

negative association with FE_{NO} . In normal weight/underweight patients with asthma, only age affected FE_{NO} ($B = -0.017$, 95% CI, -0.026 to -0.008 ; $F = 13.89$, $P < .001$).

We observed a negative association between BMI and FE_{NO} in overweight or obese patients with asthma, independent from sex, atopy, lung function, or corticosteroid use. Despite the adjusting to these main confounders, we cannot exclude the hypotheses that FE_{NO} measurement could be affected by others factors that cannot have been controlled.

Several cross-sectional and case-control studies have found obesity to be associated with asthma diagnosis, respiratory symptoms, poor lung function, and increased airway hyperreactivity. Plausible links between asthma and obesity include mechanical factors, inflammatory conditions, and stress models. The increased abdominal and chest wall mass causes decreased functional residual capacity and reduced lung and tidal volumes.^{6,7} Obesity is also a state of chronic and low-grade systemic inflammation with increased levels of the proinflammatory leptin and plasminogen activator inhibitor and decreased serum levels of the protective anti-inflammatory adiponectin.³ The relation between BMI and FE_{NO} has provided conflicting evidence. In children, BMI had no association with FE_{NO} ,⁸⁻¹⁰ whereas in 24 nonobese adults without asthma, a positive association has been reported.¹¹ Recently, a case-control study described a positive association in normal weight and obese healthy adults, but no significant association was observed in the asthma group. Although BMI was correlated with serum leptin levels in both groups, FE_{NO} was not related to serum leptin levels in patients with asthma.¹²

Our findings are based on cross-sectional data, and a causal relationship cannot be inferred. Although BMI is not the gold standard to assess body composition, it correlates with total body fat content and has been the most widely used measure to assess overweight or obesity and to monitor changes in body weight. In the future, research should include more complex anthropometric measures (eg, waist-circumference, skinfold thickness, or bioelectrical impedance analysis) that assess total body mass distribution, including body fat mass measure.

In conclusion, the observed negative association between BMI and FE_{NO} in overweight or obese patients with asthma provides additional support to the hypothesized mechanical link between obesity and asthma.

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Eosinophil β_1 integrin activation state correlates with asthma activity in a blind study of inhaled corticosteroid withdrawal

To the Editor:

Eosinophilic inflammation is a characteristic feature of asthma regulated by many factors, including treatment with corticosteroids.¹ On gradual inhaled corticosteroid (ICS) reduction, there is an increase in sputum eosinophils that often precedes changes in airway caliber and loss of asthma control.^{2,3} Conversely, ICS treatment directed toward a reduction in sputum eosinophils improves asthma control and prevents exacerbations.⁴ These and other reports suggest that eosinophils in the airway are associated with diminished asthma control and increased risk for exacerbation and that eosinophil recruitment to the airway likely is a key step in the development of bronchial inflammation and exacerbation.

Eosinophil recruitment to the airway involves integrins and their ligands.⁵ $\alpha_4\beta_1$ Integrin mediates blood eosinophil adhesion to vascular cell adhesion molecule 1, preferentially induced on endothelium in response to T_H2

immunity mediators.⁵ Integrin-mediated adhesion is a function of cell-surface integrin density, activation state, and ligand density. Although $\alpha_4\beta_1$ on purified blood eosinophils binds vascular cell adhesion molecule 1 constitutively,⁵ the possibility exists that $\alpha_4\beta_1$ is activated *in vivo* on circulating eosinophils and the activation state is a determinant of eosinophil adhesion to activated endothelium and movement to the airway. If so, blood eosinophil β_1 activation state may be a biomarker for the level of asthma activity or control.

Eosinophil integrins were therefore evaluated in patients with asthma as part of an ICS withdrawal study. The overall goal of the study was to test the hypothesis that circulating eosinophils exhibiting enhanced survival, adhesion, and migration; prime instability of airway function; and loss of asthma control. Eight subjects were recruited with a diagnosis of mild, persistent asthma ($FEV_1 \geq 80\%$ predicted), a requirement for ICSs, and parameters of asthma control indicating an effective response to current ICS treatment—that is, absence of symptoms and minimal use of inhaled β_2 -agonists (see this article's [Table E1](#) in the Online Repository at www.jacionline.org). Subjects were entered in a randomized, double-blind, placebo-controlled, 2-period crossover study (see this article's [Fig E1](#) in the Online Repository at www.jacionline.org).

We focused on reactivity of blood eosinophils with the activation-sensitive anti- β_1 (CD29) integrin mAb N29⁶ and on expression of the α_D integrin subunit.⁷ N29 was chosen because it was variably reactive with eosinophils in a preliminary study of 10 donors (not shown). α_D was chosen because it is upregulated on bronchoalveolar lavage (BAL) fluid eosinophils after antigen challenge and is low or absent on eosinophils in unchallenged blood⁷ (not shown). Total β_1 and β_2 were included as controls. When N29 and α_D expression was compared between eosinophils in whole blood and purified eosinophils from the same donors, expression was higher on purified cells, indicating that β_1 can become activated and α_D recruited to the surface on purification. To avoid such artefactual activations, we performed flow cytometry on whole blood. Eosinophils were gated based both on scattering and reaction with anti-CD14/CD16.

When the subjects underwent true ICS withdrawal, changes in some measurements indicative of asthma control, such as airway sensitivity to methacholine ($P = .008$) and fraction of exhaled nitric oxide (FENO; $P = .02$), were significantly different than during sham withdrawal (see this article's [Table E2](#) in the Online Repository at www.jacionline.org). Changes in other measurements were not significantly different between the periods. However, there were significant differences in some measurements comparing the visit after true ICS withdrawal with that before withdrawal, such as FEV_1 (mean after true ICS withdrawal = 3.9 L, 96% of baseline; mean before true ICS withdrawal = 4.2 L, 102% of baseline; $P = .05$), peak expiratory flow (mean after withdrawal = 480 L/min, before = 550 L/min, $P = .008$), symptom score (mean after withdrawal = 2.0, before = 0.6, $P =$

.03), and airway sensitivity to methacholine (mean after withdrawal = 3.1 mg/mL, before = 8.7 mg/mL, $P = .04$; see this article's [Table E2](#) in the Online Repository at www.jacionline.org). When comparing the visit after sham ICS withdrawal with that before sham withdrawal, mean FEV_1 was not different ($P = .35$), although there were differences in individual subjects, including one who had a >10% decrease (see this article's [Table E1](#) in the Online Repository at www.jacionline.org).

Comparing changes in N29 and total β_1 integrin expression on blood eosinophils during the study periods, there were no significant differences (see this article's [Table E3](#) in the Online Repository at www.jacionline.org). Comparing the visit after true ICS withdrawal with that before true withdrawal (mean N29⁺ cells was 50% and 28%, respectively; see this article's [Table E3](#) in the Online Repository at www.jacionline.org), significance was not reached ($P = .23$), although striking changes occurred in individual subjects (not shown).

Because of the considerable visit-to-visit variability in FEV_1 , FENO, and sputum eosinophils and the individual variability in N29 expression, markers of loss of asthma control were correlated with N29. Correlations after true ICS withdrawal were analyzed by using the Spearman rank correlation test. Across multiple study visits, P values were obtained for correlations using mixed-effect linear models of the ranked data, each including a fixed effect term for study visit and a random effect term for study subject, to account for treatment effects and within-subject correlation across visits.⁸ There was an inverse correlation between percent N29⁺ eosinophils and FEV_1 after ICS withdrawal (Spearman rank correlation coefficient [r_s] = -0.74 ; $P = .05$). When all visits were analyzed, N29 expression significantly correlated with FEV_1 ($P = .01$ for percent positive cells; $P = .02$ for level; [Table I](#)). N29 correlated directly with FENO after ICS withdrawal ($r_s = 0.79$; $P = .03$), but not when all visits were analyzed ([Table I](#)). N29 expression did not correlate with sputum eosinophils ([Table I](#)). Total β_1 expression did not correlate with FEV_1 but did correlate with FENO ([Table I](#)). α_D expression, β_2 expression, eosinophil percentage in BAL fluid, or concentration of eosinophils in blood did not correlate with FEV_1 (not shown).

The correlations with FEV_1 raise the possibility that N29 expression on eosinophils in peripheral blood is a possible marker for the level of asthma activity or control. Although N29 correlated with FEV_1 regardless of whether only the visits after true ICS withdrawal or all visits were analyzed, it is not possible to conclude from the current study whether it correlates with the severity of the underlying asthma and/or with the magnitude of the response to ICS withdrawal. A larger study of patients with asthma of a range of severity on or off ICS will be required to make such conclusions.

N29 expression correlated better with FEV_1 ($P = .01$) than did percentage of eosinophils in sputum ($P = .33$) or FENO ($P = .56$), 2 existing biomarkers of asthma control.¹ We performed receiver-operator characteristic (ROC) curve analyses on the ability of N29, FENO, and

TABLE I. Correlations between integrin expression on blood eosinophils and lung function, FENO, or the percentage of eosinophils in sputum using all data sets

	FEV ₁		FENO		Sputum eosinophils	
	r _s	P	r _s	P	r _s	P
N29 (gMCF)	−0.50	.02	0.49	.07	0.24	.28
(% positive cells)	−0.56	.01	0.39	.35	0.34	.29
Total β ₁ integrin (gMCF)	−0.28	.59	0.51	.02	0.14	.37
(% positive cells)	−0.28	.37	0.22	.05	0.07	.41
Sputum eosinophils (%)	−0.18	.33	0.34	.46		
FENO	−0.27	.56				

gMCF, Geometric mean channel fluorescence.

FEV₁ expressed as percent of baseline. P, repeated measures rank regression test⁸ (R software, Vienna, Austria; www.R-project.org); r_s, Prism 3.0, GraphPad, San Diego, Calif. Correlations are among data from all visits (before ICS withdrawal, after ICS withdrawal, before sham ICS withdrawal, and after sham withdrawal), where data were available. The number of visits with available data was 29 for measurement of N29 expression, 30 for total β₁ integrin expression, and 32 for sputum eosinophils and FENO. N29⁶ was from Chemicon (Temecula, Calif) and mAb MAR4 to total β₁ from Becton Dickinson (San Diego, Calif).

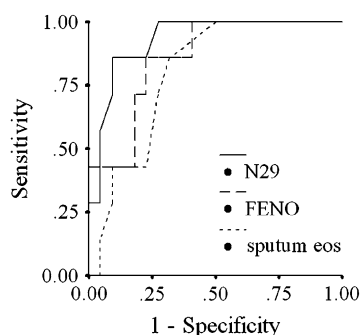


FIG 1. ROC curves (SPSS 11.0, Chicago, Ill) for the ability of blood eosinophil expression of the epitope for the activation-sensitive β₁ integrin mAb N29 (percent positive cells), FENO, or eosinophil percentage in sputum to predict FEV₁ <95% of baseline. Areas under curve: N29, 0.93; FENO, 0.86; sputum eosinophils, 0.80.

sputum eosinophils to predict FEV₁ < 95% of baseline. N29 performed well (area under curve = 0.93) and better than did FENO and sputum eosinophils (Fig 1). However, comparing these ROC curves⁹ showed that the differences in the areas under curve were not significant, given the small numbers. Thus, whether the predictive ability of N29 expression is significantly higher than that of FENO or sputum eosinophils must be tested in a larger study.

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