALF leptin levels were associated with BMI only in participants with asthma and not in nonasthma participants (Fig E3, *D*). Because eotaxin-1 is a potent eosinophil chemoattractant, we measured BALF eotaxin-1 levels in a subset of our study cohort, and we found that BALF eotaxin-1 was elevated in participants with asthma ($P = .02$), particularly in those with comorbid obesity ($P = .02$) (see Fig E4, *A*, in this article's Online Repository at www.jacionline.org). These measures were not associated with serum or BALF leptin levels (Fig E4, *B* and *C*).

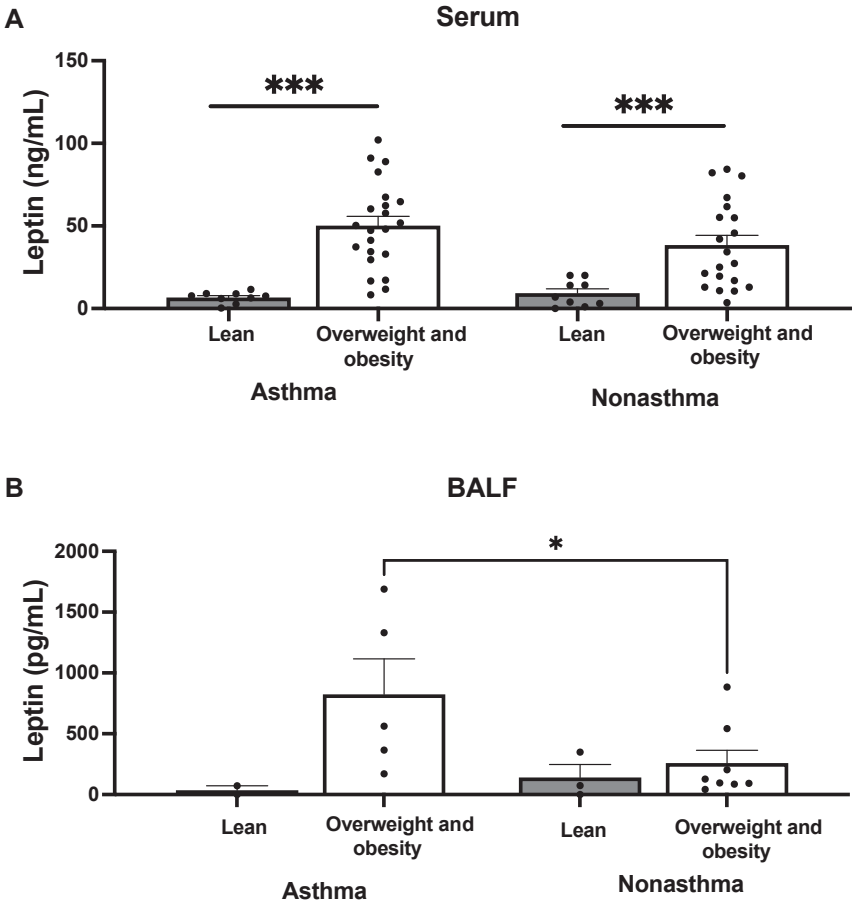


FIG 2. Leptin levels are elevated in overweight and obese participants with asthma. **A,** Mean serum leptin levels ± SEM in asthma and nonasthma participants with (n = 20-22 per group) and without (n = 9-10 per group) overweight and obesity. ***$P < .001$. **B,** Mean BALF leptin levels ± SEM in asthma and nonasthma participants with (n = 5-8 per group) and without (n = 3 per group) overweight and obesity. *$P < .05$.

Leptin augments IL-13–induced eotaxin production by human airway fibroblasts

Our studies have shown that airway fibroblasts derived from participants with asthma exhibit altered cellular functions in response to proinflammatory stimuli compared with cells derived from nonasthma participants.[31] We hypothesized that airway fibroblasts from participants with asthma respond differently to leptin than cells from nonasthma participants, and these responses may influence airway tissue eosinophilia. To test this hypothesis, primary airway fibroblasts were cultured from airway biopsy tissue of asthma and nonasthma participants and incubated with 50 ng/mL IL-13 as a positive control, increasing doses of leptin, combined IL-13 with increasing doses of leptin, or serum-free media as control for 24 hours. These concentrations of leptin are within the physiologic range of leptin levels observed in serum from study participants (Fig 2, *A*). We then measured relative mRNA and protein levels of eotaxin-1 (*CCL11*) and eotaxin-3 (*CCL26*). Incubation of airway fibroblasts of participants with asthma with 100 ng/mL leptin or combined 20 ng/mL leptin with IL-13 stimulated increased eotaxin-1 (*CCL11*) mRNA expression compared with similarly treated cells from nonasthma participants ($P = .02$ for leptin alone; $P = .02$ for combined leptin and IL-13). The combination of 100 ng/mL leptin and IL-13 induced increased eotaxin-1 (*CCL11*) mRNA expression in airway fibroblasts of participants with asthma compared with the effect of IL-13 alone ($P = .03$) (Fig 3, *A*). No differences were observed in eotaxin-1 secretion in combined leptin and IL-13 treatments relative to IL-13 alone in airway fibroblasts of either asthma or nonasthma participants ($P > .05$ for all comparisons) (Fig 3, *B*). For eotaxin-3 (*CCL26*) mRNA expression, we observed that in airway fibroblasts of both asthma and nonasthma participants, combined IL-13 and leptin (20 or 100 ng/mL) induced elevated expression levels relative to leptin alone at those doses (20 ng/mL leptin: $P = .02$ for asthma and $P = .01$ for nonasthma; 100 ng/mL leptin: $P = .01$ for asthma and $P = .004$ for nonasthma) (Fig 3, *C*). Furthermore, eotaxin-3 secretion by airway fibroblasts in asthma, but not in nonasthma, was increased by combined IL-13 and leptin (10 or 100 ng/mL) treatments relative to IL-13 alone ($P = .04$ for IL-13 + 10 ng/mL leptin; $P = .04$ for IL-13 + 100 ng/mL leptin) (Fig 3, *D*).

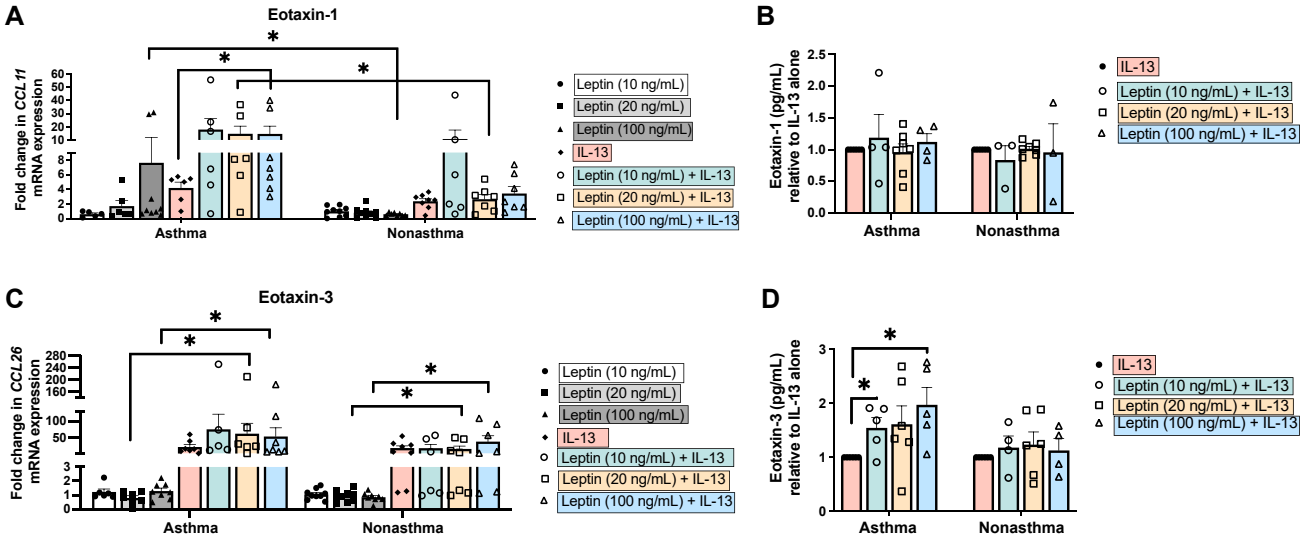


FIG 3. A-D, Leptin augments IL-13–induced eotaxin-1 and eotaxin-3 production by human airway fibroblasts. Cultured airway fibroblasts from asthma and nonasthma participants were incubated with serum-free media (untreated control), leptin (10, 20, or 100 ng/mL), IL-13 (50 ng/mL), or combined leptin and IL-13 treatments for 24 hours. Total RNA and cell supernatants were collected. Quantitative RT-PCR revealed relative expression of eotaxin-1 (*CCL11*) (Fig 3, *A*) and eotaxin-3 (*CCL26*) (Fig 3, *C*) mRNA in each treatment group (n = 6-9 for asthma; n = 7-10 for nonasthma). Data are expressed as mean fold change in *CCL11* or *CCL26* mRNA expression ± SEM normalized to *GAPDH* mRNA expression and to *CCL11* and *CCL26* expression levels of the untreated control. Eotaxin-1 (Fig 3, *B*) and eotaxin-3 (Fig 3, *D*) protein levels in cell supernatants as determined by ELISA (n = 4-7 for asthma; n = 3-11 for nonasthma per treatment group). Data are expressed as mean fold change ± SEM in eotaxin-1 or eotaxin-3 protein levels relative to the IL-13 treatment group. **P* < .05.

IL-13Rα2 expression in human airway fibroblasts is associated with body mass and enhanced with leptin and IL-13 in asthma

We hypothesized that leptin may be modulating IL-13R subunit expression on airway fibroblasts, leading to changes in IL-13 signaling and stimulation of eotaxin production. To test this hypothesis, we first evaluated mRNA levels of each of the IL-13 receptor subunits in airway fibroblasts from asthma and nonasthma participants. Airway fibroblast *IL13RA2* mRNA expression was not different between untreated control cells from asthma compared with nonasthma participants ($P = .70$) (Fig 4, *A*). However, when stratifying these data by body mass groups, *IL13RA2* mRNA expression was markedly elevated in untreated control cells from participants with overweight and obesity compared with cells from lean participants regardless of asthma status ($P = .005$ for asthma; $P = .03$ for nonasthma) (Fig 4, *B*). Indeed, an association was noted between airway fibroblast *IL13RA2* mRNA expression and BMI in both asthma and nonasthma participants (Fig 4, *C*). We previously reported that immunostaining of IL-13Rα2 in airway tissue of participants with asthma was increased relative to nonasthma participants.[31] We now report a positive association between submucosal airway tissue immunostaining of IL-13Rα2 and BMI in participants with asthma (Fig 4, *D*). This association was not apparent in nonasthma participants (Fig 4, *D*). To investigate specific effects of leptin on airway fibroblast expression of *IL13RA2*, cells from asthma and nonasthma participants were incubated with leptin, IL-13, and combined IL-13 and leptin treatments for 24 hours. Combined IL-13 and leptin stimulated increased *IL13RA2* mRNA expression compared with IL-13 alone ($P = .03$) or leptin alone ($P = .04$) in the airway fibroblasts of participants with asthma, but no effect was observed in cells from nonasthma participants ($P > .05$ for all comparisons) (Fig 4, *E*). Although airway fibroblast *IL13RA1* mRNA expression was elevated in asthma compared with nonasthma ($P = .002$), no differences were observed for airway fibroblast *IL4RA* mRNA expression in asthma versus nonasthma or for *IL13RA1* and *IL4RA* mRNA expression in either asthma or nonasthma participants with overweight and obesity versus lean participants ($P > .05$ for all comparisons), and no association was found between BMI measurements and airway fibroblast *IL13RA1* and *IL4RA* mRNA expression or IL-13Rα1 and IL-4Rα airway tissue immunostaining (see Fig E5 in this article's Online Repository at www.jacionline.org).

Exogenous leptin augments lung eosinophilia in allergen-challenged mice

To further investigate the mechanism whereby leptin mediates tissue eosinophilia and eotaxin production in the lung, we used a mouse model of chronic allergen exposure to induce allergic airways disease in lean mice concurrently with exogenous leptin administration.[45] At the end of the 6-week protocol, lung tissue, BALF, and blood were collected (Fig 5, *A*). We previously reported that IL-5 and eotaxin-1 levels in BALF were increased in HDM-challenged mice compared with saline-challenged mice, but that leptin did not enhance the airway eotaxin-1 levels of either saline- or HDM-challenged mice and reduced IL-5 levels in HDM-challenged mice.[45] Immunohistochemical staining of mouse lung tissue sections revealed that HDM-challenged mice that received leptin injections exhibited a greater number

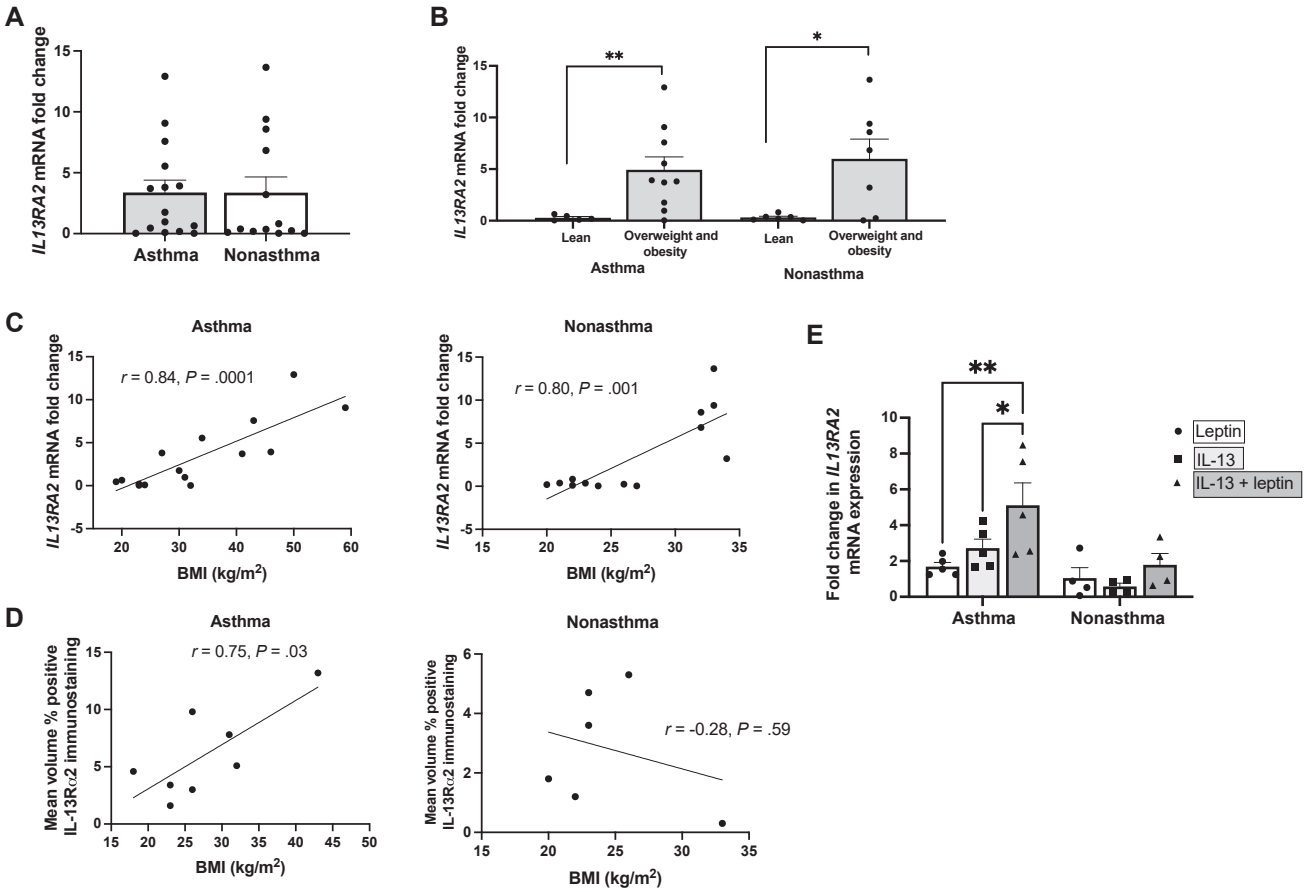


FIG 4. IL-13Rα2 expression in human airway fibroblasts is associated with body mass and enhanced with leptin and IL-13 exposure in asthma. Cultured airway fibroblasts were incubated with serum-free media (untreated control), leptin (20 ng/mL), IL-13 (50 ng/mL), or combined leptin and IL-13 for 24 hours. **A** and **B,** Mean fold change ± SEM in *IL13RA2* was determined by quantitative RT-PCR in untreated control cells of asthma (n = 15) and nonasthma (n = 13) groups (Fig 4, *A*) and of participants with and without overweight and obesity (asthma: n = 5, lean; n = 10, overweight and obesity; nonasthma: n = 6, lean; n = 7, overweight and obesity) (Fig 4, *B*). **P* < .05; ***P* < .01. Data are normalized to β-actin in individual participants and to the mean *IL13RA2* of the nonasthma participants. **C,** Relationship of fold change in *IL13RA2* in untreated control airway fibroblasts with BMI in asthma (*left*) and nonasthma (*right*) participants. Immunohistochemical staining of airway biopsy tissue for IL-13 receptor subunits was previously described.[27] **D,** Relationship of mean volume percent positive IL-13Rα2 immunostaining in airway biopsy tissue with BMI in asthma (*left*) and nonasthma (*right*) participants. **E,** Mean fold change ± SEM in *IL13RA2* expression per treatment group in airway fibroblasts from asthma (n = 5) and nonasthma (n = 4) participants. **P* < .05; ***P* < .01. Data are normalized to *GAPDH* and to *IL13RA2* of the untreated control.

of MBP-positive cells compared with HDM-challenged, saline-injected mice ($P = .04$) or mice that were administered leptin alone ($P < .0001$) (Fig 5, *B* and *C*). In addition, IL-13Rα2 (which occurs in both soluble and cell-surface tethered forms in mice[48]) levels in BALF were increased in mice administered both HDM and leptin compared with those administered leptin alone ($P = .02$) (Fig 5, *D*).

Lack of leptin signaling reduces IL-13–induced eotaxin-1 secretion and *Il13ra2* expression by mouse lung fibroblasts

To determine the effect of leptin or leptin receptor–mediated pathways on IL-13Rα2 signaling, we performed an experiment in which we administered chronic intranasal HDM allergen or saline to lean WT C57BL6 mice or to obese mice deficient in leptin (*ob/ob*) or in signaling through the long isoform of the leptin receptor (*db/db*) (Fig 6, *A*). Body weight was markedly elevated in both HDM- and saline-challenged *db/db* and *ob/ob* mice relative to WT mice at the end of the 6-week protocol ($P < .0001$ for all comparisons) (Fig 6, *B*). As expected, serum leptin levels were highly elevated in obese *db/db* mice compared with lean WT mice ($P = .02$ for HDM-challenged mice; $P = .03$ for saline-challenged mice), and allergen challenge did not influence circulating leptin levels ($P = .82$ in *db/db* mice; $P = .54$ in WT mice). Serum leptin was undetectable in *ob/ob* mice (*db/db* vs *ob/ob*: $P = .03$ for HDM-challenged mice; $P = .02$ for saline-challenged mice) (Fig 6, *C*). Also, as expected,

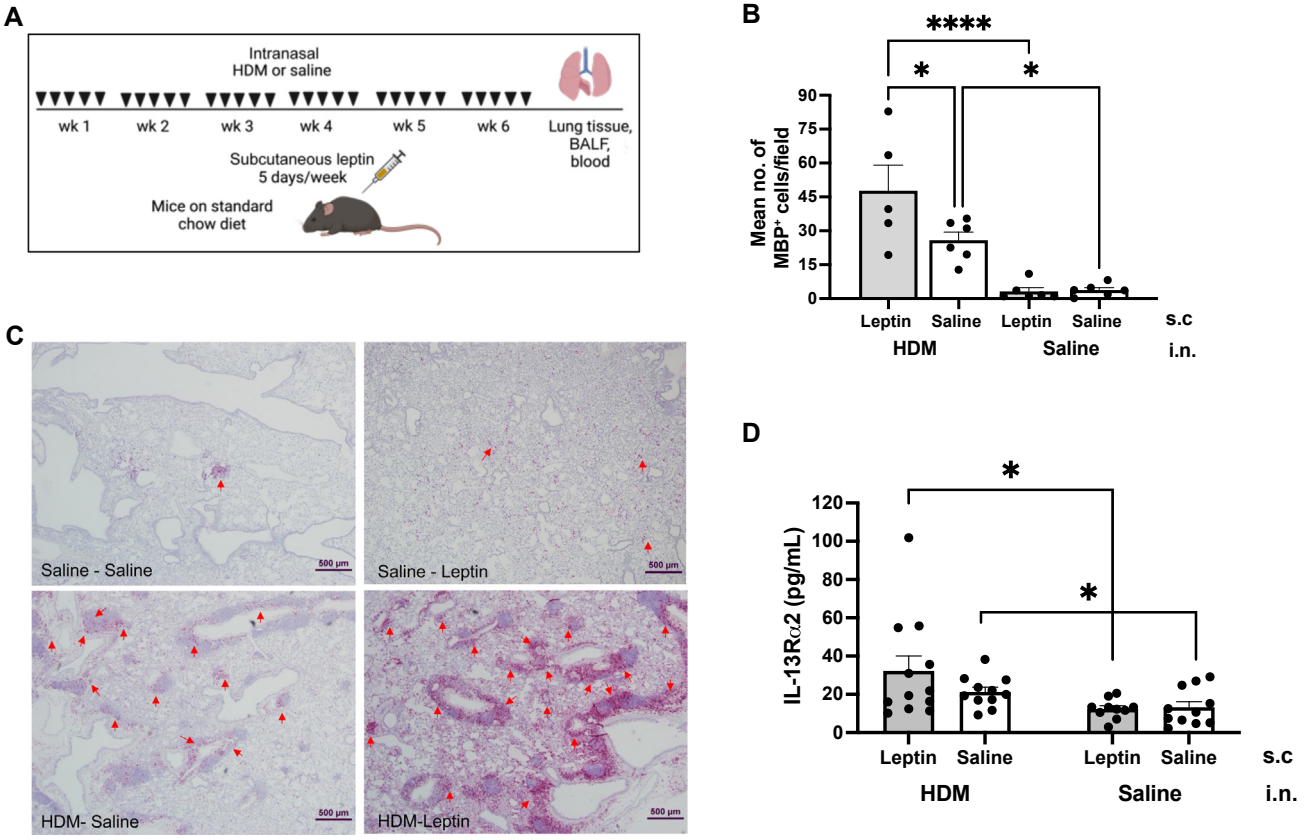


FIG 5. Chronic exogenous leptin administration in HDM-challenged mice augments lung eosinophilia. **A,** Schematic diagram of chronic HDM allergen mouse model with subcutaneous leptin administration. End points were collected 24 hours following the final dose of HDM allergen. Created with Biorender.com. **B,** Mean number of MBP-positive (MBP^+) cells/field ± SEM in lung tissue of mice per allergen/leptin treatment group (n = 5-6). *$P < .05$; ****$P < .0001$. **C,** Representative images of fields of lung tissue indicating stained MBP^+ cells in each treatment group. Arrows indicate MBP^+ cells. **D,** Mean IL-13Rα2 levels ± SEM in BALF of mice (n = 10-12) per treatment group as measured by ELISA. *$P < .05$.

overall BALF cell counts and peribronchial inflammation scores were elevated in all 3 genotypes of HDM-challenged mice compared with saline-challenged mice, indicating that the chronic allergen challenge model successfully induced allergic airways disease (hematoxylin and eosin scores: $P \leq .0005$ for HDM-challenged mice vs saline-challenged mice in all genotypes) (see Fig E6, *A* and *B*, in this article's Online Repository at www.jacionline.org). Mice deficient in leptin (*ob/ob*) exhibited increased percentages of eosinophils in BALF compared with mice with intact leptin expression (WT and *db/db*) ($P = .04$ for *ob/ob* vs WT; $P = .03$ for *ob/ob* vs *db/db*) (Fig 6, *D*). No differences were observed among the 3 genotypes of mice with regard to BALF levels of IL-13, IL-5, or eotaxin-1 or serum levels of IL-13 in either HDM- or saline-challenged mice ($P > .05$ for all comparisons) (Fig E6, *C-E*). Levels of IL-13Rα2 in BALF were not different between the mouse genotypes, regardless of allergen challenge ($P > .05$ for all comparisons), but serum IL-13Rα2 levels were elevated in HDM-challenged *db/db* mice compared with HDM-challenged WT ($P = .01$) and *ob/ob* ($P = .01$) mice as well as saline-challenged *db/db* mice ($P < .001$) (Fig 6, *E*). In lung fibroblasts cultured from saline-challenged mice, we observed a marked induction of eotaxin-1 secretion following treatment of the cells with IL-13 relative to control-treated cells only in cells from WT mice ($P = .005$), and this effect of IL-13 was reduced in lung fibroblasts from *db/db* ($P = .0006$) and *ob/ob* ($P = .001$) mice (Fig 6, *F*). Also, we found that *Il13ra2* mRNA expression in IL-13–treated lung fibroblasts from saline-challenged *db/db* mice was increased compared with *Il13ra2* expression levels in lung fibroblasts of *ob/ob* mice ($P = .04$) (Fig 6, *G*). Taken together, these data from both mouse models suggest that elevated leptin levels (as seen in *db/db* mice or mice administered with exogenous leptin) promoted airway IL-13Rα2 expression, eotaxin secretion by lung fibroblasts, and retention of eosinophils in lung tissue in the setting of chronic allergic airways disease.

Inhibition of IL-13Rα2 abrogates IL-13 and leptin-induced eotaxin-1 and eotaxin-3 secretion by human airway fibroblasts

We hypothesized that signaling through IL-13Rα2 in airway fibroblasts links leptin with stimulation of eotaxin production by airway fibroblasts. Thus, we preincubated airway fibroblasts with specific neutralizing antibodies for IL-13Rα2 for 2 hours before treating the cells with leptin, IL-13, and combined leptin and IL-13 for 24 hours. We found that the IL-13Rα2 neutralizing antibody treatment reduced leptin-induced eotaxin-1 secretion in airway fibroblasts relative to the effect of the IgG control antibody

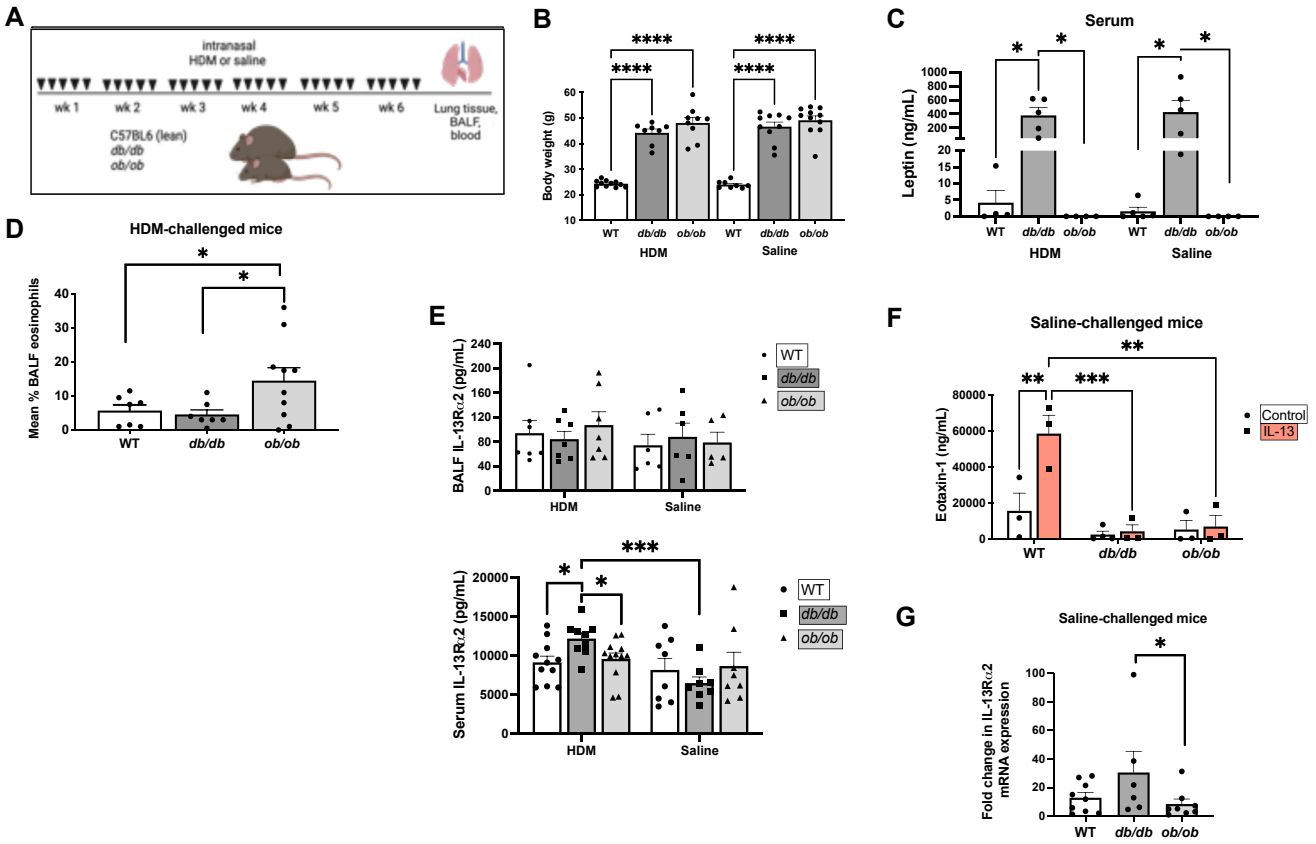


FIG 6. Leptin (*ob/ob*) or leptin receptor (*db/db)* deficiency reduces IL-13–induced eotaxin-1 secretion and *Il13ra2* expression by mouse lung fibroblasts. **A,** Diagram of chronic allergen challenge model in WT (C57BL6), *db/db*, and *ob/ob* mice. Created with Biorender.com. **B,** Mean body weight ± SEM of HDM- or saline-challenged WT (n = 8-11), *db/db* (n = 8-10), and *ob/ob* (n = 9-11) mice at the end of the protocol. ****$P < .0001$. **C,** Mean serum leptin (ng/mL) ± SEM in HDM- or saline-challenged WT (n = 4-5), *db/db* (n = 5), and *ob/ob* (n = 4) mice as measured by ELISA. *$P < .05$. **D,** Mean percent eosinophils ± SEM in BALF of HDM-challenged WT (n = 7), *db/db* (n = 7), and *ob/ob* (n = 10) mice. *$P < .05$. **E,** BALF (*top*) and serum (*bottom*) IL-13Rα2 levels ± SEM in HDM- or saline-challenged WT (n = 6-11), *db/db* (n = 6-9), and *ob/ob* (n = 5-12) mice. *$P < .05$; ***$P < .001$. Cultured lung fibroblasts of naive mice (n = 3-4 mice per genotype) were incubated with serum-free media (control) or 50 ng/mL IL-13 for 24 hours. **F,** Mean eotaxin-1 levels ± SEM in saline-challenged mouse lung fibroblast supernatants. **$P < .01$; ***$P < .001$. **G,** Mean fold change in *Il13ra2* expression ± SEM in IL-13–treated lung fibroblasts isolated from saline-challenged mice. Data were normalized to *Gapdh* and to *Il13ra2* in the untreated control in each mouse. *$P < .05$.

($P < .001$) (Fig 7, *A*). Also, in the presence of IL-13Rα2 neutralizing antibody pretreatment, combined leptin and IL-13, when normalized to the effect of IL-13 alone, reduced eotaxin-1 secretion from airway fibroblasts compared with IgG control antibody pretreatment ($P = .04$) (Fig 7, *B*). Furthermore, IL-13–stimulated eotaxin-3 production was enhanced following IL-13Rα2 antibody treatment compared with IgG control antibody ($P = .03$) (Fig 7, *C*). When normalized to the effect of IL-13 alone, the ability of leptin to augment IL-13–driven eotaxin-3 secretion was specifically inhibited by the IL-13Rα2 antibody compared with IgG control antibody ($P = .03$) (Fig 7, *D*). Therefore, IL-13Rα2 activity was required to positively regulate both eotaxin-1 and eotaxin-3 production in the presence of leptin (even when combined with IL-13) while also acting to negatively regulate IL-13–induced eotaxin secretion by airway fibroblasts (see Table E2 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Although asthma has long been associated with obesity, our study presents the first evidence linking leptin, T2 cytokine signaling, and airway mesenchymal cell production of eosinophil chemotactic factors in association with airway tissue eosinophilia in T2 asthma and obesity. We confirmed reports[12,15,16] that eosinophils localize to the airway submucosa in greater numbers in participants with asthma and obesity compared with nonobese participants with asthma. We also demonstrated that standard biomarkers of eosinophilic inflammation were not associated with measures of airway tissue eosinophilia in participants with asthma and comorbid obesity, as was previously reported.[49] Furthermore, we showed that leptin, IL-5, eotaxin-1, and IL-13Rα2 production in the airways of participants with asthma and obesity was elevated compared with that in nonasthma participants with obesity. Our *in vitro* experiments provide

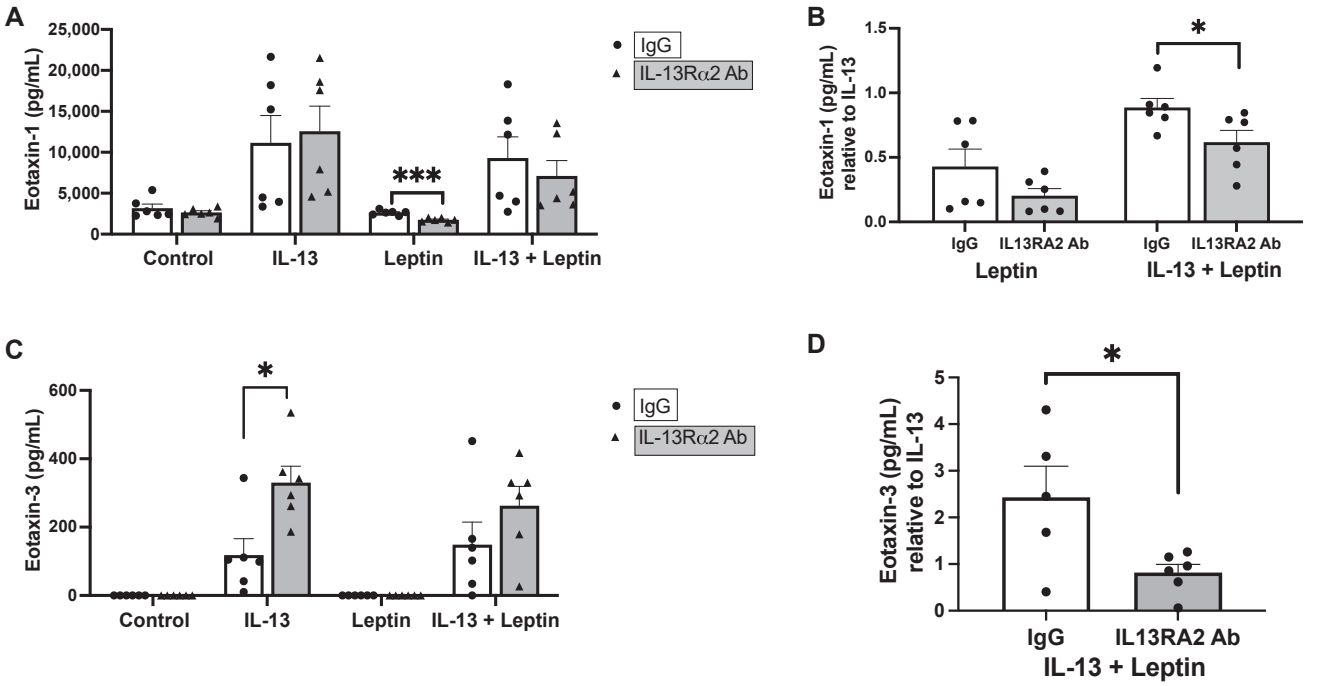


FIG 7. Inhibition of IL-13Rα2 signaling reduces combined IL-13 and leptin-induced eotaxin-1 and eotaxin-3 secretion by human airway fibroblasts. Cultured airway fibroblasts from lean nonasthma participants (n = 2) (experiments repeated 3 times for each participant) were pretreated for 2 hours with either control IgG antibody or IL-13Rα2 neutralizing antibody (25 ng/mL) and then incubated with serum-free media (untreated control), leptin (20 ng/mL), IL-13 (50 ng/mL), or combined leptin and IL-13 treatments for 24 hours. Cell supernatants were collected. **A,** Mean eotaxin-1 levels ± SEM as measured by ELISA in human airway fibroblast cell supernatants. ***$P < .001$. **B,** Mean fold change in eotaxin-1 secretion ± SEM relative to the effect of IL-13 alone. *$P < .05$. **C,** Mean eotaxin-3 levels ± SEM as measured by ELISA in human airway fibroblast cell supernatants. *$P < .05$. **D,** Mean fold change in eotaxin-3 secretion ± SEM relative to the effect of IL-13 alone. *$P < .05$.

evidence that leptin works with T2 cytokine signaling in human airway fibroblasts to promote enhanced levels of eotaxin-1 and eotaxin-3 expression or protein secretion as well as *IL13RA2* expression and that specific inhibition of IL-13Rα2 activity can downregulate eotaxin-1 and eotaxin-3 secretion, particularly eotaxin-3, by these cells in response to combined leptin and IL-13 exposure. To our knowledge, our study is the first to report a requirement of IL-13Rα2 signaling for mediating the effects of leptin and to show that IL-13Rα2 localization in the airway submucosa is elevated in individuals with obesity and asthma. Finally, in mice with chronic allergic airway disease combined with exogenous leptin administration or leptin and leptin receptor deficiency *in vivo*, we confirmed that leptin drives increased allergen-induced mouse lung tissue eosinophilia and concomitant airway IL-13Rα2 secretion and production of eotaxin-1 by lung fibroblasts.

We present data that agree with work by others who reported elevated BALF leptin in participants with asthma and obesity, despite serum leptin levels not differing between groups.[38,39] Sideleva et al[39] also reported elevated BALF leptin levels in asthma that correlated with those found in visceral adipose tissue from participants with asthma and suggested that elevated BALF leptin levels in asthma may be related to the high levels of leptin in adipose tissue draining into the portal circulation and subsequently passing through the vasculature of the lung. Moreover, Watanabe et al[40] showed that leptin-producing immune cells were more abundant in the airway submucosa of lean participants with asthma, even greater than in obese nonasthma individuals; however, these authors did not report BALF leptin levels in these participants. Further research needs to be done to correlate local airway cell expression/secretion of leptin and BALF leptin levels in asthma.

Airway fibroblasts are an important source of eotaxin, expression and secretion of which can be stimulated by leptin.[50-53] Eotaxin-1 colocalized with airway tissue fibroblasts in participants with severe asthma,[50] and in HDM-challenged mice, IL-13–induced eotaxins secreted by lung fibroblasts promoted eosinophil trafficking to pulmonary tissue.[51] In normal human lung fibroblasts, leptin dose-dependently stimulated eotaxin-1 mRNA expression and secretion, an effect that was also dependent on leptin receptor signaling.[52] Similarly, Imoto et al[53] demonstrated that leptin stimulated eotaxin-3 mRNA expression and secretion and enhanced the effects of IL-4 or IL-13 to stimulate eotaxin-3 in primary nasal polyp fibroblasts from participants with chronic rhinosinusitis. Our study extends these reports by demonstrating that leptin, at concentrations that are physiologic on the basis of our study's blood measurements, stimulates eotaxin-1 secretion and augments the effect of IL-13 to induce both eotaxin-1 and eotaxin-3 expression specifically in primary airway fibroblasts from T2 asthma and nonasthma participants. In contrast to the findings by Imoto et al (and our observations in airway fibroblasts with regard to eotaxin-1), we

found no effect of leptin on eotaxin-3 gene expression or secretion by human airway fibroblasts. These observations suggest that leptin stimulates eotaxin-1 and eotaxin-3 through different signaling pathways in the 2 tissues. Further research is warranted to discern the distinct downstream signaling mediators that facilitate leptin-induced eotaxin-1 or eotaxin-3 production.

Obesity influences eosinophil movement in the lung. In participants with asthma and obesity, eosinophils uniquely exhibit increased eotaxin-induced chemotaxis and adhesion activity, an effect associated with serum leptin levels.[54] Furthermore, in allergic participants without obesity, leptin augments eotaxin-induced eosinophil chemotaxis, calcium mobilization, and cytoskeletal rearrangement.[55] Murine obesity models show accumulation of eosinophils in lung tissue facilitated by delaying eosinophil transit to the airway lumen.[18,56] In the absence of obesity, exogenous leptin stimulates elevated eosinophil influx in pleural fluid of ovalbumin-sensitized mice.[41] Yet, we showed that BALF IL-5 levels were reduced in lean allergen-challenged mice in response to exogenous leptin.[45] Thus, leptin alone is sufficient to influence eosinophil trafficking in the lung. Our data herein support this concept as we show that exogenous leptin augments HDM allergen–induced lung tissue eosinophilia, and BALF eosinophils are elevated in obese mice lacking leptin. We also demonstrate that lung fibroblasts from leptin-deficient mice have impaired IL-13–induced eotaxin-1 secretion, suggesting that lack of leptin signaling may also alter other key factors, such as receptors, required to mediate IL-13 signaling.

IL-13Rα2 was once thought to have no signaling activity,[57] but several studies now point to the requirement of IL-13Rα2 signaling in mediating IL-13–induced cellular functions.[58-60] The specific role of IL-13Rα2 signaling in mediating obesity and leptin-influenced inflammation is currently unknown. Duffen et al[61] demonstrated upregulated expression of IL-13Rα2 in adipose tissue of obese mice and humans compared with lean counterparts. Our data show that *db/db* mice, which have a mutated, nonfunctional long isoform of the leptin receptor (Ob-Rb) and are obese with high circulating levels of leptin, have increased serum levels of IL-13Rα2 following allergen challenge and express increased *Il13ra2* in lung fibroblasts compared with WT mice. These findings suggest that the lack of signaling through the Ob-Rb unmasks a process whereby leptin stimulates IL-13Rα2 production. The *db/db* mice express short isoforms of the leptin receptor (Ob-Ra, c, d, e, or f).[62] Ob-Ra is highly expressed in lung tissue,[63] and the short leptin receptor isoforms were attributed to mediating pulmonary responses to ozone in *db/db* mice.[64] Thus, it is possible that the short isoforms of leptin receptor may mediate leptin-induced IL-13Rα2 production by lung fibroblasts. Furthermore, IL-13Rα2 expression in lung fibroblasts can be stimulated *in vitro* with prostaglandin E2 (PGE2).[65] Leptin also enhances the synthesis of PGE2 by lung immune cells,[66] and indomethacin, a PGE2 inhibitor, blocked the combined effects of leptin and IL-13 on eotaxin production in airway smooth muscle cells.[67] Thus, it is plausible to consider a factor such as PGE2 mediating the effect of leptin to augment IL-13–induced IL-13Rα2 expression and initiate downstream signaling to stimulate eotaxin secretion in airway fibroblasts or in other airway mesenchymal cells. Future studies will interrogate the cell signaling pathways involved with airway leptin/IL-13/IL-13Rα2 signaling in the setting of asthma with comorbid obesity.

The strengths of our study include the *ex vivo* investigation of cellular responses using primary human airway fibroblasts from clinically well-characterized asthma and nonasthma participants with and without obesity. Also, we complement these human cell experiments with *in vivo* studies of experimental chronic allergic airway disease in gain-of-function and loss-of-function mouse models with regard to leptin signaling. However, our study has some important limitations. First, the participants included in our study exhibited mild asthma according to the Global INitiative for Asthma guidelines.[43] Analyses of responses in cells from participants with severe asthma and obesity are warranted. Second, in our study, all participants with asthma were atopic, whereas all nonasthma participants showed no evidence of atopy. We found no association between serum IgE and leptin levels in our study cohort (data not shown). However, other reports suggest that in asthma, atopy and serum leptin concentrations are linked.[68,69] Studies that focus on comparisons of atopic and nonatopic participants with asthma in the setting of obesity are needed. Third, participants with asthma and obesity evaluated in our study were overwhelmingly female, which is typical for adult asthma because asthma is more common in women. Yet, we included only male *ob/ob* and *db/db* mice in our *in vivo* studies of chronic allergic airways disease. These obese mutant mice exhibit sexual dimorphism with regard to metabolism, with female mice exhibiting greater variation than male mice[70,71]; therefore, we decided to use male mice in our present study to reduce variability between individuals in our experiments. Future studies should include more male participants with asthma and comorbid obesity and both sexes of mice for *in vivo* studies to discern sex differences, particularly with regard to leptin signaling in directing sex-specific differences in allergen-induced airway hyperresponsiveness[45] and risk for asthma.[72,73] Fourth, we classified participants as lean, overweight, or having obesity on the basis of BMI. Waist circumference provides a more accurate measure of abdominal adiposity and is associated with visceral fat levels.[74-77] Future studies must combine multiple anthropometric indicators to account for heterogeneity in obesity phenotypes. Finally, although the levels of potentially anti-inflammatory adiponectin and other adipokines (eg, resistin) are modulated in association with leptin, obesity, and asthma,[78,79] we did not measure the levels of these adipokines in our study cohort. Future studies will address whether adiponectin or the adiponectin/leptin ratio has an impact on airway tissue eosinophilia in asthma with comorbid obesity.

Our study demonstrates a novel association whereby leptin, an adipokine that circulates in high levels in individuals with obesity, augments the effects of IL-13 in T2 asthma to promote secretion of eotaxins by mesenchymal cells in the airway submucosa. Signaling through IL-13Rα2 on airway fibroblasts mediates the effects of combined leptin and IL-13 to release eotaxins. Thus, our study suggests a new mechanism to explain how obesity can modulate and increase severity of T2 asthma phenotypes by promoting increased airway eosinophilia. IL-13Rα2 may be a potential therapeutic target for improvement of airway tissue eosinophilia in obesity-associated asthma.

DISCLOSURE STATEMENT

This study was supported by the National Institutes of Health (grant no. R01HL130234 to J.L.I., grant nos. R01HL086887 and R01HL153641 to L.G.Q., grant nos. P01HL108793,

R01HL064619, and SCCOR HL-05-009 to M.K., and grant nos. R01ES034350 and R01ES027574 to R.M.T.) and by the American Lung Association/American Academy of Allergy, Asthma & Immunology Allergic Respiratory Disease Award (to J.L.I.).

Disclosure of potential conflict of interest: J. Weiss founded iDream Enterprise LLC and Let's Diversifi PBLLC. R. M. Tighe received grants from Genentech and Boehringer Ingelheim; and completed a consulting relationship with Proctor & Gamble. J. L. Ingram received a grant from Sanofi/Regeneron. L. G. Que received grants from Sanofi/Regeneron and Boehringer Ingelheim. The rest of the authors declare that they have no relevant conflicts of interest.

We thank the following individuals for assistance with this study: Tony Church, Jeffrey Groth, Wesley Sigmon, Jordan Wong, Michael Ghio, and Akhil Hegde for technical support; Denise Beaver, Catherine Hathcock, Erika Coleman, Catherine Foss, Katherine Prince, Shelby Martindale, and Antoinette Santoro for assistance with participant recruitment and clinical measurements; and Jack Harkema for technical advice.

Key messages

- **In obesity-associated human asthma and in a mouse model of chronic allergic airways disease, leptin augments IL-13–induced pulmonary fibroblast eotaxin-1, eotaxin-3, and IL-13Rα2 expression and secretion.**
- **Inhibition of IL-13Rα2 activity reduces leptin-enhanced IL-13–induced eotaxin-1 and eotaxin-3 production by primary airway fibroblasts.**
- **Observed airway tissue eosinophilia in T2 asthma with comorbid obesity may be mediated by the excessive local eotaxin production by submucosal airway fibroblasts exposed to leptin and IL-13, through a mechanism requiring IL-13Rα2.**

REFERENCES

1. Ward ZJ, Bleich SN, Cradock AL, Barrett JL, Giles CM, Flax C, et al. Projected U.S. state-level prevalence of adult obesity and severe obesity. N Engl J Med 2019; 381:2440-50.
2. Most recent asthma data. Atlanta, Ga: Centers for Disease Control and Prevention. 2016. Available at: https://www.cdc.gov/asthma/most_recent_data.htm. Accessed August 12, 2023.
3. Scott HA, Ng SHM, McLoughlin RF, Valkenborghs SR, Nair P, Brown AC, et al. Effect of obesity on airway and systemic inflammation in adults with asthma: a systematic review and meta-analysis. Thorax 2023;78:957-65.
4. Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DYM. Body mass and glucocorticoid response in asthma. Am J Respir Crit Care Med 2008;178:682-7.
5. Sposato B, Scalese M, Milanese M, Masieri S, Cavaliere C, Latorre M, et al. Factors reducing omalizumab response in severe asthma. Eur J Intern Med 2018;52: 78-85.
6. FitzGerald JM, Bleecker ER, Menzies-Gow A, Zangrilli JG, Hirsch I, Metcalfe P, et al. Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. Lancet Respir Med 2018;6:51-64.
7. Ortega H, Li H, Suruki R, Albers F, Gordon D, Yancey S. Cluster analysis and characterization of response to mepolizumab. A step closer to personalized medicine for patients with severe asthma. Ann Am Thorac Soc 2014;11:1011-7.
8. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;181:315-23.
9. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008;178: 218-24.
10. Lefaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. J Allergy Clin Immunol 2017;139:1797-807.
11. Pakkasela J, Ilmarinen P, Honkamaki J, Tuomisto LE, Andersen H, Piirila P, et al. Age-specific incidence of allergic and non-allergic asthma. BMC Pulm Med 2020; 20:9.
12. Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. Am J Respir Crit Care Med 2013;188:657-63.
13. Marijsse GS, Seys SF, Schelpe A-S, Dilissen E, Goeminne P, Dupont LJ, et al. Obese individuals with asthma preferentially have a high IL-5/IL-17A/IL-25 sputum inflammatory pattern. Am J Respir Crit Care Med 2014; 189:1284-5.
14. Pinkerton JW, Kim RY, Brown AC, Rae BE, Donovan C, Mayall JR, et al. Relationship between type 2 cytokine and inflammasome responses in obesity-associated asthma. J Allergy Clin Immunol 2022;149:1270-80.
15. van der Wiel E, ten Hacken NHT, van den Berge M, Timens W, Reddel HK, Postma DS. Eosinophilic inflammation in subjects with mild-to-moderate asthma with and without obesity: disparity between sputum and biopsies. Am J Respir Crit Care Med 2014;189:1281-4.
16. Farhai N, Loutsios C, Tregay N, Wright AKA, Berair R, Lok LSC, et al. In vivo imaging reveals increased eosinophil uptake in the lungs of obese asthmatic patients. J Allergy Clin Immunol 2018;142:1659-62.
17. van Huisstede A, Rudolphus A, Castro Cabezas M, Biter LU, van de Geijn G-J, Taube C. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. Thorax 2015; 70:659-67.
18. Calixto MC, Lintomen L, Schenka A, Saad MJ, Zanesco A, Antunes E. Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. Brit J Pharmacol 2010;159:617-25.
19. Lintomen L, Calixto MC, Schenka A, Antunes E. Allergen-induced bone marrow eosinophilopoiesis and airways eosinophilic inflammation in leptin-deficient ob/ob mice. Obesity (Silver Spring) 2012;20:1959-65.
20. Medoff BD, Okamoto Y, Leyton P, Weng M, Sandall BP, Raher MJ, et al. Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. Am J Respir Cell Mol Biol 2009;41:397-406.
21. Rothenberg ME, MacLean JA, Pearlman E, Luster AD, Leder P. Targeted disruption of the chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. J Exp Med 1997;185:785-90.
22. Conroy DM, Williams TJ. Eotaxin and the attraction of eosinophils to the asthmatic lung. Respir Res 2001;2:150-6.
23. Pope SM, Fulkerson PC, Blanchard C, Akei HS, Nikolaidis NM, Zimmermann N, et al. Identification of a cooperative mechanism involving interleukin-13 and eotaxin-2 in experimental allergic lung inflammation. J Biol Chem 2005;280: 13952-61.
24. Shibata K, Nomiyama H, Yoshie O, Tanase S. Genome diversification mechanism of rodent and lagomorpha chemokine genes. Biomed Res Int 2013; 2013:856265.
25. Pope SM, Brandt EB, Mishra A, Hogan SP, Zimmermann N, Matthaei KI, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. J Allergy Clin Immunol 2001;108:594-601.
26. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998;282:2261-3.
27. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258-61.
28. Zhou X, Hu H, Balzar S, Trudeau JB, Wenzel SE. MAPK regulation of IL-4/IL-13 receptors contributes to the synergistic increase in CCL11/eotaxin-1 in response to TGF-b1 and IL-13 in human airway fibroblasts. J Immunol 2012; 188:6046-54.
29. Kurokawa M, Matsukura S, Kawaguchi M, Ieki K, Suzuki S, Odaka M, et al. Expression and effects of IL-33 and ST2 in allergic bronchial asthma: IL-33 induces eotaxin production in lung fibroblasts. Int Arch Allergy Immunol 2011; 155:12-20.
30. White SR, Martin LD, Stern R, Laxman B, Marroquin BA. Expression of IL-4/IL-13 receptors in differentiating human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2010;299:L681-93.
31. Ingram JL, Huggins MJ, Church TD, Li Y, Francisco DC, Degan S, et al. Airway fibroblasts in asthma manifest an invasive phenotype. Am J Respir Crit Care Med 2011;183:1625-32.
32. LaPorte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. Am J Respir Crit Care Med 2001; 164:141-8.

33. Lupardus PJ, Birnbaum ME, Garcia KC. Molecular basis for shared cytokine recognition revealed in the structure of an unusually high affinity complex between IL-13 and IL-13Ralpha2. Structure 2010;18:332-42.
34. He CH, Lee CG, Dela Cruz CS, Lee CM, Zhou Y, Ahangari F, et al. Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor α2. Cell Rep 2013;4:830-41.
35. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13α2 receptor is involved in induction of TGF-β1 production and fibrosis. Nat Med 2006;12:99-106.
36. Barderas R, Bartolome RA, Fernandez-Acenero MJ, Torres S, Casal JI. High expression of IL-13 receptor α2 in colorectal cancer is associated with invasion, liver metastasis and poor prognosis. Cancer Res 2012;72:2780-90.
37. Kiernan K, MacIver NJ. The role of the adipokine leptin in immune cell function in health and disease. Front Immunol 2021;11:622468.
38. Holguin F, Rojas M, Brown LA, Fitzpatrick AM. Airway and plasma leptin and adiponectin in lean and obese asthmatics and controls. J Asthma 2011;48:217-23.
39. Sideleva O, Suratt BT, Black KE, Tharp WG, Pratley RE, Forgione P, et al. Obesity and asthma: an inflammatory disease of adipose tissue not the airway. Am J Respir Crit Care Med 2012;186:598-605.
40. Watanabe K, Suzukawa M, Kawauchi-Watanabe S, Igarashi S, Asari I, Imoto S, et al. Leptin-producing monocytes in the airway submucosa may contribute to asthma pathogenesis. Respir Investig 2023;61:5-15.
41. Amorim NRT, Souza-Almeida G, Luna-Gomes T, Bozza PT, Canetti C, Diaz BL, et al. Leptin elicits in vivo eosinophil migration and activation: key role of mast cell-derived PGD_2. Front Endocrinol 2020;11:572113.
42. Conus S, Bruno A, Simon H-U. Leptin is an eosinophil survival factor. J Allergy Clin Immunol 2005;116:1228-34.
43. Global Initiative for Asthma. Global strategy for asthma management and prevention. Fontana, WI. 2023. Available at: https://ginasthma.org/2023-gina-main-report/. Accessed August 14, 2023.
44. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902-7.
45. Ihrie MD, McQuade VL, Womble JT, Hegde A, McCravy MS, Lacuesta CVG, et al. Exogenous leptin enhances markers of airway fibrosis in a mouse model of chronic allergic airways disease. Respir Res 2022;23:131.
46. Haley KJ, Sunday ME, Porrata Y, Kelley C, Twomey A, Shahsafaei A, et al. Ontogeny of the eotaxins in human lung. Am J Physiol Lung Cell Mol Physiol 2008;294:L214-24.
47. Motulsky HJ, Brown RE. Detecting outliers when fitting data with nonlinear regression—a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinform 2006;7:123.
48. Chen W, Sivaprasad U, Gibson AM, Ericksen MB, Cunningham CM, Bass SA, et al. IL-13 receptor α2 contributes to development of experimental allergic asthma. J Allergy Clin Immunol 2013;132:951-8.
49. Lugogo N, Green CL, Agada N, Zhang S, Meghdadpour S, Zhou R, et al. Obesity's effect on asthma extends to diagnostic criteria. J Allergy Clin Immunol 2018;141:1096-104.
50. Wenzel SE, Trudeau JB, Barnes S, Zhou X, Cundall M, Westcott JY, et al. TGF-β and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts. J Immunol 2002;169:4613-9.
51. Gazzinelli-Guimaraes PH, Golec DP, Karmele EP, Sciurba J, Bara-Garcia P, Hill T, et al. Eosinophil trafficking in allergen-mediated pulmonary inflammation relies on IL-13-driven CCL-11 and CCL-24 production by tissue fibroblasts and myeloid cells. J Allergy Clin Immunol Glob 2023;2:100131.
52. Watanabe K, Suzukawa M, Arakawa S, Kobayashi K, Igarashi S, Tashimo H, et al. Leptin enhances cytokine/chemokine production by normal lung fibroblasts by binding to leptin receptor. Allergol Int 2019;68:S3-8.
53. Imoto Y, Ueki S, Kato Y, Yoshida K, Morikawa T, Kimura Y, et al. Elevated serum leptin levels in patients with eosinophilic chronic rhinosinusitis. Front Pharmacol 2022;12:793607.
54. Grotta MB, Squebola-Cola DM, Toro AADC, Ribeiro MAGO, Mazon SB, Ribeiro JD, et al. Obesity increases eosinophil activity in asthmatic children and adolescents. BMC Pulm Med 2013;13:39.
55. Kato H, Ueki S, Kamada R, Kihara J, Yamauchi Y, Suzuki T, et al. Leptin has a priming effect on eotaxin-induced human eosinophil chemotaxis. Int Arch Allergy Immunol 2011;155:335-44.
56. Ingram JL, Kraft M. IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. J Allergy Clin Immunol 2012;130:829-42.
57. He B, Liang J, Qin Q, Zhang Y, Shi S, Cao J, et al. IL-13/IL-13RA2 signaling promotes colorectal cancer stem cell tumorigenesis by inducing ubiquitinated degradation of p53. Genes Dis 2023;11:495-508.
58. Barderas R, Bartolome RA, Fernandez-Acenero MJ, Torres S, Casal JI. High expression of IL-13 receptor a2 in colorectal cancer is associated with invasion, liver metastasis, and poor prognosis. Cancer Res 2012;72:2780-90.
59. Fujisawa T, Joshi B, Nakajima A, Puri RK. A novel role of interleukin-13 receptor alpha2 in pancreatic cancer invasion and metastasis. Cancer Res 2009;69:8678-85.
60. Duffen J, Zhang M, Masek-Hammerman K, Nunez A, Brennan A, Jones JEC, et al. Modulation of the IL-33/IL-13 axis in obesity by IL-13Rα2. J Immunol 2018;200:1347-59.
61. Lu FL, Johnston RA, Flynt L, Theman TA, Terry RD, Schwartzman IN, et al. Increased pulmonary responses to acute ozone exposure in obese db/db mice. Am J Physiol Lung Cell Mol Physiol 2006;290:L856-65.
62. Osborn O, Sanchez-Alavez M, Brownell SE, Ross B, Klaus J, Dubins J, et al. Metabolic characterization of a mouse deficient in all known leptin receptor isoforms. Cell Mol Neurobiol 2010;30:23-33.
63. Lollmann B, Gruninger S, Stricker-Krongrad A, Chiesi M. Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and e in different mouse tissues. Biochem Biophys Res Commun 1997;238:648-52.
64. Penke LR, Ouchi H, Speth JM, Lugogo N, Huang YJ, Huang SK, et al. Transcriptional regulation of the IL-13Rα2 gene in human lung fibroblasts. Sci Rep 2020;10:1083.
65. Nair P, Radford K, Fanat A, Janssen LJ, Peters-Golden M, Cox PG. The effects of leptin on airway smooth muscle responses. Am J Respir Cell Mol Biol 2008;39:475-81.
66. Moynihan BJ, Tolloczko B, El Bassam S, Ferraro P, Michoud M-C, Martin JG, et al. IFN-gamma, IL-4 and IL-13 modulate responsiveness of human airway smooth muscle cells to IL-13. Respir Res 2008;9:84.
67. Ghaffer O, Hamid Q, Renzi PM, Allakhverdi Z, Molet S, Hogg JC, et al. Constitutive and cytokine-stimulated expression of eotaxin by human airway smooth muscle cells. Am J Respir Crit Care Med 1999;159:1933-42.
68. Guler N, Kirerleri E, Ones U, Tamay Z, Salmayenli N, Darendeliler F. Leptin: does it have any role in childhood asthma? J Allergy Clin Immunol 2004;114:254-9.
69. Kilic H, Oguzulgen IK, Bakir F, Turktas H. Asthma in obese women: outcomes and factors involved. J Investig Allergol Clin Immunol 2011;21:290-6.
70. Won E-Y, Yoon M-K, Kim S-W, Jung Y, Bae H-W, Lee D, et al. Gender-specific metabolic profiling of obesity in leptin-deficient *ob/ob* mice by ^{1}H NMR spectroscopy. PLoS One 2013;8:e75998.
71. Chen W, Wilson JL, Khaksari M, Cowley MA, Enriori PJ. Abdominal fat analyzed by DEXA scan reflects visceral body fat and improves the phenotype description and the assessment of metabolic risk in mice. Am J Physiol Endocrinol Metab 2012;303:E635-43.
72. Sood A, Ford ES, Camargo CA Jr. Association between leptin and asthma in adults. Thorax 2006;61:300-5.
73. Ziyab AH, Mukherjee N, Kurukulaaratchy RJ, Zhang H, Ewart S, Arshad H, et al. Leptin receptor gene polymorphisms and sex modify the association between acetaminophen use and asthma among young adults: results from two observational studies. Respir Res 2018;19:179.
74. Pray R, Riskin S. The history and faults of the body mass index and where to look next: a literature review. Cureus 2023;15:e48230.
75. Bray GA. Beyond BMI. Nutrients 2023;15:2254.
76. Salmon-Gomez L, Catalan V, Fruhbeck G, Gomez-Ambrosi J. Relevance of body composition in phenotyping the obesities. Rev Endocr Metab Disord 2023;24:809-23.
77. Ping Z, Pei X, Xia P, Chen Y, Guo R, Hu C. Anthropometric indices as surrogates for estimating abdominal visceral and subcutaneous adipose tissue: a meta-analysis with 16,129 participants. Diabetes Res Clin Pract 2018;143:310-9.
78. Yamamoto R, Ueki S, Moritoki Y, Kobayashi Y, Oyamada H, Konno Y, et al. Adiponectin attenuates human eosinophil adhesion and chemotaxis: implications in allergic inflammation. J Asthma 2013;50:828-35.
79. Leivo-Korpela S, Lehtimaki L, Vuolteenaho K, Nieminen R, Kankaanranta H, Saarelainen S, et al. Adipokine resistin predicts anti-inflammatory effect of glucocorticoids in asthma. J Inflamm (Lond) 2011;8:12.

METHODS

Human study exclusion criteria

Exclusion criteria for this study included postbronchodilator FEV_1 less than 50% predicted, inpatient status, pregnancy, use of anticoagulants, upper or lower respiratory tract infection within 1 month of the study, use of antibiotics within 2 months of the study, use of systemic corticosteroids within 4 weeks of the study, use of glucagon-like peptide-1 receptor agonists or dipeptidyl peptidase-4 inhibitors within 2 weeks of the study, a smoking history of more than 5 pack years or any cigarette use within the previous 6 months, significant uncontrolled nonasthma pulmonary disease, or other medical problems.

Bronchoscopy

Participants underwent bronchoscopy with endobronchial biopsy and bronchoalveolar lavage as previously described.[E1] Before the bronchoscopy, spirometry was performed and participants with asthma received 0.18 mg of inhaled albuterol. Biopsy sites were randomized to the right or left lower lobe and 4 to 5 endobronchial tissue samples were collected. Supplemental oxygen was administered throughout the procedure along with monitoring of heart rate, blood pressure, and oxygen saturation. On completion of the procedure, participants were carefully monitored and then discharged once their FEV_1 reached 90% of their prebronchoscopy, postbronchodilator value.

Human BALF processing

Fresh BALF was centrifuged at 1200 revolutions per minute for 10 minutes at 4°C. The supernatant was removed and stored at −80°C, whereas the pellet was resuspended in 1 mL PBS. Total cells in the BALF pellet were counted with the Scepter cell counter (PHCC00000, MilliporeSigma, Burlington, Mass), and then 25,000 cells were centrifuged in a cytospin at 800 revolutions per minute for 3 minutes to transfer the cells to slides. The cytospin slides were then dried overnight in room air and stained with Easy III (Azer Scientific, Morgantown, Pa) by submerging the slides in fixative for 15 seconds, in stain 1 for 30 seconds, and then in stain 2 for 15 seconds before rinsing with water. Images of the stained BALF cells were acquired at 20× magnification and manual differential cell counts were obtained for 250 cells per slide.

Pulmonary fibroblast culture

Human airway biopsy tissue was minced into several cartilage-free pieces. The right lung of each mouse was digested and homogenized.[E2] The human airway or mouse lung tissue digest cells were seeded in 60-mm collagen-coated dishes. At 70% confluency, cultures were passaged into 75-cm^2 flasks to promote growth and eventually split into 12-well plates. All samples were cultured at 5% CO_2 and 37°C in low-glucose Dulbecco modified Eagle medium (Gibco) supplemented with 10% FBS, 5% antibiotic-antimycotic (Gibco), and 5% gentamicin. To passage cells, trypsin (MilliporeSigma) was used in a 1:2 ratio with media as per manufacturer's protocol. Cultures were passaged up to 4 times before experiments, and asthma or nonasthma cultures were cultured identically. The serum-free media consisted of Ham F12 media supplemented with 0.25% BSA, 5% antibiotic-antimycotic, and 5% gentamicin.

Mouse lung lavage and inflammation scoring

At the termination of the protocol, mice were sacrificed and lungs were lavaged with saline. Differential cell counts were determined as previously described.[E3] The percent of airway cell types in lavage fluid was calculated by differentiating (according to standard morphological criteria) 200 cells on slides stained using the Hema-3 staining kit (Thermo Fisher Scientific, Waltham, Mass). Peribronchial inflammation was semiquantitatively determined in a blinded examination of hematoxylin and eosin–stained sections using a 4-tiered scoring system of inflammation severity as previously described.[E4]

Quantitative RT-PCR

Total RNA from human airway and mouse lung fibroblasts was extracted using TRIzol reagent (Gibco) after exposure to mediators for 24 hours. After precipitation with isopropanol, the RNA was washed several times with 75% ethanol and then resuspended in 30 μL RNase-free deionized water. Total RNA was quantified by determining absorbance at 260 nm using a Nanodrop spectrophotometer (ThermoFisher Scientific). First-strand cDNA preparation was performed using 1 μg of total RNA and random hexamers in a 50-μL reaction according to the manufacturer's protocol (Applied Biosystems, Waltham, Mass). The 50-μL PCR reaction contains 50 ng cDNA, 100 nM fluorogenic probe, 200 nM primers, and other components contributed from Applied Biosystems 2× RT-PCR Master Mix. Human eotaxin-1 (*CCL11*), eotaxin-3 (*CCL26*), IL-13Rα2 (*IL13RA2*), β-actin, and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as well as mouse IL-13Rα2 (*Il13ra2*) and *GAPDH* (*Gapdh*) Taqman primers (Applied Biosystems) were used to evaluate relative gene expression (see Table E1 in this article's Online Repository at www.jacionline.org).

The specificity of PCR was verified by lack of a signal in no-template controls or reverse transcriptase negative RNA samples. The threshold cycle (C_T) was recorded for each sample, in each condition. Fold changes in *CCL11, CCL26, IL13RA2*, or *Il13ra2* mRNA expression were calculated by normalization of expression levels to *GAPDH*, *Gapdh*, or β-actin mRNA levels and then compared with those of untreated or IL-13–treated cells by the $\Delta\Delta C_T$ method.

ELISA

Soluble protein detection in serum, BALF, or pulmonary fibroblast cell culture supernatants of human and mouse eotaxin-1 and IL-5, human leptin and eotaxin-3, and mouse IL-13 and IL-13Rα2 was performed using sandwich ELISA assays (R&D Systems) according to the manufacturer's protocol. The absorbance at 450 nm from all ELISA tests was measured using a FLUOstar Omega plate reader (BMG Labtech, Cary, NC).

Immunohistochemistry

Detection and quantification of IL-13Rα2 in human airway biopsy tissue were described previously.[E5] For detection of MBP-positive eosinophils, human airway biopsy tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were baked and dehydrated with xylene, ethanol (100%, 95%, and 70%), and PBS, respectively. A proteinase treatment with 0.1% trypsin was performed for 15 minutes, and the sections were blocked in 10% normal goat serum (Vector Labs, Newark, Calif) for 1 hour at room temperature. After blocking, sections were stained with MBP antibody (AbD Serotec, Hercules, Calif) at 1:30 dilution (33.3 μg/mL) and incubated overnight at 4°C. Sections were incubated with biotinylated goat anti-mouse antibody (Vector Labs) at 1:200 dilution for 1 hour at room temperature,

and then incubated with 0.3% hydrogen peroxide before treatment with Avidin-Biotin Complex (Vector Labs) for 1 hour at room temperature. Diaminobenzidine tetrahydrochloride (MilliporeSigma) was applied to visualize peroxidase activity, and 2% methyl green counterstain was applied before rehydration with water, ethanol, and xylene. Cover slips were applied.

For detection of MBP-positive eosinophils in mouse lung, the left lung of each mouse was preserved in 4% paraformaldehyde. After 24 hours, the paraformaldehyde was removed and replaced with 70% ethanol and stored at 4°C. Lung tissue was embedded in paraffin, sectioned at 5-μm thickness, and mounted on slides. Immunohistochemical staining was performed using an antibody specific for murine MBP (Rat anti-mouse MBP clone MT-14.7, Mayo Clinic, Scottsdale, Ariz) at 1:500 in normal antibody diluent (ScyTek Labs, Logan, Utah) as described previously[E6] using the Biocare intelliPath Flex (Biocare Medical, Pacheco, Calif) automated immunostaining platform with ProMARK detection reagents and AutoWash buffer rinses between each step.

REFERENCES

E1. Kraft M, Lewis C, Pham D, Chu HW. IL-4, IL-13, and dexamethasone augment fibroblast proliferation in asthma. J Allergy Clin Immunol 2001;107:602-6.

E2. Ihrie MD, McQuade VL, Womble JT, Hegde A, McCravy MS, Lacuesta CVG, et al. Exogenous leptin enhances markers of airway fibrosis in a mouse model of chronic allergic airways disease. Respir Res 2022;23:131.

E3. Lin R, Degan S, Theriot BS, Fischer BM, Strachan RT, Liang J, et al. Chronic treatment *in vivo* with β-adrenoceptor agonists induces dysfunction of airway β2-adrenoceptors and exacerbates lung inflammation in mice. Br J Pharmacol 2012;165:2365-77.

E4. Hollingsworth JW, Theriot BS, Li Z, Lawson BL, Sunday ME, Schwartz DA, et al. Both hematopoietic-derived and non-hematopoietic-derived {beta}-arrestin-2 regulates murine allergic airway disease. Am J Respir Cell Mol Biol 2010;43:269-75.

E5. Ingram JL, Huggins MJ, Church TD, Li Y, Francisco DC, Degan S, et al. Airway fibroblasts in asthma manifest an invasive phenotype. Am J Respir Crit Care Med 2011;183:1625-32.

E6. Harkema JR, Eldridge EA, Lewandowski RP, Wagner JG. Influx, persistence, and recall of eosinophils and GATA-3+ innate lymphoid cells in the nasal mucosa of mice exposed and reexposed to the gaseous air pollutant ozone. Toxicol Pathol 2020;48:323-37.

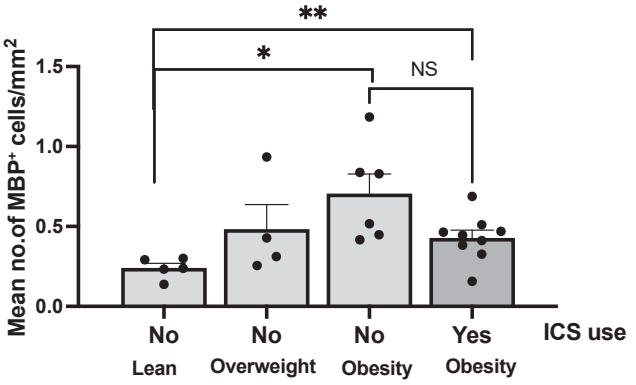


FIG E1. Airway tissue eosinophilia is elevated in participants with asthma and obesity regardless of ICS use. Mean number of MBP-positive (MBP^+) cells/mm^2 ± SEM in airway biopsy tissue of participants with asthma by body mass group, with participants with obesity stratified by ICS use (yes/no) (n = 4-9 per group). *NS*, Not significant. **P* < .05; ***P* < .01.

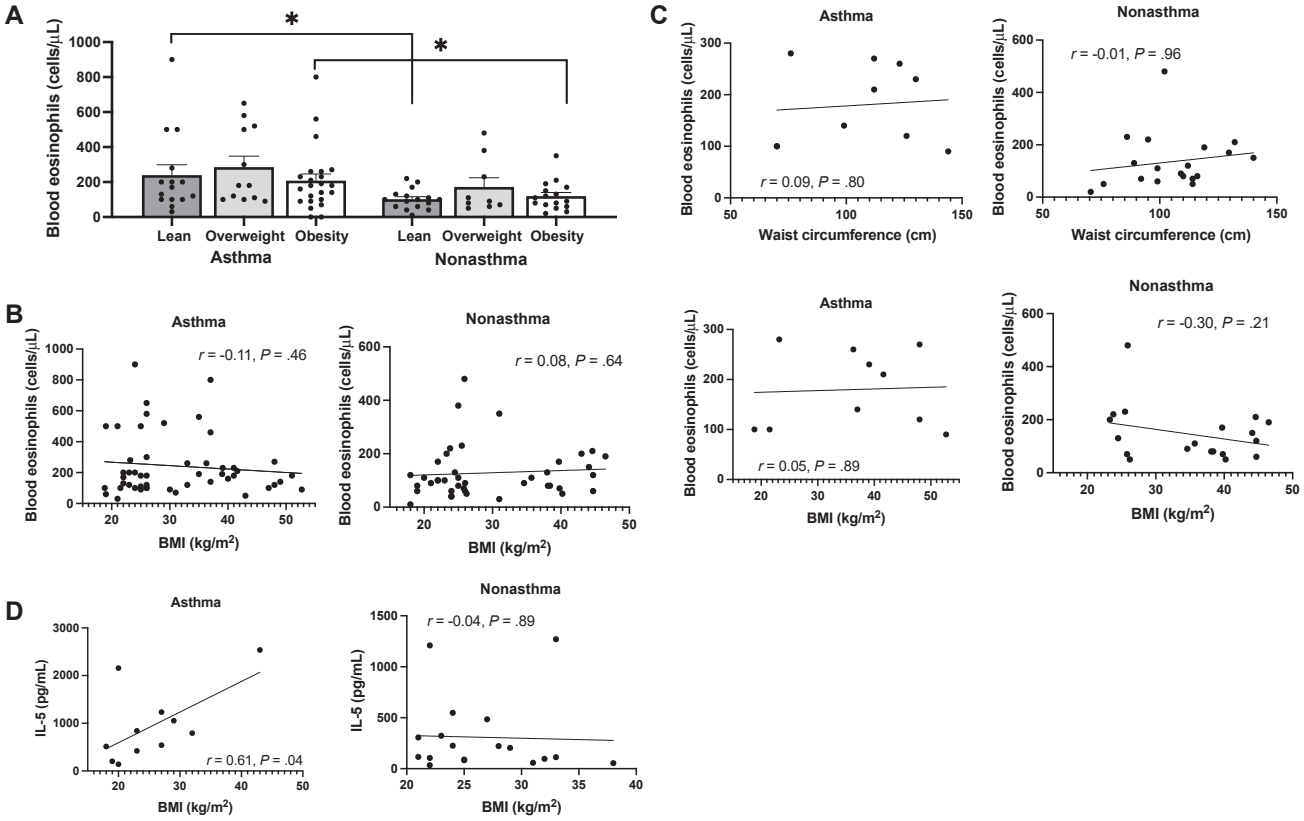


FIG E2. Peripheral eosinophil counts are elevated in participants with asthma and not associated with parameters of obesity. **A,** Mean blood eosinophil counts (cells/μL) ± SEM by body mass group in asthma (n = 10-23 per group) and nonasthma (n = 9-15 per group) participants. **P* < .05. **B,** Relationship of blood eosinophil counts with BMI in asthma (*left*) and nonasthma (*right*) participants. **C,** Relationship of blood eosinophil counts with measures of waist circumference (*top*) or BMI (*bottom*) in a subset of asthma (*left*) and nonasthma (*right*) participants for which both measures were available. **D,** Relationship of BALF IL-5 levels with BMI in asthma (*left*) and nonasthma (*right*) participants.

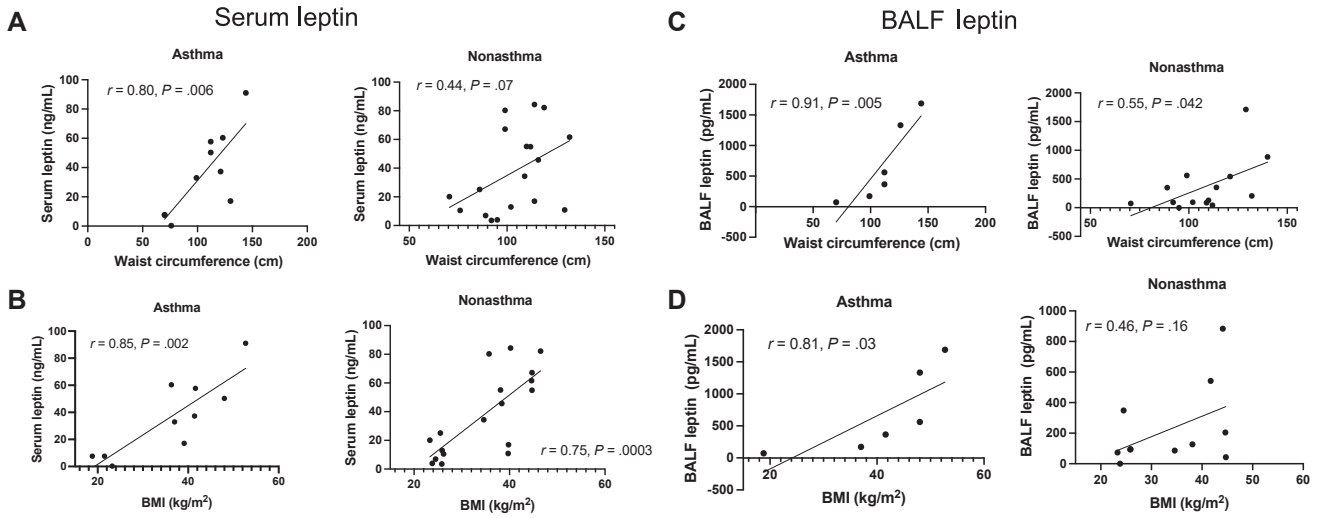


FIG E3. Serum and BALF leptin levels are associated with waist circumference and BMI in participants with asthma for which both measures were available. **A,** Relationship of serum leptin levels with waist circumference in asthma (*left*) and nonasthma (*right*) participants. **B,** Relationship of serum leptin levels with BMI in asthma (*left*) and nonasthma (*right*) participants for which waist circumference was also measured. **C,** Relationship of BALF leptin levels with waist circumference in asthma (*left*) and nonasthma (*right*) participants. **D,** Relationship of BALF leptin levels with BMI in asthma (*left*) and nonasthma (*right*) participants for which waist circumference was also measured.

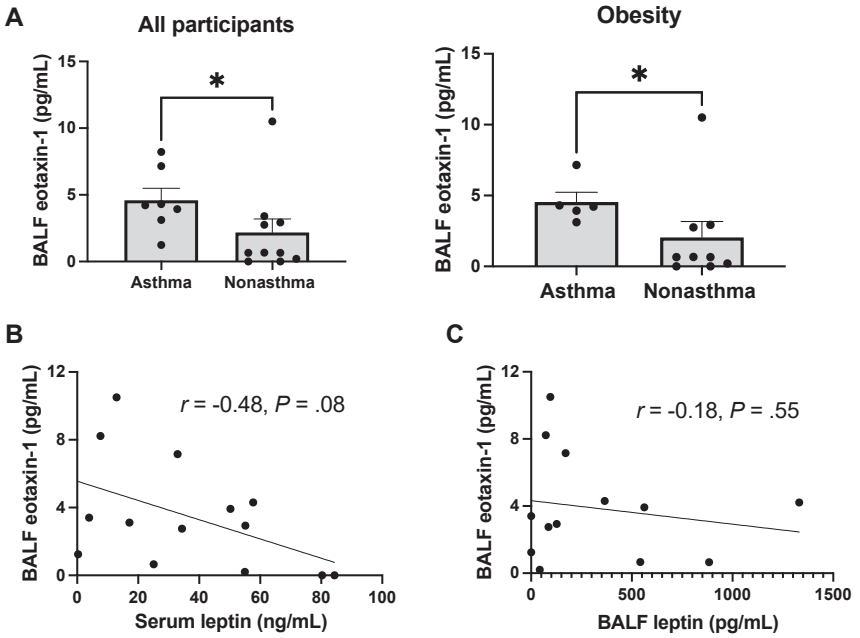


FIG E4. Eotaxin-1 levels in BALF are elevated in asthma compared with nonasthma and not associated with serum or BALF leptin levels. **A,** Mean eotaxin-1 levels ± SEM as measured using ELISA in BALF from all participants (*left*) or specifically in participants with obesity (*right*). **P* < .05 (n = 5-7 for asthma; n = 9-10 for nonasthma). **B,** Relationship of BALF eotaxin-1 levels with serum leptin levels in all participants. **C,** Relationship of BALF eotaxin-1 levels with BALF leptin levels in all participants.

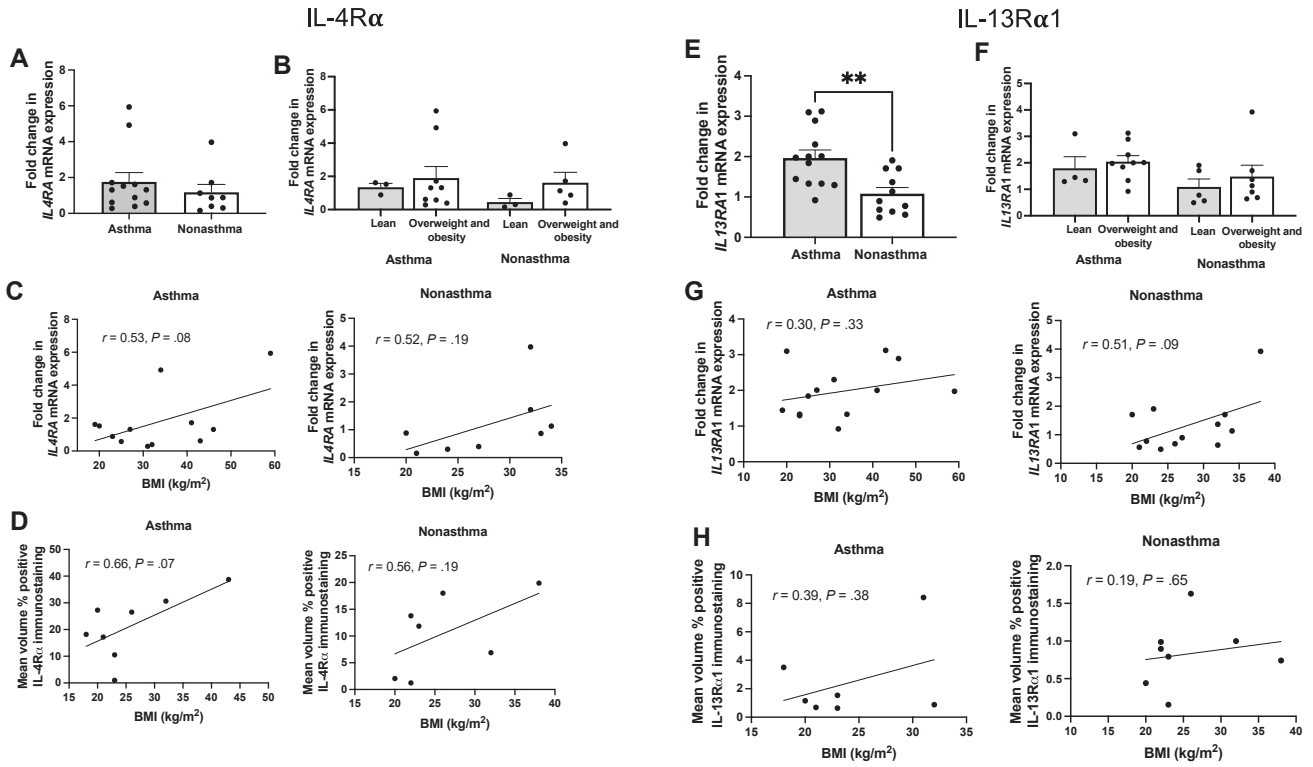


FIG E5. IL-4Rα and IL-13Rα1 expression in human airway fibroblasts is not associated with body mass. Cultured airway fibroblasts derived from asthma and nonasthma participants were incubated with serum-free media alone (untreated control) for 24 hours. Total RNA was collected. Mean fold change ± SEM in *IL4RA* and *IL13RA1* mRNA expression was determined by quantitative RT-PCR in asthma (n = 12 for *IL4RA*; n = 13 for *IL13RA1*) and nonasthma (n = 8 for *IL4RA*; n = 11 for *IL13RA1*) groups (**$P < .01$) (Fig E5, *A* and *E*) and in participants with and without obesity (*IL4RA*: asthma, n = 3 for lean and n = 9 for overweight and obesity; nonasthma, n = 3 for lean and n = 5 for overweight and obesity; *IL13RA1*: asthma, n = 4 for lean and n = 9 for overweight and obesity; nonasthma, n = 5 for lean and n = 7 for overweight and obesity) (Fig E5, *B* and *F*). Data are normalized to β-actin mRNA expression in individual participants and to the mean *IL4RA* or *IL13RA1* expression level of the nonasthma participants. **C** and **G,** Relationship of fold change in *IL4RA* or *IL13RA1* mRNA expression in untreated airway fibroblasts with BMI in asthma (*left*) and nonasthma (*right*) participants. Immunohistochemical staining of airway biopsy tissue for IL-4Rα and IL-13Rα1 was as described previously.[E5] **D** and **H,** Relationship of mean volume percent positive IL-4Rα or IL-13Rα1 immunostaining in airway biopsy tissue with BMI in asthma (*left*) and nonasthma (*right*) participants.

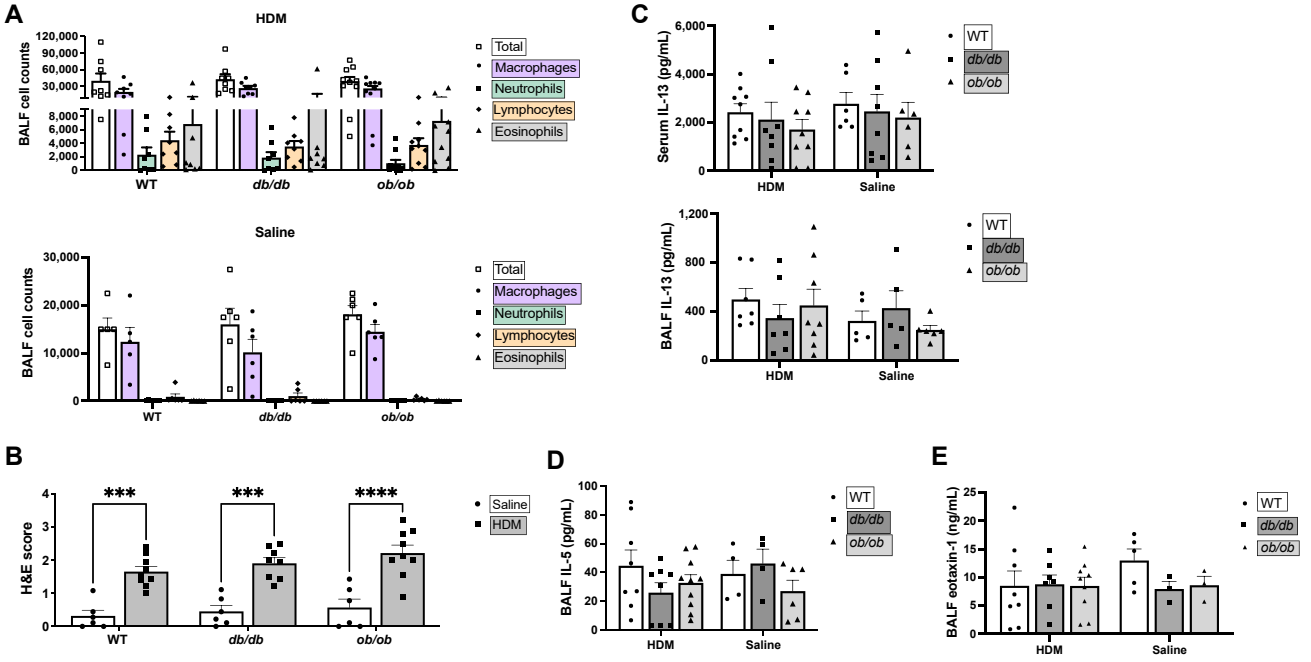


FIG E6. BALF cytokines, serum IL-13, and peribronchial inflammation do not differ between chronically HDM-challenged C57BL6 (WT), *db/db*, and *ob/ob* mice. **A,** Mean differential BALF cell counts ± SEM in HDM-challenged (*top*) and saline-challenged (*bottom*) WT, *db/db*, and *ob/ob* mice (n = 8-10 for HDM; n = 5-6 for saline per genotype). **B,** Peribronchial inflammation scores in HDM- and saline-challenged WT, *db/db*, and *ob/ob* mice (n = 8-9 for HDM; n = 6 for saline per genotype). Data are expressed as mean H&E score ± SEM per mouse. ***$P < .001$; ****$P < .0001$. **C,** Mean IL-13 levels ± SEM in serum (*top*) and BALF (*bottom*) as measured by ELISA in HDM- and saline-challenged WT, *db/db*, and *ob/ob* mice (n = 5-8 mice per genotype for BALF; n = 6-9 mice per genotype for serum). **D,** Mean IL-5 levels ± SEM in BALF as measured by ELISA in HDM- and saline-challenged WT, *db/db*, and *ob/ob* mice (n = 8-10 mice for HDM; n = 4-6 mice for saline per genotype). **E,** Mean eotaxin-1 levels ± SEM in BALF as measured by ELISA in HDM- and saline-challenged WT, *db/db*, and *ob/ob* mice (n = 7-9 mice for HDM; n = 3-5 mice for saline per genotype). *H&E*, Hematoxylin and eosin.

TABLE E1. Human- and mouse-specific Taqman (Applied Biosystems) assays used in the study

Gene name	Species	Applied Biosystems no.
CCL11	Human	Hs00237013_m1
CCL26	Human	Hs00171146_m1
IL13RA2	Human	Hs00152924_m1
GAPDH	Human	Hs99999005_m1
ACTB	Human	Hs01060665_g1
Il13ra2	Mouse	Mm00515166_m1
Gapdh	Mouse	Mm99999915_g1

ACTB, β-Actin.

TABLE E2. Summary of IL-13Rα2 neutralizing antibody experiments shown in Fig 7

IL-13Rα2 neutralizing antibody vs IgG control	Eotaxin-1	Eotaxin-3
IL-13 alone	→	↑
Leptin alone	↓↓	→
IL-13 + leptin/IL-13 alone	↓	↓

Eotaxin-1 and eotaxin-3 secretion levels in airway fibroblasts were derived from lean, nonasthma participants following incubation with IL-13 alone, leptin alone, or combined IL-13 and leptin (normalized to IL-13 alone) in the presence of IL-13Rα2 neutralizing antibody. Up arrows (↑) indicate increased eotaxin production; down arrows (↓) indicate reduced eotaxin production; and horizontal arrows (→) indicate no change in eotaxin production relative to the effect of IgG control antibody.