Elevated Sputum Interleukin-5 and Submucosal Eosinophilia in Obese Individuals with Severe Asthma

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Rationale: **The relationship between airway inflammation and obesity in severe asthma is poorly understood.**

Objectives: **We sought to determine the relationship between sputum mediator profiles and the distribution of eosinophilic inflammation and obesity in people with severe asthma.**

Methods: **Clinical parameters and eight mediators in sputum were assessed in 131 subjects with severe asthma from a single center categorized into lean, overweight, and obese groups defined by their body mass index. In an independent group of people with severe asthma (n = 45) and healthy control subjects (n = 19) eosinophilic inflammation was enumerated in bronchial submucosa, blood, and sputum and related to their body mass index.**

Measurements and Main Results: **Sputum IL-5 geometric mean (95% confidence interval) (pg/ml) was elevated in the obese (1.8 [1.2–2.6]) compared with overweight (1.1 [0.8–1.3]; $P = 0.025$) and lean (0.9 [0.6–1.2]; $P = 0.018$) subjects with asthma and was correlated with body mass index ($r = 0.29$; $P < 0.001$). There was no relationship among body mass index, the sputum cell count, or other sputum mediators. In the bronchoscopy group the submucosal eosinophil number in the subjects with asthma was correlated with body mass index (Spearman rank correlation, $r_s = 0.38$; $P = 0.013$) and the median (interquartile range) number of submucosal eosinophils was increased in obese (19.4 [11.8–31.2]) (cells per square millimeter) versus lean subjects (8.2 [5.4–14.6]) ($P = 0.006$). There was no significant association between sputum or peripheral blood eosinophil counts and body mass index.**

Conclusions: **Sputum IL-5 and submucosal eosinophils, but not sputum eosinophils, are elevated in obese people with severe asthma. Whether specific antieosinophilic therapy is beneficial, or improved diet and lifestyle in obese asthma has antiinflammatory effects beyond weight reduction, requires further study.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Obesity and severe asthma are commonly associated. The relationship between airway inflammation and obesity in severe asthma is poorly understood.

What This Study Adds to the Field

Sputum IL-5 and submucosal eosinophils, but not sputum eosinophils, are elevated in obese people with severe asthma. Whether specific antieosinophilic therapy is beneficial, or improved diet and lifestyle in obese asthma has antiinflammatory effects, requires further study.

Keywords: asthma; obesity; cytokines; phenotypes; eosinophil

Asthma is a common, complex inflammatory disorder affecting about 5% of adults in the general population, of which approximately 5–10% suffer from severe disease (1, 2). Severe disease is often associated with comorbidities, such as obesity, and is particularly important because these patients suffer from substantial morbidity and consume a disproportionately high amount of the overall healthcare resources spent on asthma management (3–6).

Asthma, and in particular severe asthma, is a heterogeneous disease as highlighted by the different phenotypes identified using cluster analysis of clinical data (7–10) and cytokine profiles of airway samples (11–13). The key benefit of dividing a multidimensional disease, such as asthma, into distinct phenotypes is expected to be more effective treatment targeting. This has been effectively shown with the success of eosinophilic airway inflammation–directed corticosteroid (14–16) and anti–IL-5 treatment (17–19) to prevent asthma exacerbations in eosinophilic subjects with asthma. The association of obesity and asthma has been evident

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Author Contributions: D.D., M.B., A.S., P.H., S. Siddiqui, and S. Shah were involved in the recruitment of volunteers and in data collection. F.A.S., D.D., and C.E.B. were involved in the immunohistochemistry. J.W. undertook the sputum mediator assessment. N.K. and C.E.B. were involved in the macrophage color assessment. C.N., A.H., D.D., P.H., and C.E.B. were involved in statistical analyses and interpretation. D.D., S.G., M.B., P.H., A.S., P.B., R.G., N.K., I.P., R.D.M., A.J.W., and C.E.B. were involved in the design of the study and interpretation. D.D., A.J.W., and C.E.B. were involved in the study design, volunteer recruitment, data collection, data interpretation, and data analysis, and had full access to the data and are responsible for the integrity of the data and final decision to submit. All authors contributed to the writing of the manuscript and have approved the final version for submission.

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TABLE 1. CHARACTERISTICS OF SUBJECTS WITH ASTHMA CLASSIFIED BY THEIR BODY MASS INDEX

	All Subjects (*n = 131*)	Lean (*n = 28*)	Overweight (*n = 48*)	Obese (*n = 55*)	*P* Value
Current age	50 (1)	51 (3)	49 (2)	51 (2)	0.63
Male, n (%)	58 (44)	12 (43)	26 (54)	20 (36)	0.19
Age of onset	26 (2)	24 (4)	23 (3)	29 (3)	0.23
BMI, kg/m^2	30 (0.6)	22.0 (0.4)	27.5 (0.2)	36.2 (0.8)	<0.001
Smoking history, pack-years	5.1 (1.3)	7.4 (2.1)	2.9 (1.4)	5.9 (2.7)	0.42
Severe exacerbations	3.5 (0.3)	3.6 (0.5)	3.3 (0.4)	3.6 (0.5)	0.87
ACQ_6 score*	2.4 (0.13)	2.6 (0.26)	2.4 (0.21)	2.3 (0.22)	0.82
Oral corticosteroid use, n (%)	70 (53)	15 (54)	27 (56)	28 (51)	0.75
Daily prednisolone dose, mg	6.0 (0.7)	5.7 (1.2)	6.6 (1.3)	5.6 (1.0)	0.78
ICS dose†	1,600 (1,000–2,000)	1,600 (1,000–2,000)	1,600 (1,265–2,000)	2,000 (1,200–2,000)	0.37
Atopy present, n (%)	59 (45)	11 (39)	23 (48)	25 (46)	0.76
Total IgE, IU/L‡	156 (121–201)	168 (84–339)	172 (116–256)	137 (95–200)	0.77
Pre-BD FEV_1	2.1 (0.1)	2.2 (0.2)	2.0 (0.1)	2.0 (0.1)	0.54
Pre-BD FEV_1% predicted	70.0 (1.9)	76.2 (4.1)	64.6 (3.3)	71.6 (2.7)	0.06
Pre-BD FEV_1/FVC ratio	68.1 (1.2)	65.6 (3.4)	64.5 (2.0)	72.3 (1.3)	0.007
BD reversibility %FEV_1	8.8 (1.2)	7.1 (2.5)	11 (2.2)	7.8 (1.8)	0.39
Post-BD FEV_1% predicted	75.3 (1.9)	81.1 (4.3)	70.9 (3.4)	76.2 (2.7)	0.14
Eosinophils, %‡	3.1 (2.3–4.2)	2.5 (1.3–4.8)	3.0 (1.8–5.0)	3.6 (2.3–5.7)	0.70
Neutrophils, %‡	55.6 (49.2–62.8)	57.0 (44.0–74.0)	58.3 (47.2–72.0)	52.8 (43.4–64.2)	0.77
Total cell count (10^6/g sputum)‡	1.6 (1.2–2.1)	1.2 (0.6–2.5)	1.5 (0.9–2.5)	1.9 (1.4–2.6)	0.47

Definition of abbreviations: ACQ = Asthma Control Questionnaire; BD = bronchodilator; BMI = body mass index; ICS = inhaled corticosteroids.
Data expressed as mean (SEM) unless otherwise stated.
* ACQ score was adjusted to remove effect of FEV_1% predicted domain.
† Doses of all inhaled corticosteroids were converted to the equivalent dose of beclomethasone dipropionate and expressed here as median dose (interquartile range).
‡ Data expressed as geometric mean (95% confidence interval). *P* value based on analysis of variance for continuous variables.

from epidemiologic studies, including case control studies and cross-sectional studies that have shown an increased risk of asthma in obese individuals based on the body mass index (BMI) (20–23). Emerging evidence suggests that obesity might represent a distinct severe asthma phenotype (6) with changes in exhaled nitric oxide metabolism (24) and macrophage function (25, 26). Its association with eosinophilic inflammation is contentious with some reports suggesting no association with sputum (27) or blood cell counts (6) and others suggesting there is a noneosinophilic obese phenotype (7).

We hypothesized that sputum mediator profiles and eosinophilic inflammation are differentially expressed among subjects with severe asthma categorized into normal, overweight, and obese subgroups. To test our hypothesis we measured in two independent groups of subjects with severe asthma stratified by their BMI sputum mediators and eosinophilic inflammation in the peripheral blood, bronchial submucosa, and sputum.

METHODS

Subjects

We recruited three independent subject groups. The severe asthma "sputum cytokine profiling" group (n = 131) were recruited from a single center, the Difficult Asthma Clinic, Leicester, United Kingdom. All subjects had a clinician's diagnosis of asthma requiring treatment step 4 or 5 according to GINA guidelines (2). A second group, the "bronchoscopy" group, included subjects with severe asthma recruited from the Difficult Asthma Clinic (n = 45) and healthy control subjects (n = 19). Sputum cytokine data from a group of patients with chronic obstructive pulmonary disease described previously (n = 34) was used as a disease control group (28). Written informed consent was obtained from all subjects and the study was approved by the Leicestershire, Northamptonshire, and Rutland ethics committee.

Protocol

Demographics and spirometry were recorded for all subjects. Reversibility was assessed after administration of 400 μg albuterol by a spacer device. Atopy was defined as a wheal 3 mm greater than control on skin-prick testing or specific IgE (Pharma CAP; ALK-Abelló, Madrid, Spain) to one or more of *Dermatophagoides pteronyssinus*, grass, tree, cat, dog, or *Aspergillus fumigatus* allergens. Sputum samples were collected in those subjects with asthma after spontaneous expectoration.

In the "sputum cytokine profiling" group asthma control was determined by the Asthma Control Questionnaire (29). Severe exacerbations were defined as worsening of symptoms requiring greater than or equal to 3 days of high-dose systemic corticosteroids (30). In the "bronchoscopy" group subjects underwent bronchoscopy in accordance with the British Thoracic Society guidelines within 1 week of their baseline assessment.

TABLE 2. SPUTUM MEDIATOR LEVELS IN THE GROUPS CLASSIFIED BY BODY MASS INDEX

Mediators	All Subjects (*n = 131*)	Lean (*n = 28*)	Overweight (*n = 48*)	Obese (*n = 55*)	*P* Value*
Cytokines/receptors					
IL-1β	127 (94–171)	128 (83–199)	159 (88–288)	105 (67–165)	0.46
IL-5	1.3 (1.0–1.5)	0.9 (0.6–1.2)	1.1 (0.8–1.3)	1.8 (1.2–2.6)	0.011
IL-6	63 (48–84)	68 (39–120)	65 (39–108)	60 (38–94)	0.93
IL-8	3,318 (2,280–4,830)	4,945 (3,066–7,974)	3,658 (2,028–6,596)	2,525 (1,260–5,063)	0.39
TNF-α	4.0 (2.9–5.3)	3.5 (2.1–6.0)	5.4 (2.9–9.8)	3.2 (2.2–4.8)	0.31
Chemokines					
CCL4	210 (150–293)	215 (109–423)	272 (156–474)	167 (97–289)	0.44
CXCL10	481 (342–677)	610 (302–1,234)	481 (278–832)	429 (241–766)	0.75
Growth factors					
VEGF	1,521 (1,258–1,840)	1,670 (1,086–2,568)	1,787 (1,334–2,394)	1,272 (929–1,740)	0.26

Definition of abbreviations: TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
All data represented as geometric mean (95% confidence interval), all values are in pg/ml.
* *P* value based on analysis of variance for continuous variables on log data.

Sputum Processing and Cytokine Assessment

Sputum was selected, dispersed using mucolytic dithiothreitol, and processed to generate a sputum differential cell count and cell-free supernatants (31). Sputum mediator measurements were performed using the sensitive Meso Scale Discovery Platform (Meso Scale Discovery, Gaithersburg, MD) for eight mediators that we had previously established to be valid after spiking experiments (28). In half of the bronchoscopy subjects (n = 23) eosinophil proteins in sputum macrophages were assessed by quantifying the red hue of sputum macrophages on Romanovsky-stained cytospins as described previously (32).

Immunohistochemistry

The 2-μm sections from glycomethacrylate-embedded bronchial biopsies were stained using major basic protein monoclonal antibody (clone BMK13, Monosan; Caltag MedSystems, Buckingham, UK) or isotype control (DAKO, Ely, UK) and enumerated as eosinophils per square millimeter submucosa (33).

Statistical Analysis

Statistical analysis was performed using PRISM version 4 (GraphPad Software, San Diego, CA); SAS version 8.02 (SAS Institute, Inc., Cary, NC); and SPSS version 16 (SPSS, Inc., Chicago, IL). Data were independently coded and verified using R. Parametric and nonparametric data are presented as mean (standard error of the mean) and median (interquartile range), respectively. Log-transformed data are presented as geometric mean (95% confidence interval). Sputum cytokine concentrations and eosinophilic inflammation in different compartments were presented in the subjects with asthma stratified by BMI (<25 lean, 25 to <30 overweight, >30 obese). One-way analysis of variance with Tukey correction and Kruskal-Wallis test with Dunn intergroup comparison or unpaired *t* test and Mann-Whitney test were used to compare across groups for parametric and nonparametric data, respectively, as appropriate. A *P* value of less than 0.05 was taken as the threshold of statistical significance.

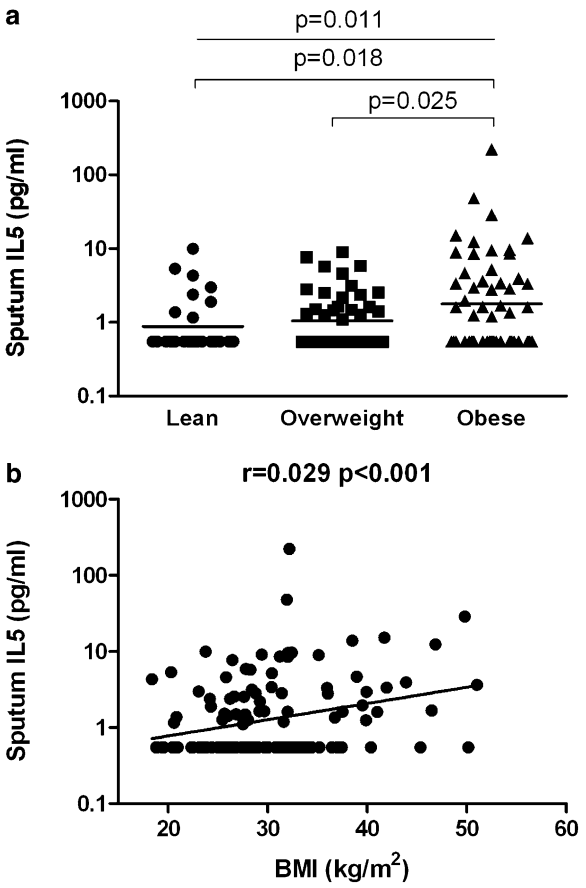

Figure 1. The relationship of sputum IL-5 and obesity in severe asthma. (*a*) Subjects are classified by their body mass index (BMI) (kg/m^2) into lean (<24.9); overweight (⩾25–29.9); and obese (⩾30). The *bar* represents the geometric mean sputum IL-5 concentration (pg/ml). *P* value across the groups (ANOVA) is given above the *solid line*. For pairwise comparisons, *P* values are given above the *line* with *markings* at each end (unpaired *t* tests for exact *P* values, when Tukey's *post hoc* test $P < 0.05$). (*b*) Correlation between sputum IL-5 and BMI.

RESULTS

In the severe asthma "sputum cytokine profiling" group the clinical characteristics of the lean, overweight, and obese groups were not significantly different except for a small difference in airflow obstruction, which was less evident in the obese group (Table 1). The sputum cytokine profiles were similar across the groups except for sputum IL-5 (Table 2) without a significant difference in the sputum eosinophil or neutrophil counts (Table 1). Sputum IL-5 geometric mean (95% confidence interval) (pg/ml) was elevated in the obese (1.8 [1.2–2.6]) compared with overweight (1.1 [0.8–1.3]; $P = 0.025$) and lean (0.9 [0.6–1.2]; $P = 0.018$) subjects with asthma (analysis of variance across groups, $P = 0.011$) (Table 2 and Figure 1a). Sputum IL-5 was correlated with BMI ($r = 0.29$; $P < 0.001$) (Figure 1b). There was no association between sputum IL-5 and other clinical parameters including age, sex, atopy, or medication. Similarly, in chronic obstructive pulmonary disease the sputum IL-5 concentration was correlated with BMI ($r = 0.55$; $P < 0.001$) (*see* Figure E1a in the online supplement) and significantly increased in those overweight and obese subjects compared with those with lean BMI (*see* Figure E1B).

We then considered whether the elevated sputum IL-5 in the overweight and obese subjects with severe asthma, in the absence of an increased sputum eosinophil count, might reflect eosinophilic inflammation in another compartment, such as the bronchial submucosa. We therefore stratified an independent population of subjects with severe asthma that underwent bronchoscopy (n = 45) into three groups based on their BMI and assessed the burden of eosinophilic inflammation in these groups and healthy control subjects (n = 19) in (*1*) bronchial submucosa, (*2*) peripheral blood, (*3*) sputum, and (*4*) red hue of eosin-stained sputum macrophages as a marker of eosinophil clearance. There were no significant differences in the clinical characteristics among the asthma groups (Table 3). The median (interquartile range) number of submucosal eosinophils was increased in severe asthma compared with healthy control subjects as a whole (Table 3). A representative example of submucosal eosinophil staining in an obese subject with asthma is as shown (Figure 2a). Submucosal eosinophil number were increased in the obese (19.4 [11.8–31.2]) (cells per square millimeter) versus lean subjects with asthma (8.2 [5.4–14.6]; $P = 0.006$) (Figure 2b). The submucosal eosinophil number in the subjects with asthma was correlated with BMI (Spearman rank correlation, $r_s = 0.38$; $P = 0.013$) (*see* Table E1). Likewise, the peripheral blood eosinophil (Figure 3) count and sputum eosinophil count (Figure 4) were increased in severe asthma compared with healthy control subjects as a whole and in all three BMI-defined groups, but were not significantly different among the lean, overweight, and obese severe asthma groups. BMI was correlated with peripheral blood ($r_s = 0.32$; $P = 0.040$), but not sputum eosinophil count ($r_s = -0.17$; $P = 0.30$) (*see* Table E1). A smaller number of patients (n = 23) had eosinophilic protein measurement in their airway macrophages and there was no significant difference among groups, although the red hue was lowest in the obese severe asthma group (*see* Figure E2). In the healthy control subjects there were no significant correlations between the eosinophil number and BMI in any of the compartments (data not shown).

TABLE 3. CHARACTERISTICS OF BRONCHIAL BIOPSY FROM SUBJECTS WITH ASTHMA AND HEALTHY CONTROL SUBJECTS

	Healthy Control Subjects ($n = 19$)	All Asthma Subjects ($n = 45$)	*P* Value	Lean ($n = 15$)	Overweight ($n = 11$)	Obese ($n = 19$)	*P* Value
Current age	40 (4)	49 (1)	0.012	49 (1)	48 (1)	50 (1)	0.9
Male, n (%)	11 (57)	20 (44)	0.36	4 (26)	7 (63)	9 (47)	0.29
BMI, kg/m^2	25.5 (0.9)	29.7 (0.9)	0.014	23.1 (0.4)	27.1 (0.4)	35.7 (1.1)	<0.001
Smoking history, pack-years	0	1.5 (2)	—	1 (0.5)	3 (2)	0.5 (1.5)	0.44
Oral corticosteroid use, n (%)	—	14 (30)	—	2 (15)	5 (45)	7 (20)	0.28
Daily prednisolone dose, mg	—	11.5 (2)	—	6.5 (1.5)	13.5 (4.5)	13 (3)	0.39
ICS dose*	—	1,600 (640–4,000)	—	1,440 (1,000–4,000)	1,600 (1,000–4,000)	1,600 (640–4,000)	0.90
Atopy present, n (%)	4 (21)	21 (45)	0.06	6 (40)	5 (45)	10 (52)	0.80
Total IgE, IU/L†	—	163 (86–310)	—	185 (80–427)	203 (31–1,306)	128 (53–284)	0.43
Pre-BD FEV_1	3.3 (0.2)	2.37 (0.11)	<0.005	2.4 (0.2)	2.4 (0.2)	2.3 (0.2)	0.83
Pre-BD FEV_1% predicted	99.8 (3.6)	80.8 (3.1)	<0.005	87 (5.5)	78 (7.4)	77 (4.4)	0.43
Pre-BD FEV_1/FVC ratio	79 (0.01)	72 (0.3)	<0.005	78 (0.6)	64 (0.8)	71 (0.5)	0.37
Total cell count (10^6/g sputum)	1.3 (0.8–2.2)	2.3 (1.5–3.4)	0.79	2.5 (0.8–5.4)	1.6 (0.5–4.4)	2.7 (1.6–4.6)	0.60
Sputum eosinophils, %‡	0.5 (0–1)	4 (1.35–17)	0.001	10.25 (1.5–23)	4.2 (0.25–20.5)	3 (1.25–8.5)	0.33
Sputum neutrophils, %	56.6 (40.7–74.3)	48.1 (37.6–61.5)	0.43	40.5 (27.4–65.1)	51 (36.3–71.7)	59.3 (46.3–75.8)	0.26
Eosinophils/mm^2 submucosa‡	4.6 (0.5–7.5)	11.7 (6–25.6)	<0.005	8.2 (5.4–14.6)	8.8 (3.9–37.7)	19.4 (11.8–31.2)	0.025
Peripheral blood eosinophil count ($\times 10^9$cells/L)‡	0.13 (0.09–0.23)	0.29 (0.2–0.51)	0.001	0.24 (0.19–0.54)	0.35 (0.21–0.52)	0.36 (0.21–0.5)	0.78

Definition of abbreviations: BD = bronchodilator; BMI = body mass index; ICS = inhaled corticosteroids.
Subjects with asthma stratified by their BMI.
Data expressed as mean (SEM) unless otherwise stated.
* Doses of all inhaled corticosteroids were converted to the equivalent dose of beclomethasone dipropionate and expressed here as median dose (range).
† Data expressed as geometric mean (95% confidence interval).
‡ Data expressed as median (interquartile range).

DISCUSSION

Here we have identified for the first time that obesity in severe asthma is associated with an elevated bronchial submucosal eosinophil number, and sputum IL-5 in the absence of an increased sputum eosinophil count. Our findings suggest that eosinophilic inflammation may play an important role in a group of obese subjects with asthma that hitherto have been labeled as noneosinophilic. This underscores the importance of the combination of clinical and biologic phenotyping with the inclusion of inflammatory profiles in different compartments in severe asthma to further understand the complexity of the disease.

In the sputum mediator profiling our most striking observation was the surprising finding that the overweight and obese groups with severe asthma were paradoxically associated with the highest sputum IL-5 concentration without a significant increase in the sputum eosinophil count. We believe this observation is robust and unlikely to be caused by chance because in addition to elevated sputum IL-5 in the obese subjects with severe asthma we found it was also elevated in a disease control group of obese subjects with chronic obstructive pulmonary disease, notwithstanding the heterogeneity in the sputum IL-5 concentration and relatively small numbers in each subgroup. Interestingly, an association between sputum Th2 cytokines and obesity in asthma has been challenged (34). However, in this earlier study sputum IL-5 was increased in obese subjects with asthma and control subjects combined compared with those subjects with a lean BMI and demonstrated a nonsignificant increase in sputum IL-5 in the obese subjects with asthma versus those with a lean BMI ($P = 0.052$) (34). Similarly, serum eotaxin levels were correlated with obesity and weight loss after bariatric surgery (35). In a recent 10-week intervention study of 38 subjects with asthma that underwent either weight reduction or exercise alone or in combination with weight reduction, subjects in the exercise-only arm had a significant reduction in sputum eosinophils (36). Taken together these small studies do begin to suggest a relationship between changes in body habitus and eosinophilic airway

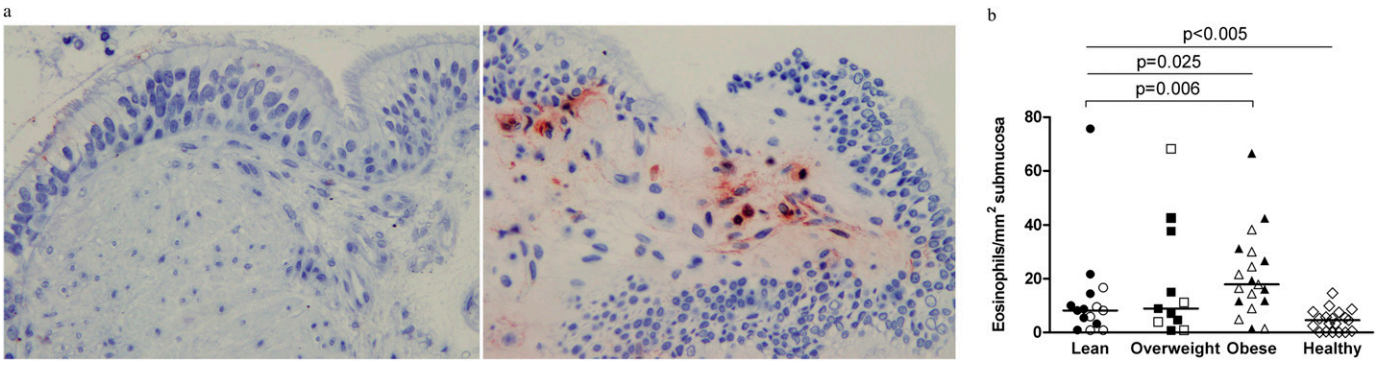

Figure 2. Bronchial submucosal eosinophil count in subjects with severe asthma stratified by body mass index. (*a*) Photomicrograph of a bronchial biopsy from an obese subject with severe asthma showing isotype control (*left*) and major basic protein-stained eosinophils (*right*) (original magnification, ×200). (*b*) Subjects with asthma are classified by their body mass index (kg/m^2) into lean (<24.9), overweight (⩾25–29.9), and obese (⩾30) and compared with healthy control subjects. The *horizontal bar* is the median. *Open symbols* denote subjects with sputum eosinophilia less than 3%; *closed symbols* denote subjects with sputum eosinophilia greater than or equal to 3%. *P* values for across and between group comparisons are as shown.

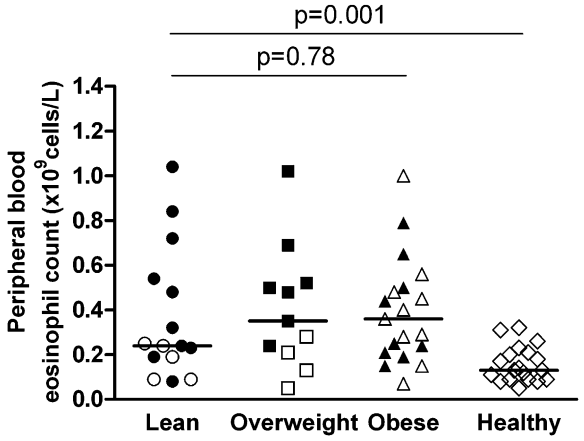

Figure 3. Peripheral blood eosinophil count in subjects with severe asthma stratified by body mass index. Subjects with asthma are classified by their body mass index (kg/m^2) into lean (<24.9), overweight (≥25–29.9), and obese (≥30) and compared with healthy control subjects. The *horizontal bar* is the median. *Open symbols* denote subjects with sputum eosinophilia less than 3%; *closed symbols* denote subjects with sputum eosinophilia greater than or equal to 3%. *P* values for across and between group comparisons are as shown.

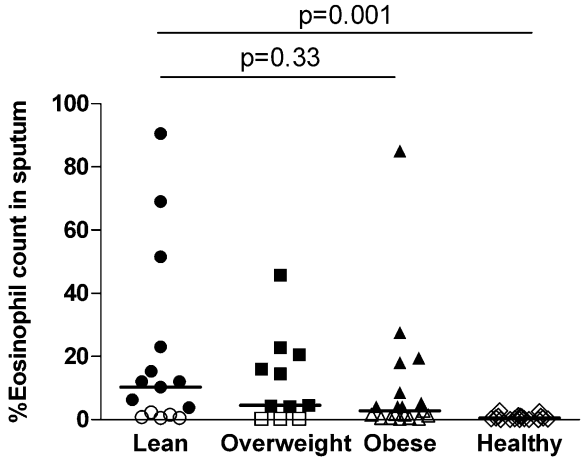

Figure 4. Sputum eosinophil count in subjects with severe asthma stratified by body mass index. Subjects with asthma are classified by their body mass index (kg/m^2) into lean (<24.9), overweight (≥25–29.9), and obese (≥30) and compared with healthy control subjects. The *horizontal bar* is the median. *Open symbols* denote subjects with sputum eosinophilia less than 3%; *closed symbols* denote subjects with sputum eosinophilia greater than or equal to 3%. *P* values for across and between group comparisons are as shown.

inflammation. Our findings might seem contrary to the current dogma of an association between obesity in asthma and noneosinophilic inflammation. However, studies have reported in asthma a decrease (37, 38) and no difference in the proportion of people with asthma that have a sputum eosinophilia (27, 39) between those with and without obesity. Here we again show that a sputum and peripheral blood eosinophil count is not elevated in obese subjects with severe asthma, but for the first time report an increase in submucosal eosinophils and sputum IL-5 in obese subjects with asthma. This apparent anomaly between an increased Th2 sputum cytokine profile in the absence of a sputum eosinophilia suggests that either eosinophil function is altered in obesity, such that response to CCR3 chemokines and Th2 cytokines is impaired, or that eosinophils are retained in the airway wall and possibly have an altered survival or adhesion within the airway wall.

To test this hypothesis we enumerated the number of eosinophils in the peripheral blood, bronchial submucosa, sputum, and eosinophil uptake by sputum macrophages in an independent group of subjects with severe asthma. Indeed, the number of submucosal eosinophils was increased in the obese subjects with severe asthma. This was associated with a trend to a reduction in the eosinophil clearance by macrophages, although this did not reach statistical significance. This increase in tissue, but not luminal airway eosinophilia, in obesity is entirely consistent with animal models. Murine models of asthma have shown that CC chemokines are up-regulated in obesity (40) with increased eosinophilia in lung tissue, but not bronchoalveolar lavage. Whether these tissue eosinophils are activated in obese people with asthma and contribute to disease needs to be further investigated.

Noneosinophilic asthma defined by sputum cell counts is associated with a poor response to corticosteroids and obesity itself is associated with corticosteroid insensitivity. Therefore, it is recognized that this group responds poorly to inhaled or systemic corticosteroid therapy. This questions the clinical importance of a bronchial submucosal eosinophilia in obese asthma. However, after corticosteroid therapy and specific anti–IL-5 monoclonal therapy submucosal eosinophils are incompletely attenuated (41) suggesting that this important immunopathologic feature of asthma is refractory to current therapy. Given that the presence of airway eosinophilia has convincingly been shown to drive exacerbation frequency, further randomized controlled trials are indicated to assess whether the target population for novel eosinophil-targeted therapies is much greater than first anticipated. Thus, it remains a possibility that the tissue eosinophilia in this group is clinically important and their reduction may translate into meaningful clinical outcomes. Whether alternative strategies to reduce eosinophilic inflammation by targeting the IL-5 receptor (42), CRTh2 (43), or important eosinophil-derived cytokines, such as IL-13 (44, 45), have efficacy in obese people with asthma needs to be tested.

Our findings also underscore the importance of obesity as a driver for the production of numerous proinflammatory cytokines and suggest that obesity may contribute to the inflammatory burden in asthma and cause increased work of breathing, as a consequence of extrathoracic restriction. Therefore, strategies targeted at diet and lifestyle to reduce obesity including bariatric surgery may indeed have an antiinflammatory role beyond simply weight reduction and may contribute to the benefits in lung function observed after successful weight reduction (46, 47).

Our study has a number of potential limitations. We have focused on severe asthma because this group represents the patients with the greatest unmet need. Whether our findings are consistent across disease severity needs to be studied. Adherence to therapy was not systematically assessed (48) and therefore we cannot completely exclude the possibility that some of the differences observed among the groups stratified by BMI are in part caused by differences in adherence to therapy. Our findings would have been strengthened by further increasing the granularity of our assessments to include additional mediators, molecular phenotyping, such as genomic, epigenomic, and transcriptomic analysis (49, 50). We have not assessed fully the environmental exposure to allergens, occupational sensitizers and irritants, pollutants, or pathogens. Our study also only includes measures at stable visits and does not include assessments at exacerbations. The integration of transcriptomic, proteomic, and cellular data, longitudinally and at times of instability, will provide further insight into the phenotypic complexity of severe asthma. The specificity of BMI as a measure of fat-free mass has been questioned, but we

have previously reported that BMI and fat-free mass index are very closely related in severe asthma (27). Recent studies have suggested that a fatty diet might promote neutrophilic inflammation (51). The subjects' diets were not formally assessed in this study and future studies should relate body habitus and diet to airway inflammation.

In conclusion, we have undertaken a comprehensive analysis of the clinical, cellular, and sputum mediator profiles of severe asthma. Our most important observation was that in the absence of a sputum eosinophilia obese subjects with severe asthma have elevated sputum IL-5 and eosinophils in their airway wall. This has important implications in the understanding of the impact of obesity in the immunopathology of asthma. It questions the view on the choice of current and future biologic therapy in this group, and highlights the importance of strategies to improve diet and lifestyle that may provide benefits in terms of weight reduction, reduced risk of comorbidities, and as suggested here eosinophilic airway inflammation.

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