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High sputum total adiponectin is associated with low odds for asthma

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Abstract

Objective: Adipose tissue produces adiponectin, an anti-inflammatory protein. High systemic total adiponectin is associated with a low risk for incident asthma but the association with lung adiponectin is not known. Our objective was to evaluate the association between sputum total adiponectin and asthma.

Methods: This case-control study included 44 cases with objectively-confirmed asthma and an equal number of body mass index (BMI) and sex-matched controls. Serum and sputum adiponectin were estimated by ELISA and Western Blot technique, respectively. While Fisher's exact test, *t*-test and Spearman's correlations were used for univariate analyses, Spearman and regression analyses were performed for multivariable analyses

Results: While high-molecular-weight adiponectin was the dominant isoform in serum, medium-molecular-weight isoform was dominant in sputum. Sputum total adiponectin was not correlated with serum adiponectin or BMI. Sputum total adiponectin was lower among asthmatics than controls ($p = 0.03$), although individual sputum isoforms were not similarly associated. High sputum total adiponectin was associated with lower odds for asthma (OR 0.33, 95% C.I. 0.12, 0.91), even after adjustment for systemic adiposity measures including serum adiponectin.

Conclusions: High sputum total adiponectin predicted lower odds for asthma, even after adjustment for serum adiponectin. Although not studied, it is possible that pharmacological modulation of sputum adiponectin may suggest new ways to prevent and/or treat asthma.

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Keywords

Adiponectin; adiposity; airway inflammation; asthma; obesity; oxidant stress

Introduction

Adiponectin, a protein produced primarily by adipose tissue, has a largely anti-inflammatory role that involves inhibition of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 and promotion of anti-inflammatory mediators (IL-10 and IL-1 receptor antagonist). Despite being produced by adipose tissue, systemic adiponectin concentrations are inversely correlated with body mass index (BMI) [1]. An explanation for this finding is that adipose tissue in the obese produces less adiponectin due to the paracrine inhibition by excess TNF-α and IL-6 [2]. Additionally, impaired mitochondrial function in the obese induces an endoplasmic reticulum stress response, resulting in inadequate processing of the mature adiponectin protein in adipocytes [3]. Circulating adiponectin includes three distinct isoforms – low-molecular weight (LMW) (trimers); medium-molecular weight (MMW) (hexamers); and high-molecular weight (HMW or higher order multimers). The various isoforms may vary in their potency of effect. For instance, HMW adiponectin may be the most biologically active in regulating insulin resistance [4]. Both adiponectin and its multiple receptors are expressed in various cell types in the lung, including the bronchial epithelium [5–7]. The lung is therefore a target organ for adiponectin effect and consequently, adiponectin abnormalities may be associated with lung disease such as asthma.

Murine studies establish a causal protective association between systemic total adiponectin and airway changes of asthma [8,9]. Murine data are confirmed by limited human studies including a recent study by our group that demonstrated high serum total adiponectin is associated with low risk for incident asthma among women, particularly among current smokers but sputum adiponectin was not determined and the potential confounding effect of fat mass was not adjusted for in that study [10]. Further, since smoking affects both systemic and lung adiponectin concentrations as well as risk for asthma [11–13], the described association between serum adiponectin and asthma in smokers [10] may simply represent residual confounding from smoking. We therefore minimized the confounding effect of current smoking in the current study by examining the association of adiponectin with asthma among non-current smokers. Additionally, we minimized the confounding effect of BMI and sex on this association by comparing our asthma cases with BMI-and sex-matched controls. We further studied sophisticated measures of regional and global adiposity as potential confounders in our study. We now hypothesize that high sputum and serum total adiponectin concentrations (i.e. primary predictor variables) would be associated with lower odds for asthma (primary outcome variable) as well as with lower levels of airway hyperresponsiveness, eosinophilic inflammation, and systemic or airway oxidant stress among subjects with asthma (secondary outcome variables). Oxidant stress was chosen as a secondary asthma outcome since it is increased in obesity-associated asthma and systemic adiponectin deficiency [14,15]. We further expected that sputum adiponectin would better

predict asthma than serum adiponectin because the former better reflects the local environment of the lung.

Methods

Study design

Forty-four subjects with asthma and an equal number of BMI- and sex-matched healthy controls were examined in a case-control study. Asthma was defined by a physician diagnosis PLUS, a confirmatory methacholine challenge test ($PC_{20} \leq 16$ mg/mL). Controls were defined by the absence of physician diagnosis of asthma PLUS a negative methacholine challenge ($PC_{20} > 16$ mg/mL). Subjects were recruited by newspaper or radio advertisements from the Albuquerque metropolitan area community during the period 2007–2010. Study population characteristics are provided in Table 1. Sample size estimates are discussed in the Online Supplementary data. Other than the study coordinator, all technicians were blinded to the case status of the subjects.

Inclusion criteria

The study included English-speaking subjects at least 18 years of age.

Exclusion criteria

Subjects with systemic conditions that affected serum adipokine concentrations or increased risk for methacholine challenge testing were excluded [1,16]. The exclusion criteria were – (1) history of diabetes mellitus, atherosclerotic cardiovascular disease, chronic kidney disease or anorexia nervosa; (2) use of statin class of drugs; (3) current smoking or having quit smoking within previous two months; (4) pregnancy and nursing state; (5) presence of lung diseases other than asthma; (6) stroke in prior 3 months; (7) aortic aneurysm; and (8) failure to expectorate adequate-quality sputum in response to induction. In addition, all tests were delayed in the event of an acute infection within the prior 4 weeks and respiratory tract infections or asthma exacerbations within the prior 8 weeks. For menstruating women, testing was done within 3–14 days following the cessation of menstrual flow (a period of high estrogen and low progesterone) to standardize the effect of sex hormones on test variables. None of the subjects in the study were on systemic corticosteroids at the time of the study.

Questionnaires

Standardized questionnaires were administered by the same trained interviewer to obtain information on smoking history, respiratory health [17], asthma severity [18], gastroesophageal reflux [19] and habitual physical activity [20]. Details of these questionnaires are available in the Online Supplementary data.

Blood analysis

Peripheral blood was obtained in a fasting state in the morning. Serum concentrations of leptin, total and isoforms of adiponectin, and immunoglobulin E were estimated using the ELISA technique (ALPCO Immunoassays, Salem, NH for leptin/adiponectin and Bethyl

Labs, Inc., Montgomery, TX for IgE). Plasma was analyzed for lectin-type oxidized low-density lipoprotein receptor-1 (LOX-1) using the ELISA technique (R&D Systems, Minneapolis, MN) to assess systemic oxidant stress. We chose this biomarker of oxidation of proteins since its basal expression is very low and it is potently induced by oxidant species [21].

Skin prick test

Eleven common aeroallergens were tested in duplicate by a skin prick test. These aeroallergens included Cottonwood, Mountain cedar, Bermuda grass, Kentucky bluegrass, Meadow fescue grass, Russian thistle, Kochia, Western ragweed, cat hair and dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. The occurrence of a wheal of at least 3 mm on both tests for any one antigen after a 10-min period was used to define atopy.

Induced sputum analysis

Sputum was induced at mid-day by nebulization of 5% hypertonic saline aerosol using a small volume ultrasonic nebulizer and processed using the technique described by Hargreave [22], whereby dense portions ("sputum plugs") were selected and divided into two parts – the first part was liquefied with 0.1% dithiothreitol (DTT; Calbiochem, La Jolla, CA) and the second part treated with protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). All samples were stored at −70° C. Total adiponectin and adiponectin isoforms were measured in the protease inhibitor-processed sputum samples by the Western blot technique (ALPCO Diagnostics, Salem, NH; no kits were used but details of antibodies used; blotting conditions; and spike and recovery experiment are provided in the Online Supplementary data). Adiponectin was measured in arbitrary units for the total (relative to the provided standard from the same blot) and percent of total for the individual isoforms in sputum. Sputum leptin and eosinophil-derived neurotoxin (EDN) [23–25] levels were measured by ELISA technique from the DTT-treated samples (RnD Systems, Minneapolis, MN and ALPCO Immunoassays, Salem, NH, respectively), as detailed in the Online Supplementary data.

Exhaled breath condensate

About 2–3 mL of exhaled breath condensate (EBC) was collected, as per the American Thoracic Society (ATS) guidelines [26], using an R-tube device (Respiratory Research Inc.). The condensate was snap frozen and stored immediately after collection at −70 °C to prevent auto-peroxidation of lipids and artifactual elevation of 8-isoprostanes (a biomarker of oxidation of lipids), as published previously by our team and others [27–29]. EBC was concentrated 14-fold by lyophilization [30] and subsequently resuspended in sterile deioinized water for measurement of 8-isoprostanes using the previously-described radioimmunoassay technique at the Montuschi laboratory (Rome, Italy) [31].

Spirometry and methacholine bronchoprovocation

Spirometry tests were conducted as per the 2005 ATS guidelines [32] and results were expressed as percent of the ethnicity-specific predicted values [33]. Methacholine challenge

test was also performed per the ATS guidelines [16] with the Cockcroft modification of the methods of Chai et al. [34] and PC_{20} was defined by the concentration of methacholine associated with an FEV_1 decrease by at least 20% from post-diluent values.

Body fat determination

Detailed measures of total and regional adiposity were obtained using anthropometry, bioelectrical impedance and dual energy X-ray absorptiometry (DEXA) techniques by a trained dietician. Additional details for these techniques are provided in the Online Supplementary data.

Statistical methods

Distributions of all continuous variables were examined. When a variable was not normally distributed, logarithmic or square root transformations were performed to normalize the data. Missing data were excluded from analysis. Fisher's exact test, *t*-test and Spearman's correlations were used for univariate analysis. Spearman and regression analyses were performed for multivariable analyses. A two-sided p value <0.05 was considered statistically significant. SAS 9.3 (SAS Institute Inc, Cary, NC) was used for statistical analysis.

The study was approved by the University of New Mexico's Institutional Review Board (Human Research Protection Office, Albuquerque, NM; Protocol number 06–296). All subjects signed informed consent prior to their participation.

Results

Comparison of demographic and adiposity characteristics

Of all asthmatics, 38.6, 22.7, 27.3 and 11.4% were rated as intermittent, mild persistent, moderate persistent and severe persistent, respectively [18]. Tables 1 and 2 describe the distribution of demographic and adiposity variables, respectively, in the study population. These tables demonstrate no significant difference in these variables between subjects with asthma and controls. Subjects with asthma were, however, more likely to be atopic; have greater percent counts of blood eosinophils and concentrations of sputum EDN; lower levels of prebronchodilator FEV_1 and greater airway hyperresponsiveness (Table 1).

Distribution of serum and sputum adipokines

While HMW adiponectin was the dominant isoform in serum, the MMW isoform was dominant in sputum (Table 4). Sputum concentrations of total adiponectin were not correlated with their serum concentrations or with BMI (Tables 3 and E-V in the text and Online Supplementary data, respectively). Serum HMW adiponectin concentrations were inversely correlated with BMI (Spearman's r of −0.31; $p = 0.004$). In contrast, sputum HMW adiponectin concentrations were positively correlated with BMI (Spearman's r of 0.25; $p = 0.02$). Women had higher serum total adiponectin concentrations than men but there were no sex-related differences in sputum concentrations of total adiponectin or of individual adiponectin isoforms (Table E-II, Online Supplementary data). Subjects reporting a high risk for having gastroesophageal reflux had lower sputum total adiponectin concentrations than those reporting a low risk ($p = 0.02$ adjusted for age, sex and BMI) but

there were no differences in concentrations of sputum adiponectin isoforms or of serum total adiponectin.

High sputum total adiponectin was associated with lower odds for asthma

Sputum total adiponectin concentrations were lower in subjects with asthma than controls ($p = 0.03$ in both univariate and multivariable analyses in Tables 4 and E-III, in the text and Online Supplementary data, respectively), although individual sputum adiponectin isoforms were not associated with asthma (Table 4). High sputum total adiponectin concentrations were associated with lower odds for asthma (OR 0.33, 95% C.I. 0.12, 0.91), even after adjustment for potentially confounding systemic adiposity measures (such as serum total adiponectin and serum leptin, chosen based upon our previous work [10,35–37]; Table 5). Additional adjustment for percent body fat or lean mass in the multivariable models did not change the above results (Tables 5 and E-III in the text and Online Supplementary data, respectively). Although sputum total adiponectin concentrations tended to be lower in subjects with moderate/severe asthma, as compared to intermittent/mild persistent disease, this association did not reach statistical significance (adjusted $p = 0.10$). The use of the ratio of total adiponectin to leptin instead of total adiponectin alone (as studied by some authors [38]), diminished the effect size between groups. There were no significant two-way interactions between obese status (BMI ≥ 30 kg/m^2); BMI; sex or atopic status, and sputum total adiponectin on asthma status (unadjusted $p \geq 0.48$ for all analyses).

Select adiponectin isoforms were inversely correlated with sputum eosinophilic inflammation and oxidant stress

Sputum total adiponectin was not associated with any physiological, inflammatory or oxidant stress measures of asthma severity in our study (Table 6). Sputum LMW adiponectin was inversely correlated with sputum EDN and airway oxidant stress (exhaled breath condensate 8-isoprostanes) among controls but not asthmatics (Table 6, group interaction adjusted $p = 0.07$ and 0.08, respectively). In addition, sputum LMW adiponectin was inversely correlated with systemic oxidant stress (plasma LOX-1) in all subjects (adjusted Spearman's r of 0.41; $p = 0.002$). On the other hand, sputum HMW adiponectin was inversely correlated with systemic oxidant stress among subjects with asthma but not among controls (group interaction significant with adjusted $p = 0.008$). Sputum adiponectin measures were not correlated with lung function or airway hyperresponsiveness.

Discussion

High sputum total adiponectin concentrations were associated with lower odds for asthma in this BMI- and sex-matched case-control study drawn from a carefully selected population of primarily women non-current smokers. Sputum total adiponectin may be more strongly associated with asthma than serum total adiponectin or serum leptin, after matching on BMI. Sputum total adiponectin is not correlated with the corresponding serum value or with BMI. Although MMW isoform is the dominant isoform of adiponectin in the sputum, the HMW and the LMW (i.e. non-dominant) isoforms in sputum are inversely associated with systemic oxidant stress among subjects with asthma, after adjustment for BMI.

Murine studies establish a causal protective association between systemic adiponectin and airway changes of asthma [8,9]. Allergen bronchoprovocation of sensitized BALB/cJ mice reduces adiponectin production and expression of pulmonary adiponectin receptors [8]. On the other hand, systemic adiponectin infusion attenuates allergic airway inflammation and hyperresponsiveness in mice [8]. Allergen-induced airway inflammation is greater among genetically adiponectin-deficient mice than wild-type mice [9]. The adiponectin–airway relationship in mice is therefore *bidirectional*.

Human data regarding the association between adiponectin and asthma is still evolving. In a small study of morbidly obese women undergoing bariatric surgery, visceral abdominal adipose tissue from subjects with asthma showed a lower expression of total adiponectin than controls, after adjustment for BMI [39]. The strongest supporting evidence for the adiponectin–asthma association comes from a longitudinal cohort that showed high serum total adiponectin was associated with decreased risk for *incident* asthma among women and that this association was stronger among currently smoking women than women not currently smoking [10]. Our case-control study that focused on sputum measures, however, did not show an association between serum adiponectin and asthma, possibly due to a smaller sample size and exclusion of current smokers. Limited data also suggest that an increase in serum total adiponectin by a multidisciplinary weight reduction intervention may favorably influence asthma severity outcomes [40].

Like mice, the systemic adiponectin–asthma relationship is bidirectional in humans but the primary direction of this association is that adiponectin affects asthma, except in severe conditions when the reverse direction is also true. Thus, prevalent asthma has no chronic effects on serum adiponectin [10] and transient bronchoprovocation from experimental allergen inhalation does not acutely affect serum adiponectin concentrations among subjects with asthma [41]. Yet, severe asthma exacerbations requiring hospitalization result in a transient decrease in serum adiponectin [38]. It is possible that systemic spill-over of intense local inflammation in a severe asthma exacerbation inhibits the secretion of adiponectin from adipose tissue [38]. In order to minimize the confounding effect of asthma exacerbation on adiponectin, we delayed testing in our study by at least 8 weeks following any asthma exacerbation, none of which required hospitalization.

We demonstrate that sputum total adiponectin may better predict asthma status than other adiposity variables such as serum total adiponectin, serum leptin or DEXA measures of fat and lean mass. Airway adiponectin was not correlated with systemic adiponectin, as also described by others [39,42]. Potential explanations for this finding are that adiponectin may be degraded within the lung itself and/or different isomers may be transported at different rates from blood into the airway. It is also possible that adiponectin produced by intrathoracic visceral fat or by various cells in the lung such as airway epithelium [12] may have paracrine effects that may be stronger than the endocrine effects of systemic adiponectin. While it may seem surprising to find group differences in sputum total adiponectin but no differences in individual isoforms, this may be explained by the larger variance of the isoform concentrations.

Although the published data are limited and confusing, high serum adiponectin is associated with less clinical asthma severity and high lung function measures among boys and women [38,43–45] but surprisingly associated with more clinical severity among men [43]. On the contrary, serum adiponectin is not associated with eosinophilic inflammatory asthma measures in any population group [42]. In our study, we did not find sputum total adiponectin to be associated with physiological, eosinophilic inflammatory or oxidant stress measures of asthma severity (Table 6). On the other hand, the non-dominant isoforms of sputum adiponectin (i.e. HMW and LMW isoforms) were associated with low systemic oxidant stress among subjects with asthma. It is possible that low HMW and LMW adiponectin isoforms in asthma are associated with non-eosinophilic airway inflammation potentiated by systemic oxidant stress.

The strengths of our study include an innovative and topical research question, a well-characterized study population, focus on airway adiponectin and its isoforms, ability to control for systemic and regional adiposity measures, and study of physiological, inflammatory and oxidant stress-related asthma outcomes. Use of self-report or questionnaires may result in information bias which was minimized by the use of standard questionnaires administered by the same interviewer. Information bias was further minimized by the use of trained study personnel, standard test protocols, strict quality control for assays and blinding of study personnel towards case status of study subjects. Selection bias arises from the recruitment process but was minimized by recruitment from the community, instead of the hospital. Self-report of physician diagnosis of asthma is subject to misclassification bias [46] and was therefore minimized by confirmatory use of the methacholine challenge test. Since cigarette smoke exposure increases lung adiponectin in mice [47] but reduces lung adiponectin in otherwise healthy current smokers [12], we minimized the variable and confounding effect of cigarette smoke by carefully focusing on non-current smokers. Additional limitations include a relatively small sample size that reduces the confidence in the strength of our associations as well as our ability to examine interactions; lack of longitudinal data to establish causality or temporal sequence of association; and lack of mechanistic data.

Conclusions

Although limited data suggest that an increase in serum total adiponectin by weight reduction may favorably influence asthma severity outcomes [40], it remains unclear whether therapeutic strategies to increase serum total adiponectin also raise sputum total adiponectin and whether the latter in turn decrease asthma risk and/or severity. We demonstrate in this study that sputum total adiponectin predicts asthma but future studies confirming our findings are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Declaration of interest

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Table 1.

Unadjusted comparison of characteristics between subjects with asthma and controls.

Characteristic	Asthma (*n* = 44) % or mean ± S.D.	Controls (*n* = 44) % or mean ± S.D.	*p* Value
Age (in years)	34.7 ± 13.9	32.0 ± 10.8	0.32
Female sex	68.2%	68.2%	1.00
White race	86.4%	73.8%	0.18
Hispanic ethnicity	27.3%	27.9%	1.00
If woman, premenopausal status	70%	83.3%	0.36
Former smokers	34.1%	31.8%	1.0
Current environmental tobacco smoke exposure	22.7%	18.2%	0.79
Atopic state by skin prick test	88.4%	60.5%	0.006
Serum Immunoglobulin E (IU/mL)	288.6 ± 360.3	124.6 ± 260.1	0.02
% Eosinophil in blood cells	3.9 ± 7.1	1.8 ± 1.1	0.06[a]
Sputum eosinophil derived neurotoxin (ng/mL)	58.6 ± 95.7	31.5 ± 33.9	0.09[a]
Pre-bronchodilator FEV_1 (L)	3.1 ± 0.8	3.5 ± 0.8	0.02
Pre-bronchodilator FVC (L)	4.2 ± 1.1	4.3 ± 1.1	0.45
% predicted pre-bronchodilator FEV_1	88.9 ± 13.3	101.1 ± 11.3	<0.001
% predicted pre-bronchodilator FVC	99.6 ± 12.5	105.0 ± 12.8	0.05
Methacholine PC_{20} (mg/mL)	4.4 ± 4.7	16.2 ± 1.4	<0.001
Inhaled corticosteroid use	38.6%	0%	<0.001
Self-reported physical activity (METS per week)	71.5 ± 76.1	83.3 ± 60.1	0.42

FVC: forced vital capacity; FEV_1: Forced expiratory volume in one second; METS: metabolic equivalents. Student's í-test was used for analyzing continuous variables and Fisher's exact test for categorical variables.

[a]Sputum EDN and percent eosinophil in blood cells, when square root transformed, were different between the two groups (p = 0.004 and 0.002, respectively).

Table 2.

Unadjusted comparison of adiposity characteristics between subjects with asthma and controls.

Adiposity characteristic	Asthma (n = 44) % or mean ± S.D.	Controls (n = 44) % or mean ± S.D.	p Value
Body mass index (in kg/m^2)	30.7 ± 9.6	30.8 ± 8.9	0.95
Waist circumference (in cm)	98.5 ± 22.4	98.0 ± 19.8	0.92
Hip circumference (in cm)	114.1 ± 19.1	112.5 ± 16.1	0.67
Waist to hip ratio	0.86 ± 0.09	0.87 ± 0.09	0.66
Subscapular skinfold thickness (in mm)	28.7 ± 15.6	27.1 ± 14.4	0.62
Triceps skinfold thickness (in mm)	27.0 ± 12.8	26.2 ± 13.2	0.77
Chest wall skinfold thickness (in mm)	18.8 ± 9.8	16.9 ± 9.5	0.36
Midaxillary skinfold thickness (in mm)	22.9 ± 11.5	20.5 ± 11.2	0.33
Suprailiac skinfold thickness (in mm)	29.9 ± 14.7	26.3 ± 13.0	0.22
Thigh skinfold thickness (in mm)	36.4 ± 18.3	31.8 ± 16.5	0.21
% total body fat, using anthropometric measurements[a]	30.5 ± 11.4	28.5 ± 11.4	0.42
% total body fat, using bioelectrical impedance[a]	38.2 ± 11.5	36.5 ± 12.3	0.50
% total body fat, using DEXA	38.9 ± 11.7	38.1 ± 13.1	0.77
% arm fat (DEXA)	34.7 ± 14.8	33.5 ± 15.5	0.73
% leg fat (DEXA)	40.2 ± 12.6	39.2 ± 14.2	0.73
% trunk fat (DEXA)	41.3 ± 11.6	40.6 ± 12.9	0.79
% android fat (DEXA)	34.9 ± 19.2	39.7 ± 19.0	0.26
% gynoid fat (DEXA)	43.7 ± 10.7	42.5 ± 13.5	0.66
% total body lean mass (DEXA)	57.0 ± 11.1	57.7 ± 12.6	0.80

DEXA: Dual energy X-ray absorptiometry. Gray 1989 reference equations were used to calculate percent body fat using bioelectrical impedance data; Caucasian reference equations were used to estimate percent body fat from skinfold thickness measures.

[a]Student's t-test was used for analyzing continuous variables and Fisher's exact test for categorical variables.

Table 3.

Unadjusted correlations between sputum total adiponectin and other sputum and serum measures in all subjects ($n = 88$).

Major predictor	Other sputum and serum measures	Spearman's correlation coefficient	*p* Value
Sputum total adiponectin concentrations	Serum total adiponectin	−0.19	0.08
	Serum HMW adiponectin	−0.15	0.16
	Serum leptin	−0.07	0.55
	Sputum leptin	−0.05	0.66
	Sputum EDN	0.13	0.24

HMW: high-molecular weight isoform; MMW: mid-molecular weight isoform; LMW: low-molecular weight isoform; EDN: eosinophil derived neurotoxin. These correlations were similar within individual groups (44 subjects with asthma and 44 controls).

Table 4.

Unadjusted comparison of sputum and serum adipokines between subjects with asthma and controls.

	Asthma (*n* = 44)	Controls (*n* = 44)		
Adipokine characteristics	**Mean ± S.D.**	**Mean ± S.D.**	***p* Value for raw data without transformation**	***p* Value for data square-root transformed**
Sputum adipokines				
Sputum total adiponectin (relative units)	62.9 ± 17.4	71.3 ± 19.0	0.03	0.03
Sputum HMW adiponectin (% of total)	29.2 ± 28.8	32.8 ± 31.0	0.57	–
Sputum MMW adiponectin (% of total)	62.3 ± 32.5	55.5 ± 27.8	0.30	–
Sputum LMW adiponectin (% of total)	8.6 ± 13.7	10.9 ± 13.8	0.43	–
Sputum leptin (pg/mL)	32.4 ± 51.3	33.8 ± 72.5	0.91	0.89
Serum adipokines				
Serum total adiponectin (ng/mL)	4180.6 ± 2671.1	3987.4 ± 3106.0	0.76	0.61
Serum HMW adiponectin (ng/mL)	4426.1 ± 2742.2	3689.1 ± 2586.4	0.20	0.13
Serum leptin (pg/mL)	34032.8 ± 27 597.8	33 263.4 ± 27 874.9	0.90	0.85

HMW: high-molecular weight isoform; MMW: mid-molecular weight isoform; LMW: low-molecular weight isoform.Student's *t*-test was used for analyzing all variables. Among asthmatics, mean serum HMW adiponectin concentrations were higher than the serum total adiponectin concentrations. This is only possible since the two measures were obtained from different assays. Between-group comparisons are however still valid. Upon comparing asthmatics with and without inhaled corticosteroids showed no significant differences between the two groups with respect to sputum or serum adipokine measures (as discussed in the Online Supplementary data).

Table 5.

Multivariable analysis for asthma status as outcome in all subjects ($n = 88$).

Characteristic	Odds ratio (95% C.I.)	*p* Value
Sputum total adiponectin concentrations	0.33 (0.12, 0.91)	0.03
Age (in years)	2.05 (0.75, 5.59)	0.16
Female sex	2.01 (0.49, 8.25)	0.33
BMI (in kg/m^2)	0.38 (0.06, 2.51)	0.31
Serum total adiponectin (relative units)	1.01 (0.39, 2.61)	0.99
Serum leptin (pg/mL)	2.40 (0.31, 18.8)	0.40

BMI: body mass index. Concentrations of sputum/serum total adiponectin and serum leptin were square root transformed. Odds ratios are presented for all continuous measures as a step of two S.D. Alternate multivariable analyses additionally adjusted for percent body fat, estimated from anthropometry, bioelectrical impedance and DEXA techniques, in three separate models and found similar results as above for the relationship of asthma status on sputum total adiponectin ($p = 0.03$, 0.03 and 0.046, respectively). Alternate analysis excluded BMI and sex (since they were matching variables) and found similar results as above for the relationship of asthma status on sputum total adiponectin ($p = 0.03$).

Table 6.

Adjusted Spearman correlations between sputum adiponectin and inflammatory/oxidant stress measures among subjects with asthma and controls.

Sputum adiponectin measure	Oxidant stress measure	Asthma (n = 44; 39 for plasma and 41 for EBC)	Controls (n = 44; 41 for plasma and 39 for EBC)
Sputum total adiponectin concentrations	Plasma LOX-1	−0.19 (p = 0.26)	−0.10 (p = 0.53)
	EBC 8-isoprostanes (R-tube)	0.04 (p = 0.80)	0.16 (p = 0.35)
	Sputum EDN	0.17 (p = 0.31)	0.23 (p = 0.14)
Sputum HMW adiponectin isoform	Plasma LOX-1	−0.40 (p = 0.02)	0.19 (p = 0.26)
	EBC 8-isoprostanes (R-tube)	0.06 (p = 0.70)	0.18 (p = 0.29)
	Sputum EDN	0.10 (p = 0.55)	0.05 (p = 0.78)
Sputum MMW adiponectin isoform	Plasma LOX-1	0.24 (p = 0.16)	−0.21 (p = 0.20)
	EBC 8-isoprostanes (R-tube)	−0.12 (p = 0.48)	−0.19 (p = 0.26)
	Sputum EDN	0.01 (p = 0.96)	0.03 (p = 0.88)
Sputum LMW adiponectin isoform	Plasma LOX-1	−0.39 (p = 0.02)	−0.36 (p = 0.03)
	EBC 8-isoprostanes (R-tube)	0.08 (p = 0.64)	−0.38 (p = 0.02)
	Sputum EDN	0.25 (p = 0.13)	−0.32 (p = 0.04)

HMW: high-molecular weight isoform; MMW: mid-molecular weight isoform; LMW: low-molecular weight isoform; EDN: eosinophil-derived neurotoxin; EBC: exhaled breath condensate; LOX-1: Lectin-type oxidized low-density lipoprotein receptor 1. Spearman correlations are provided in the table after adjustment for age, sex and BMI. Unadjusted correlations (data not provided) are similar to the adjusted values provided in Table 6. The analyses in Table 6 were no longer significant after Bonferroni's correction for four predictors (p = 0.013) for each of our outcomes.

Interactions: While LMW adiponectin in sputum is inversely correlated with plasma LOX-1 in all subjects, HMW and MMW adiponectin isoforms in sputum are differently associated with plasma LOX-1 (logarithmically transformed) between subjects with asthma and controls; adjusted group interaction p = 0.008 and 0.051, respectively. The adjusted interactions between LMW sputum adiponectin and group type is weakly significant on R-tube-measured EBC isoprostanes, logarithmically transformed (p = 0.08). Interaction between LMW sputum adiponectin and group type was weakly significant on sputum EDN, square-root transformed (p = 0.07). All adiponectin levels were square root transformed for this analysis. Sputum adiponectin measures were not significantly correlated with prebronchodilator FEV_1, methacholine PC_{20} or the two-point overall methacholine dose-response ratio. There were no significant sex interactions for the above associations. Missing data explains why the numbers of plasma and EBC samples measured were less than 44 in each group and subjects with missing data were excluded from analysis.