Obesity and Asthma

An Inflammatory Disease of Adipose Tissue Not the Airway

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Rationale: Obesity is a major risk factor for asthma; the reasons for this are poorly understood, although it is thought that inflammatory changes in adipose tissue in obesity could contribute to airway inflammation and airway reactivity in individuals who are obese.

Objectives: To determine if inflammation in adipose tissue in obesity is related to late-onset asthma, and associated with increased markers of airway inflammation and reactivity.

Methods: We recruited a cohort of obese women with asthma and obese control women. We followed subjects with asthma for 12 months after bariatric surgery. We compared markers in adipose tissue and the airway from subjects with asthma and control subjects, and changes in subjects with asthma over time.

Measurements and Main Results: Subjects with asthma had increased macrophage infiltration of visceral adipose tissue ($P < 0.01$), with increased expression of leptin ($P < 0.01$) and decreased adiponectin ($p < 0.001$) when controlled for body mass index. Similar trends were observed in subcutaneous adipose tissue. Airway epithelial cells expressed receptors for leptin and adiponectin, and airway reactivity was significantly related to visceral fat leptin expression ($\rho = -0.8$; $P < 0.01$). Bronchoalveolar lavage cytokines and cytokine production from alveolar macrophages were similar in subjects with asthma and control subjects at baseline, and tended to increase 12 months after surgery.

Conclusions: Obesity is associated with increased markers of inflammation in serum and adipose tissue, and yet decreased airway inflammation in obese people with asthma; these patterns reverse with bariatric surgery. Leptin and other adipokines may be important mediators of airway disease in obesity through direct effects on the airway rather than by enhancing airway inflammation.

Keywords: asthma; obesity; adipokine; airway hyperreactivity

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Obesity is a major risk factor for asthma, and it has been hypothesized that inflammation in adipose tissue could lead to airway inflammation causing asthma in the setting of obesity.

What This Study Adds to the Field

Proinflammatory adipokines are increased in visceral adipose tissue of obese people with asthma, and visceral fat leptin is directly correlated with airway reactivity. However, this is not associated with airway inflammation. Airway epithelial cells express receptors for adipokines, suggesting that direct effects of adipokines on the airway may be important in the pathogenesis of asthma in obesity.

Obesity is a major risk factor for the development of asthma (1, 2). Asthma in obesity is often characterized by severe, poorly controlled disease (3–5), which tends to be relatively resistant to standard controller therapies (6–8). Understanding why asthma develops in the setting of obesity is essential for the formulation of effective treatments for this difficult subset of patients.

There has been intense interest in the potential role of adipose tissue in the development of asthma in obesity. Adipose tissue is an active endocrine organ elaborating cytokines and hormones that regulate metabolism and immune responses. In the lean state, adipose tissue typically secretes low levels of proinflammatory cytokines (e.g., IL-6, IL-8, tumor necrosis factor [TNF]- α) and adipokines (e.g., leptin), and produces high levels of the antiinflammatory adipokine adiponectin. In the obese state, adipose tissue hypertrophies and becomes infiltrated with proinflammatory macrophages. These activated macrophages and hypertrophic adipocytes produce increased proinflammatory cytokines and adipokines, and decreased adiponectin; this “metabolic inflammation” is thought to produce the systemic complications of obesity, such as type 2 diabetes, steatohepatitis, and the metabolic syndrome (9). This has led to speculation that proinflammatory adipokines in obesity may augment airway inflammation producing asthma in obesity.

The appreciation that asthma includes different phenotypes of disease provides a critical framework for the study of asthma in obesity. Asthma, even in lean individuals, is a syndrome comprised of distinct phenotypes that can be recognized by characteristics, such as the presence of allergy, the age of onset of disease, and the nature of airway inflammation (10, 11). This is an important concept, because phenotype of disease predicts response to therapy, as illustrated by a recent study on the efficacy of anti-IL-13 therapy in “T_H2-high” disease (12).

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Similar phenotypic heterogeneity seems to exist in obese asthma. Holguin and coworkers (13) described one group with early onset disease, a high prevalence of atopy, and high serum IgE levels; and a second, predominantly female group with later-onset disease, lower prevalence of atopy, and lower serum IgE. Sutherland and coworkers (14) have reported similar findings in a different cohort of people with asthma. We have reported that people with late-onset asthma with low serum IgE (i.e., a low T_H2 phenotype) experience significant improvements in symptoms and airway hyperreactivity (AHR) with bariatric surgery, whereas individuals with early onset disease and high IgE (a high T_H2 phenotype) experience improved symptoms, but not improved AHR. Thus, there seem to be two distinct phenotypes of asthma occurring in the obese: one type with early onset disease complicated by obesity, and a second with late-onset asthma likely developing consequent to obesity. This T_H2 -low, late-onset group has also been identified in cluster analyses studies of asthma phenotype (10, 11, 15). We reasoned that focusing on this T_H2 -low, late-onset group could reveal fundamental insights into the pathogenesis of airway disease arising secondary to obesity.

The purpose of this study was to determine if markers of metabolic inflammation in adipose tissue distinguished between obese, late-onset, T_H2 -low subjects with asthma and obese subjects without asthma, because we reasoned that metabolic inflammation would drive airway inflammation leading to airway reactivity in obese people with asthma. We included only women, because this is the group with the highest prevalence of this phenotype of asthma (10, 11, 13, 15). We sought to determine if metabolic inflammation was associated with increased inflammation in the airway, if this was related to airway reactivity, and whether this would improve with bariatric surgery-induced weight loss. Some of the results of these studies have previously been reported in abstract form (16).

METHODS

Participant Selection and Study Design

The study was approved by the University of Vermont Institutional Review Board. All participants provided written informed consent. The effects of bariatric surgery on lung function and asthma control have been included in a previous report that included early and late onset asthma in obese individuals (17). Participants were recruited before bariatric surgery. A group of women with a history of adult-onset asthma, IgE less than 100 IU/ml, and physiologic evidence of asthma (with a positive bronchodilator response or AHR to methacholine) and control women (with low IgE, no history of asthma or other lung disease) were included in this study. We included only women because women seem to have a particularly high risk of developing late-onset T_H2 -low asthma in the setting of obesity. The following participants were excluded: those with a greater than 20 pack-year smoking history or with a history of smoking within the last 6 months; on systemic steroids in the prior 6 weeks; or on thiazolidinedione medication (which may affect adipokines).

Lung Function Tests

Lung function tests were performed as previously described (17).

Adipose Tissue Sampling

Visceral adipose tissue was obtained from the omentum (and so could only be collected at the time of surgery). Subcutaneous adipose tissue was collected by percutaneous needle biopsies before surgery from all participants, and 12 months later from participants with asthma.

Blood Samples

Serum samples were obtained and stored at -80°C for later analysis.

Bronchoscopy

Participants underwent bronchoscopy with bronchoalveolar lavage (BAL) and brush biopsy under general anesthesia immediately before bariatric surgery. Consenting participants with asthma had a second bronchoscopy 12 months after surgery under conscious sedation.

Laboratory Methods

Laboratory methods are described in the online supplement.

Statistical Analyses

We compared baseline differences between participants with asthma and obese control participants, and also followed the cohort of participants with asthma over time, before and after gastric-bypass surgery. Data were summarized by descriptive statistics, with average values, and variation described using mean and standard deviation. We initially used a t test to compare differences in subjects with and without asthma, and Fisher exact test to examine differences in proportions between subjects with asthma and control subjects. Nonnormally distributed data were log-transformed before analysis. We performed multiple linear regression analysis to control for effects of body mass index (BMI) when comparing subjects with asthma and control subjects at baseline. Paired t tests were used to compare changes in measures from baseline to 12 months after surgery. Throughout figures and tables, 0 indicates baseline and 12 indicates 12 months after bariatric surgery. Spearman correlation was used to determine the relationship between BAL levels of adipokines and adipokines in serum and both visceral and subcutaneous adipose tissue. Given the exploratory nature of this study, we did not attempt to control for multiple comparisons. Statistical tests were performed with GraphPad Prism (San Diego, CA) and STATA 10.0 (College Station, TX).

RESULTS

Demographics and Weight Loss

Fifteen obese control women and 11 women with asthma participated in this study. All participants with asthma had adult-onset asthma. Participants with asthma tended to be older and significantly heavier than control subjects (Table 1).

Twelve months after bariatric surgery participants with asthma had an average weight loss of 28.4 ± 16.5 kg, and asthma control and AHR improved significantly (Table 2).

Relationship of Adipose Tissue Markers to Asthma

Baseline levels. Visceral adipose tissue from obese subjects with asthma isolated at the time of bariatric surgery had significantly lower adiponectin, but higher leptin and CD68 (a macrophage marker) expression by real-time polymerase chain reaction compared with control participants, and this was independent of BMI (Table 3). Other proinflammatory mediators, such as monocyte chemotactic protein-1 (MCP-1), IL-6, IL-8, and plasminogen

TABLE 1. DEMOGRAPHIC DATA OF PARTICIPANTS WITH ASTHMA AND CONTROL PARTICIPANTS

	Obese Control	Asthma	<i>P</i> Value
N	15	11	
Age, yr	43 ± 7	48 ± 6.7	0.08
Age of asthma onset, yr		$36.8 \pm 10.5^*$	
Serum IgE, IU/ml	24.5 ± 24	25.5 ± 26.6	0.92
Diabetes mellitus	5	5	0.53
BMI before surgery, kg/m^2	43.3 ± 5.3	52 ± 11.2	0.01
BMI after surgery, kg/m^2		40.8 ± 1.9	

Definition of abbreviation: BMI = body mass index.

Values shown are mean $\pm$ SD, *P* value shown for t test for continuous variables, and Fisher exact test for proportions.

* Minimum age reported for asthma onset was 22 years.

TABLE 2. LUNG FUNCTION, ASTHMA SYMPTOMS, AND MEDICATION USE BEFORE AND AFTER BARIATRIC SURGERY

	Asthma Visit 0	Asthma Visit 12	P Value
FEV ₁ , % predicted	79.8 ± 10.6	91.8 ± 14.2	0.001
FVC, % predicted	83.2 ± 13.6	92.3 ± 16.2	0.03
FEV ₁ /FVC, % predicted	96.7 ± 9.1	99.9 ± 6.4	0.337
PC ₂₀ , mg/ml methacholine*	3.42 ± 2.50	9.94 ± 6.40	0.0007
Exhaled nitric oxide, ppb	4.53 ± 2.89	6.98 ± 5	0.10
Juniper Asthma Control Score	1.79 ± 1.32	0.87 ± 1.13	0.11
Inhaled corticosteroid	9	6	0.36
Long-acting β agonist	6	5	0.59
Leukotriene modifier	2	1	0.54
Short-acting β agonist	11	5	0.01

Values shown are mean ± SD for lung function measures, and n for medication use.

P values are for paired *t* test for lung function measures and Fisher exact test for medication use.

*n = 9.

activator inhibitor-1, tended to be higher in subjects with asthma, although this was not significant when adjusted for BMI.

At baseline, visceral fat leptin expression was significantly related to airway reactivity to methacholine ($P = 0.0096$; $\rho = -0.8$) (Figure 1). There was no significant relationship between other adipokines and AHR (data not shown). There was a non-significant trend toward a relationship between AHR and BMI ($\rho = -0.41$; $P = 0.09$), and when BMI and visceral fat leptin expression were included in a multivariate regression analysis, AHR was more significantly related to visceral fat leptin ($P = 0.08$) than to BMI ($P = 0.83$).

Adipokine mRNA expression was also measured in subcutaneous fat. Patients with asthma had significantly higher expression of leptin in subcutaneous fat, even when controlled for BMI ($P = 0.05$). There were no significant differences in other adipokines when controlled for BMI, although all proinflammatory cytokines and adipokines tended to be higher in subjects with asthma than obese control subjects (Table 4).

Changes in adipokines in subcutaneous adipose tissue with weight loss. Only subcutaneous tissue could be collected at 12 months. Twelve months after bariatric surgery there was a trend toward increased adiponectin and decreased leptin mRNA expression, with a significant decrease in CD68 expression (Table 5). Expression of a number of proinflammatory markers was significantly decreased in patients with asthma after surgery, with a trend toward decreased expression of IFN- γ , TNF- α , and leptin. Decreased expression of the macrophage marker CD-68 in subcutaneous adipose tissue was confirmed by immunohistochemistry ($P < 0.01$) (Figure 2).

Relationship between Serum Markers of Metabolic Inflammation and Asthma

Baseline serum adipokine (IL-8, MCP-1, IL-6, leptin, and adiponectin) levels were not significantly different between obese participants with asthma and obese control participants when controlled for BMI (Figure 3). In the obese subjects with asthma, concentrations of the proinflammatory cytokines IL-6 and IL-8 decreased with weight loss (Figure 3).

Markers of Asthmatic and Metabolic Inflammation in the Lung

Airway epithelium. Epithelial cells isolated from bronchial brushing of patients with asthma had significantly higher expression of adiponectin receptor-2 and T-cadherin (which also binds adiponectin) mRNA at baseline (Table 6). Leptin expression tended to increase and adiponectin receptor-2 expression to decrease, without changes in adiponectin receptor-1 or leptin receptor 12 months after bariatric surgery (Table 7).

BAL. Changes in leptin and adiponectin protein levels in airways were similar to those found in adipose tissue: at baseline leptin levels were higher, and adiponectin lower in BAL of participants with asthma compared with control subjects (Figure 4). There were no significant differences in the levels of other proinflammatory cytokines measured at baseline between subjects with asthma and control subjects, although IL-8, MCP-1, and TNF- α tended to be higher in control subjects. There was a modest correlation between BAL leptin and airway reactivity (Spearman $\rho = -0.55$; $P = 0.16$). After weight loss, BAL IL-8 and MCP-1 increased significantly and there was a trend toward increased TNF- α (Figure 4).

Correlation between BAL, serum, and fat levels of adipokines and cytokines. To determine factors that may influence lung levels of these mediators we analyzed the correlation of BAL content of adipokines with the expression level in the two adipose different tissue depots and serum levels at the baseline visit. The correlation between adipokines in tissue and serum with BAL differed between obese control subjects and subjects with asthma, and so are reported separately (Tables 8 and 9).

In obese control subjects, but not subjects with asthma, visceral fat adiponectin was significantly related to BAL levels of adiponectin ($\rho = 0.58$; $P = 0.02$). In subjects with asthma, there was a trend toward a significant correlation between visceral fat and BAL leptin ($\rho = 0.61$; $P = 0.06$), but none between subcutaneous leptin and BAL leptin, or between serum and BAL leptin. In subjects with asthma, there were also strong correlations of BAL IL-6 with visceral, subcutaneous,

TABLE 3. ADIPOKINE GENE EXPRESSION AND CELL SIZE OF VISCERAL ADIPOSE TISSUE AT BASELINE

Adipokine	Obese Control 0	Asthma 0	P*	Adjusted P [†]
Leptin	0.125 ± 0.016	0.305 ± 0.047	0.002	0.026
Adiponectin	0.866 ± 0.134	0.347 ± 0.037	<0.001	<0.001
CD-68	0.629 ± 0.048	0.979 ± 0.109	0.015	0.012
MCP-1	0.848 ± 0.159	1.867 ± 0.433	0.08	0.49
IL-6	2.847 ± 0.910	5.883 ± 1.103	0.16	0.71
IL-8	0.924 ± 0.355	2.173 ± 1.076	0.13	0.86
PAI-1	0.532 ± 0.132	0.948 ± 0.243	0.15	0.63
TNF- α	1.275 ± 0.182	1.908 ± 0.681	0.66	0.44
IFN- γ	3.232 ± 0.640	3.385 ± 0.767	0.57	0.78
Cell size, μ m	60.9 ± 9.48	59.8 ± 6.47	0.82	0.80

Definition of abbreviations: MCP = monocyte chemotactic protein; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor.

Values shown are mean and SD.

*Analysis is unpaired *t* test of log-transformed values.

[†]P value shown for multiple regression analysis comparing subjects with asthma and control subjects when controlled for body mass index.

Figure 1. Relationship between visceral fat leptin expression and airway reactivity in participants with asthma at baseline (nine participants had measurement of methacholine reactivity and visceral fat isolation at baseline). $\rho = -0.8$, $P = 0.0096$ for Spearman's correlation. Repeat analysis dropping outlier with highest PC_{20} : $\rho = -0.74$; $P = 0.037$.

and serum IL-6, and of BAL IL-8 with visceral and subcutaneous IL-8.

Correlations at 12 months in subjects with asthma are shown in the online supplement.

BAL cell counts and macrophages. Differential cell counts of eosinophils (subjects with asthma, $0.2 \pm 0.4\%$; control subjects, $0 \pm 0\%$) and neutrophils (subjects with asthma, $2.5 \pm 1.6\%$; control subjects, $5.7 \pm 6.1\%$) were low at baseline, and did not change significantly with weight loss.

At baseline macrophages from subjects with asthma tended to produce lower levels of proinflammatory cytokines (IL-6, IL-8, IL-1 β , and TNF- α) than control subjects (Figure 5A). Response to LPS stimulation was similar between subjects with asthma and control subjects (Figure 5B). BAL macrophages spontaneously produced higher levels of cytokines after bariatric surgery (Figure 5A), whereas response to LPS was similar to that seen before surgery (Figure 5B).

TABLE 4. ADIPOKINE GENE EXPRESSION AND CELL SIZE OF SUBCUTANEOUS ADIPOSE TISSUE AT BASELINE

Adipokine	Obese Control 0	Asthma 0	P^*	Adjusted $P^\dagger$
Leptin	0.295 ± 0.032	0.439 ± 0.052	0.12	0.05
Adiponectin	1.042 ± 0.118	0.980 ± 0.073	0.99	0.27
CD-68	0.765 ± 0.072	1.069 ± 0.158	0.19	0.72
MCP-1	0.620 ± 0.146	0.881 ± 0.183	0.11	0.14
IL-6	1.705 ± 1.018	2.274 ± 0.985	0.57	0.40
IL-8	0.517 ± 0.162	1.751 ± 0.651	0.08	0.56
PAI-1	0.468 ± 0.090	0.983 ± 0.202	0.05	0.25
TNF- α	0.714 ± 0.104	1.136 ± 0.304	0.21	0.13
IFN- γ	1.270 ± 0.238	1.457 ± 0.514	0.83	0.32
Cell size, μm	54 ± 5.47	66.5 ± 9.42	0.01	0.07

Definition of abbreviations: MCP = monocyte chemotactic protein; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor.

Values shown are mean and SD.

* P value for unpaired t test of log-transformed values.

† P value for linear regression analysis comparing subjects with asthma and control subjects when controlled for body mass index.

TABLE 5. ADIPOKINE GENE EXPRESSION AND CELL SIZE OF SUBCUTANEOUS ADIPOSE TISSUE OF PARTICIPANTS WITH ASTHMA AT BASELINE AND 12 MONTHS AFTER BARIATRIC SURGERY

Adipokine	Asthma 0	Asthma 12	P^*
Leptin	0.439 ± 0.052	0.366 ± 0.038	0.11
Adiponectin	0.980 ± 0.073	1.298 ± 0.170	0.20
CD-68 (mRNA)	1.069 ± 0.158	0.435 ± 0.072	0.001
MCP-1	0.881 ± 0.183	0.278 ± 0.064	0.002
IL-6	2.274 ± 0.985	0.175 ± 0.036	0.07
IL-8	1.751 ± 0.651	0.171 ± 0.043	0.01
PAI-1	0.983 ± 0.202	0.252 ± 0.057	0.01
TNF- α	1.136 ± 0.304	0.543 ± 0.144	0.02
IFN- γ	1.457 ± 0.514	0.625 ± 0.273	0.07
Cell size, μm	66.5 ± 9.42	63.9 ± 8.20	0.61

Definition of abbreviations: MCP = monocyte chemotactic protein; PAI = plasminogen activator inhibitor; TNF = tumor necrosis factor.

Values shown are mean and SD.

* P value shown for paired t test of log-transformed values.

DISCUSSION

This study shows that markers of metabolic inflammation, particularly in visceral adipose tissue, are significantly higher in obese people with asthma compared with obese control subjects. Airway epithelial cells express receptors for the adipokines leptin and adiponectin, suggesting that airway epithelium may

Figure 2. Immunohistochemistry showing expression of the macrophage marker CD68 (red) in adipose tissue. Blue staining is DAPI and green staining is BS-1 lectin. (a) CD68 staining alone in visceral adipose tissue of participant with asthma. (b) Composite figure of visceral adipose tissue of participant with asthma. (c) Subcutaneous adipose tissue from participant with asthma before surgery. (d) Subcutaneous adipose tissue from participant with asthma after surgery. Also shown are representative sections from (e) control participants and (f) CD68 staining control.

Figure 3. Serum adipokines in 15 control subjects and 11 participants with asthma at baseline, and in 11 participants with asthma at 12 months. * $P < 0.05$. MCP = monocyte chemotactic protein.

respond to these mediators. Indeed, visceral fat leptin expression was significantly related to AHR. We speculate that metabolic mediators in obesity have direct effects on the airway, rather than increasing airway inflammation, to produce airway reactivity in obesity.

Visceral adipose tissue is strongly associated with increased risk of metabolic diseases, such as steatohepatitis and diabetes (18). We found that adipokines and inflammatory markers, particularly in visceral adipose tissue, were significantly higher in subjects with asthma than control subjects. BAL levels of leptin, IL-6, and IL-8, but not adiponectin, tended to correlate with those found in visceral adipose tissue of subjects with asthma. Others have reported discordance between serum and BAL adiponectin (19); this could be related to transport of adiponectin isoforms from serum into the lung (20). The reason that BAL leptin, IL-6, and IL-8 tend to correlate with those in visceral adipose tissue in people with asthma is not known, but could relate to the relatively high level of these mediators in asthmatic visceral adipose tissue, which drains into the portal circulation and subsequently passes through the vasculature of the lung. This association warrants further study. A number of epidemiologic studies have described associations between serum adipokines and asthma, but the data do not provide a consistent pattern regarding these relationships (21). The lack of correlation between the lung adipokines suggests that it may be difficult to interpret epidemiologic studies linking serum adipokines with asthma.

Previous studies have investigated the functional role of adiponectin and leptin in asthma: in allergic mouse models of asthma, leptin increases and adiponectin decreases airway reactivity (22, 23). We found a significant relationship between visceral fat leptin expression and airway reactivity. Although this association obviously does not show that leptin causes AHR, multiple factors could contribute to AHR in obesity (17). Shore and coworkers (22) have shown that leptin infusion increases airway reactivity in a mouse model of allergic asthma, and that this is not related to increased allergic airway inflammation. This suggests that leptin and leptin receptor signaling may affect airway function through mechanisms other than allergic airway inflammation. We found evidence of leptin and leptin receptor expression within airway epithelium. Previous reports have also shown that leptin and leptin receptor are expressed in airway epithelium (24), and bronchial expression of leptin is increased in chronic obstructive pulmonary disease (25, 26). Leptin seems to be involved in embryonic lung growth and maturation (27, 28), and in

fibroproliferative responses in the lung to bleomycin (29). Furthermore, polymorphisms in leptin and leptin receptor have been associated with differences in lung function (30, 31). Leptin contributes to remodeling in the kidney, liver, and heart (32–34). Our data suggest that visceral and locally released leptin could be acting on the airway; given the known effects of leptin on remodeling in other organs and the airway, it may contribute to remodeling of the airway in obese people with asthma.

Adiponectin may also affect airway function. Miller and coworkers (35) previously reported that airway epithelial cells of smokers expressed AdipoR1, but not AdipoR2, whereas Daniele and coworkers (36) reported lower expression of AdipoR2 compared with AdipoR1 in chronic obstructive pulmonary disease. We are not aware of any previous reports on the expression of adiponectin receptors in humans with asthma. Our data suggest that AdipoR2 is more highly expressed in the airway epithelium of people with asthma, and tends to decrease with weight loss. In contrast, T-cadherin, another putative receptor for adiponectin (37), is increased in control subjects, and tends to decrease with weight loss. Shore and coworkers (23) have previously shown that allergen challenge decreases pulmonary expression of adiponectin receptors 1 and 2 and T-cadherin in mice, and that T-cadherin may be involved in transport of adiponectin into the lungs. The lower expression of T-cadherin in people with asthma may point to an abnormality in adiponectin trafficking in the airway of obese people with asthma compared with obese control subjects, although the functional significance of this and the increased AdipoR2 expression clearly require further study (20).

TABLE 6. GENE EXPRESSION PROFILE IN BRONCHIAL EPITHELIAL CELLS AT BASELINE

Adipokine	Obese Control	Asthma 0	P
Leptin	1.459 ± 0.430	1.179 ± 0.256	0.263
Leptin receptor	2.856 ± 1.186	3.617 ± 1.359	0.215
Adiponectin receptor-1	1.008 ± 0.050	1.107 ± 0.102	0.495
Adiponectin receptor-2	1.047 ± 0.101	3.643 ± 5.570*	0.030
T-cadherin	1.065 ± 0.129	0.714 ± 0.081	0.014

Data are for 10 obese control subjects and 10 obese subjects with asthma.

P value shown for unpaired *t* test of log-transformed values.

*SD related to presence of one outlier, analysis repeated with exclusion of outlier, $P = 0.03$.

TABLE 7. GENE EXPRESSION PROFILE IN BRONCHIAL EPITHELIAL CELLS IN PARTICIPANTS WITH ASTHMA BEFORE AND AFTER BARIATRIC SURGERY

Adipokine	Asthma 0	Asthma 12	P
Leptin	0.940 ± 0.549	4.121 ± 2.159	0.23
Leptin receptor	3.715 ± 2.616	2.968 ± 1.170	0.72
Adiponectin receptor-1	0.993 ± 0.294	1.190 ± 0.144	0.06
Adiponectin receptor-2	2.098 ± 0.417	1.407 ± 0.083	0.12
T-cadherin	0.638 ± 0.115	0.869 ± 0.165	0.13

Data shown are for five subjects with asthma before and after surgery on stable regimen of inhaled corticosteroid.

P value shown for paired *t* test of log-transformed values.

Although adipokines may directly affect the airway, obesity also causes profound changes in immune cell function (38). Little is known about macrophage function in obese people with asthma. Macrophages in adipose tissue play an important role in the pathogenesis of metabolic inflammation: obesity is associated with a shift from an alternatively activated M2 macrophage to a classically activated proinflammatory M1 phenotype, and accumulation of macrophages in adipose tissue plays an important role in the production of proinflammatory cytokines involved in metabolic inflammation (39). Thus, we anticipated we might find enhanced production of proinflammatory cytokines from airway macrophages in our obese subjects. However, we found evidence of reduced cytokine production from alveolar macrophages isolated from obese control subjects and participants with asthma at the time of bariatric surgery, compared with those isolated 12 months later. We also found reduced levels of proinflammatory cytokines in BAL fluid further suggesting that alveolar macrophage function is altered in obesity. Previous studies have reported abnormalities of nonadipose tissue macrophage function in obesity. Mancuso and coworkers (40) have reported impaired function of alveolar macrophages function in obese leptin-deficient mice (specifically, impaired phagocytosis and leukotriene production), associated with increased mortality in a model of *Klebsiella pneumoniae*. Similarly, Loffreda and coworkers (41) reported that peritoneal macrophages isolated from leptin-deficient rats have impaired phagocytosis and blunted cytokine response to LPS. Amar and coworkers (42) reported impaired peritoneal macrophage function in a mouse

Figure 4. Adipokines and cytokines in bronchoalveolar lavage (BAL) fluid from control subjects (*n* = 15) and participants with asthma at baseline (*n* = 11) and participants with asthma 12 months after surgery (*n* = 11). **P* < 0.05. Of note, y axis is on a log scale. MCP = monocyte chemotactic protein; TNF = tumor necrosis factor.

TABLE 8. RELATIONSHIP OF BRONCHOALVEOLAR LAVAGE ADIPOKINES AND CYTOKINES WITH LEVELS IN VISCERAL ADIPOSE TISSUE, SUBCUTANEOUS ADIPOSE TISSUE, AND SERUM IN CONTROL SUBJECTS AT BASELINE

	BAL Leptin	BAL Adiponectin	BAL MCP-1	BAL IL-6	BAL IL-8
Visceral fat					
<i>r</i>	−0.36	0.58	0.06	0.19	0.03
<i>P</i>	0.19	0.02	0.84	0.51	0.93
Subcutaneous fat					
<i>r</i>	−0.50	0.39	0.51	0.58	0.18
<i>P</i>	0.06	0.15	0.05	0.02	0.53
Serum					
<i>r</i>	0.04	−0.19	0.38	−0.12	0.23
<i>P</i>	0.88	0.49	0.16	0.67	0.41

Definition of abbreviations: BAL = bronchoalveolar lavage; MCP = monocyte chemotactic protein.

Correlation coefficient shown is for Spearman correlation, *P* value shown without adjustment for multiple comparisons.

model of diet-induced obesity, apparently related to high levels of circulating free fatty acids and TNF-α, which increase intracellular levels of carboxyl-terminal modulator protein, a key innate immune inhibitory molecule (43). Although little is known about alveolar macrophage function in obesity, impaired alveolar macrophage phagocytosis has been reported in severe asthma (44, 45), and impaired alveolar macrophage function could interfere with remodeling in the airway (46). Lugogo and coworkers (47) recently reported differential responses of alveolar macrophages isolated from obese subjects with asthma compared with obese control subjects and lean subjects with asthma. Our data also suggest that alveolar macrophage function is altered in obesity, and this may contribute to remodeling and abnormal airway function in obese people with asthma.

It should be noted that changes in asthma medications occurred after bariatric surgery, so the macrophage data presented include only those individuals who were on a stable regimen of inhaled corticosteroids before and after surgery. Macrophages isolated at the time of bariatric surgery were from individuals under general anesthesia, and these lipophilic agents may affect macrophage function. However, bronchoscopy was performed as soon as the airway was secured, before surgery, so duration of exposure to anesthesia was short. Furthermore, volatile anesthetic exposure would not explain the concordant cytokine levels in BAL fluid.

TABLE 9. RELATIONSHIP OF BRONCHOALVEOLAR LAVAGE ADIPOKINES AND CYTOKINES WITH LEVELS IN VISCERAL ADIPOSE TISSUE, SUBCUTANEOUS ADIPOSE TISSUE, AND SERUM IN SUBJECTS WITH ASTHMA AT BASELINE

	BAL Leptin	BAL Adiponectin	BAL MCP-1	BAL IL-6	BAL IL-8
Visceral fat					
<i>r</i>	0.61	−0.36	0.28	0.79	0.77
<i>P</i>	0.06	0.34	0.42	0.006	0.009
Subcutaneous fat					
<i>r</i>	−0.02	−0.13	0.47	0.70	0.68
<i>P</i>	0.96	0.75	0.17	0.02	0.03
Serum					
<i>r</i>	−0.13	0.16	0.39	0.72	0.3689
<i>P</i>	0.70	0.66	0.27	0.02	0.29

Definition of abbreviations: BAL = bronchoalveolar lavage; MCP = monocyte chemotactic protein.

Correlation coefficient shown is for Spearman correlation, *P* value shown without adjustment for multiple comparisons.

Figure 5. Cytokine production from (A) untreated and (B) LPS-stimulated airway macrophages before surgery (controls subjects, n = 11; subjects with asthma, n = 5) and 12 months after surgery (participants with asthma, n = 5). * $P < 0.05$, ** $P < 0.01$. Of note, y axis is on a log scale. TNF = tumor necrosis factor.

Certain features of the current study cohort should be noted. The cohort included only women. Women seem to have a slightly higher risk of developing asthma in the setting of obesity suggesting that there may be factors related to sex affecting this pathophysiologic relationship. We had very few men in our cohort (reflecting the relative proportion of men seeking bariatric surgery at our institution), and so excluded them from the current analysis because we had insufficient subjects to address sex effects. Similarly, this cohort included only those with late-onset T_H2 -low asthma because of limited number of subjects with early onset T_H2 -high asthma in the cohort. In future studies it will also be important to compare the relationship of visceral adipose tissue inflammation with asthma in those with early onset T_H2 -high and late-onset T_H2 -low asthma.

In conclusion, our data suggest that visceral adipose tissue in obese women with late-onset T_H2 -low asthma produces high levels of adipokines; this is associated with airway reactivity but not airway inflammation. The fact that airway epithelial cells bear receptors for adipokines, which promote remodeling, and that alveolar macrophage function is altered suggests that noninflammatory factors, such as airway remodeling, may be contributing to airway reactivity in obesity.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Camargo CA Jr, Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. *Arch Intern Med* 1999;159:2582–2588.
- Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am J Respir Crit Care Med* 2007;175:661–666.
- Mosen DM, Schatz M, Magid DJ, Camargo CA Jr. The relationship between obesity and asthma severity and control in adults. *J Allergy Clin Immunol* 2008;122:507–511.
- Vortmann M, Eisner MD. BMI and health status among adults with asthma. *Obesity (Silver Spring)* 2008;16:146–152.
- Taylor B, Mannino D, Brown C, Crocker D, Twum-Baah N, Holguin F. Body mass index and asthma severity in the national asthma survey. *Thorax* 2008;63:14–20.
- Peters-Golden M, Swern A, Bird SS, Hustad CM, Grant E, Edelman JM. Influence of body mass index on the response to asthma controller agents. *Eur Respir J* 2006;27:495–503.
- Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DY. Body mass and glucocorticoid response in asthma. *Am J Respir Crit Care Med* 2008;178:682–687.
- Dixon AE, Shade DM, Cohen RI, Skloot GS, Holbrook JT, Smith LJ, Lima JJ, Allayee H, Irvin CG, Wise RA. Effect of obesity on clinical

- presentation and response to treatment in asthma. *J Asthma* 2006;43:553–558.
9. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest* 2011;121:2111–2117.
 10. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, D'Agostino R Jr, Castro M, Curran-Everett D, Fitzpatrick AM, et al. Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *Am J Respir Crit Care Med* 2010;181:315–323.
 11. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008;178:218–224.
 12. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, Harris JM, Scheerens H, Wu LC, Su Z, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–1098.
 13. Holguin F, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, Fitzpatrick AM, Gaston B, Israel E, Jarjour NN, et al. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 2011;127:1486–1493.
 14. Sutherland ER, Goleva E, King TS, Lehman E, Stevens AD, Jackson LP, Stream AR, Fahy JV. Cluster analysis of obesity and asthma phenotypes. *PLoS ONE* 2012;7:e36631.
 15. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, Fahy JV. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 2012;185:612–619.
 16. Sideleva O, Garudathri J, Pratley RE, Griffes LA, Black KE, Suratt BT, Poynter ME, Irvin CG, Dixon AE. Airway disease is significantly related to visceral adipose tissue inflammation in obese asthmatics [abstract]. *Am J Respir Crit Care Med* 2012;185:A4116.
 17. Dixon AE, Pratley RE, Forgione PM, Kaminsky DA, Whittaker-Leclair LA, Griffes LA, Garudathri J, Raymond D, Poynter ME, Bunn JY, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. *J Allergy Clin Immunol* 2011;128:508–515.
 18. Wree A, Kahrman A, Gerken G, Canbay A. Obesity affects the liver: the link between adipocytes and hepatocytes. *Digestion* 2011;83:124–133.
 19. Holguin F, Rojas M, Brown LA, Fitzpatrick AM. Airway and plasma leptin and adiponectin in lean and obese asthmatics and controls. *J Asthma* 2011;48:217–223.
 20. Zhu M, Hug C, Kasahara DI, Johnston RA, Williams AS, Verbout NG, Si H, Jastrab J, Srivastava A, Williams ES, et al. Impact of adiponectin deficiency on pulmonary responses to acute ozone exposure in mice. *Am J Respir Cell Mol Biol* 2010;43:487–497.
 21. Sood A. Obesity, adipokines, and lung disease. *J Appl Physiol* 2010;108:744–753.
 22. Shore SA, Schwartzman IN, Mellema MS, Flynt L, Imrich A, Johnston RA. Effect of leptin on allergic airway responses in mice. *J Allergy Clin Immunol* 2005;115:103–109.
 23. Shore SA, Terry RD, Flynt L, Xu A, Hug C. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol* 2006;118:389–395.
 24. Bruno A, Pace E, Chanez P, Gras D, Vachier I, Chiappara G, La Guardia M, Gerbino S, Profita M, Gjomarkaj M. Leptin and leptin receptor expression in asthma. *J Allergy Clin Immunol* 2009;124:230–237.
 25. Vernooy JH, Drummen NE, van Suylen RJ, Cloots RH, Moller GM, Bracke KR, Zuyderduyn S, Dentener MA, Brusselle GG, Hiemstra PS, et al. Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. *Thorax* 2009;64:26–32.
 26. Bruno A, Chanez P, Chiappara G, Siena L, Giammanco S, Gjomarkaj M, Bonsignore G, Bousquet J, Vignola AM. Does leptin play a cytokine-like role within the airways of COPD patients? *Eur Respir J* 2005;26:398–405.
 27. Huang K, Rabold R, Abston E, Schofield B, Misra V, Galdzicka E, Lee H, Biswal S, Mitzner W, Tankersley CG. Effects of leptin deficiency on postnatal lung development in mice. *J Appl Physiol* 2008;105:249–259.
 28. Kirwin SM, Bhandari V, Dimatteo D, Barone C, Johnson L, Paul S, Spitzer AR, Chander A, Hassink SG, Funanage VL. Leptin enhances lung maturity in the fetal rat. *Pediatr Res* 2006;60:200–204.
 29. Jain M, Budinger GR, Lo A, Urich D, Rivera SE, Ghosh AK, Gonzalez A, Chiarella SE, Marks K, Donnelly HK, et al. Leptin promotes fibroproliferative acute respiratory distress syndrome by inhibiting peroxisome proliferator-activated receptor-gamma. *Am J Respir Crit Care Med* 2011;183:1490–1498.
 30. van den Borst B, Souren NY, Loos RJ, Paulussen AD, Derom C, Schols AM, Zeegers MP. Genetics of maximally attained lung function: a role for leptin? *Respir Med* 2012;106:235–242.
 31. Hansel NN, Gao L, Rafaels NM, Mathias RA, Neptune ER, Tankersley C, Grant AV, Connett J, Beaty TH, Wise RA, et al. Leptin receptor polymorphisms and lung function decline in COPD. *Eur Respir J* 2009;34:103–110.
 32. Cao Q, Mak KM, Ren C, Lieber CS. Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells: respective roles of the jak/stat and jak-mediated h2o2-dependant MAPK pathways. *J Biol Chem* 2004;279:4292–4304.
 33. Zibadi S, Cordova F, Slack EH, Watson RR, Larson DF. Leptin's regulation of obesity-induced cardiac extracellular matrix remodeling. *Cardiovasc Toxicol* 2011;11:325–333.
 34. Wolf G, Ziyadeh FN. Leptin and renal fibrosis. *Contrib Nephrol* 2006;151:175–183.
 35. Miller M, Cho JY, Pham A, Ramsdell J, Broide DH. Adiponectin and functional adiponectin receptor 1 are expressed by airway epithelial cells in chronic obstructive pulmonary disease. *J Immunol* 2009;182:684–691.
 36. Daniele A, De Rosa A, Nigro E, Scudiero O, Capasso M, Masullo M, de Laurentiis G, Oriani G, Sofia M, Bianco A. Adiponectin oligomerization state and adiponectin receptors airway expression in chronic obstructive pulmonary disease. *Int J Biochem Cell Biol* 2012;44:563–569.
 37. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of acrp30/adiponectin. *Proc Natl Acad Sci USA* 2004;101:10308–10313.
 38. Mathis D, Shoelson SE. Immunometabolism: an emerging frontier. *Nat Rev Immunol* 2011;11:81.
 39. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol* 2011;11:738–749.
 40. Mancuso P, Gottschalk A, Phare SM, Peters-Golden M, Lukacs NW, Huffnagle GB. Leptin-deficient mice exhibit impaired host defense in gram-negative pneumonia. *J Immunol* 2002;168:4018–4024.
 41. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, et al. Leptin regulates proinflammatory immune responses. *FASEB J* 1998;12:57–65.
 42. Amar S, Zhou Q, Shaik-Dasthagirisahab Y, Leeman S. Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. *Proc Natl Acad Sci USA* 2007;104:20466–20471.
 43. Zhou Q, Leeman SE, Amar S. Signaling mechanisms in the restoration of impaired immune function due to diet-induced obesity. *Proc Natl Acad Sci USA* 2011;108:2867–2872.
 44. Fitzpatrick AM, Holguin F, Teague WG, Brown LA. Alveolar macrophage phagocytosis is impaired in children with poorly controlled asthma. *J Allergy Clin Immunol* 2008;121:1372–1378.
 45. Huynh ML, Malcolm KC, Kotaru C, Tilstra JA, Westcott JY, Fadok VA, Wenzel SE. Defective apoptotic cell phagocytosis attenuates prostaglandin e2 and 15-hydroxyicosatetraenoic acid in severe asthma alveolar macrophages. *Am J Respir Crit Care Med* 2005;172:972–979.
 46. Peters-Golden M. The alveolar macrophage: the forgotten cell in asthma. *Am J Respir Cell Mol Biol* 2004;31:3–7.
 47. Lugogo NL, Hollingsworth JW, Howell DL, Que LG, Francisco D, Church TD, Potts-Kant EN, Ingram JL, Wang Y, Jung S-H, et al. Alveolar macrophages from overweight/obese asthmatic subjects demonstrate a pro-inflammatory phenotype. *Am J Respir Crit Care Med* 2012;186:404–411.