Published in final edited form as:
J Asthma. 2011 April ; 48(3): 217–223. doi:10.3109/02770903.2011.555033.

Airway and plasma leptin and adiponectin in lean and obese asthmatics and controls

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Abstract

Introduction—Obesity-mediated changes in plasma adipokines have been associated with increased systemic inflammation and oxidative stress. However, it is unknown whether obesity induces similar changes in airway levels of these adipokines and whether these changes are associated with increased airway biomarkers of inflammation and oxidative stress.

Methods—Lean and obese asthmatics and controls underwent bronchoscopy with bronchoealveaolar lavage (BAL), spirometry, and provided fasting plasma leptin and adiponectin. Biomarkers of oxidation and inflammation in the BAL included exhaled nitric oxide, 8-isoprostanes, pH and nitrogen oxide products (NOx).

Results—Out of a total of 48 subjects 44% had asthma and 56% were healthy controls. Among subjects with asthma 66% were obese, 10% overweight, and 24% were lean; in the controls these proportions were respectively 63%, 11% and 26%. After adjusting for age, sex, smoking history, ethnicity, pre-bronchodilator forced exhalation in one second (FEV_1), obesity was associated with higher BAL and plasma leptin levels in asthmatics and controls. Increasing BMI was associated with increased BAL leptin and was marginally and inversely associated with BAL adiponectin. Significant associations between BAL and plasma levels were only observed for leptin. No significant associations were observed between BAL or plasma adipokines with the airway biomarkers of oxidation and inflammation.

Conclusion—Increasing BMI is associated with changes in the concentrations of airway adipokines in asthmatics and healthy controls; however, these associations are not related with biomarkers of airway oxidation or inflammation.

Keywords

Asthma; obesity; leptin adiponectin; oxidative stress

Introduction

In healthy adults obesity has been associated with increased risk of asthma incidence and greater bronchial hyperresponsiveness (BHR) whereas in subjects with asthma, it has been associated with increased symptom severity, higher rates of healthcare utilization, and

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reduced inhaled corticosteroid effectiveness[1–4]. These associations may be partly explained by obesity-mediated changes in adipokines, such as increased leptin and/or reduced adiponectin[5]. Leptin increases satiety yet as a pleitropic adipokine it can also promote inflammation and oxidative stress[6, 7]. Leptin receptors are involved in the regulation of innate and adaptive immune responses[8] and are present in the respiratory epithelium, submucosal space and activated lymphocytes in the lung[9]. Specific to asthma, leptin infusion has been shown to increase bronchial hyperresponsiveness (BHR) and systemic IgE in ovalbumin (OVA) sensitized mice[10]. In contrast, Adiponectin, which also has receptors in the respiratory epithelium and airway smooth muscle cells[11, 12], has been shown to have predominant antinflammatory and antioxidative effects[13] and to prevent OVA-mediated BHR, and airway inflammation[14].

In the absence of allergic sensitization obese leptin-receptor deficient mice (*db*-/*db*-), have increased leptin and reduced adiponectin in the bronchioalveolar lavage (BAL) when compared to lean wild-type mice[15]. This suggests that the effect of obesity on these adipokines is similar in the plasma and in the airways. However, it is unknown whether obesity-mediated changes in the BAL concentration of leptin and adiponectin translate into changes in airway inflammation and/or airway oxidative stress. In addition, it is unknown whether any relationship exists between airway and plasma levels of leptin and adiponectin. This information may be important as inferences on the pathogenesis of obesity and asthma are being made using plasma levels of these adipokines, which may not necessarily reflect their corresponding airway concentrations[16]. We hypothesized that obesity-mediated changes in BAL leptin and adiponectin would be associated with increased airway inflammation and or airway oxidative stress. Additionally, the objectives of the study were to determine how BMI and plasma levels influence the concentration of these BAL adipokines in asthmatics and controls.

Methods

Study population

This study was conducted at Grady Memorial Hospital in Atlanta, Georgia and the Clinical Research Center of Crawford Long Hospital of Emory University with the approval of the institutional review board. A convenience sample of study subjects and controls were recruited through local media advertisement, hospital clinics and university websites.

Inclusion criteria for asthmatics included having the following: physician-diagnosed asthma at least 1 year previous to study enrollment, being older than 18 years of age, meeting criteria for moderate to severe persistent asthma (Global Initiative for Asthma (GINA) class III – IV)[10] and having a post bronchodilator FEV_1/FVC (Forced exhalation volume in one second / Forced vital capacity) ratio greater than 0.7. Exclusion criteria included: current smokers, having a positive urine test for cotinine, ex-smokers who stopped smoking at least one year prior to study enrolment, or total-life smoking history > 10 pack-year, and evidence of other lung diseases or any other significant non-pulmonary co-morbidities such as congestive heart failure with ejection fraction < 50%, stable angina and disorders requiring systemic steroid treatment. Subjects were also excluded if they had an asthma exacerbation or were receiving a steroid taper for at least 1 month prior to enrolment. In addition, healthy obese subjects without any history of underlying lung disease or known allergies and meeting the same exclusion for tobacco use were recruited as the obese control group.

Study measures

All participants underwent height and body weight measurements and BMI was calculated as (body weight in Kg/ (height in cm)^2. BMI was divided into lean ($18 < BMI \leq 25$),

overweight (25 < BMI < 30) and obese (BMI ≥ 30) categories. In addition, participants answered a general questionnaire regarding past medical history, use of asthma medications and asthma morbidity. We used the Juniper asthma control questionnaire (ACQ) to determine the degree of asthma control in the week prior to study enrollment[11]. An ACQ score ≤ 0.75 is considered well-controlled asthma whereas an ACQ > 1.5 is compatible with poor asthma control[12]. Spirometry was done following the American Thoracic Society (ATS) recommendations[17]. Exhaled NO was measured with in-line sampling chemyluminescence (Eco-Medics CLD 88 sp, Michigan Ann Arbor) following ATS recommendations[18].

Fiberoptic bronchoscopy and bronchoalveolar lavage

All participants underwent bronchoscopy in the morning after an overnight fasting at the Endoscopy Unit of Crawford Long Hospital and followed establish hospital protocols for sedation and recovery. Venipuncture was performed in all participants prior to bronchoscopy. Topical lidocaine was applied to the nostrils and sprayed into the throat. After an adequate level of conscious sedation was achieved with Fentanyl and Versed, a Fiberoptic bronchoscope was inserted nasally or orally and a 150 cc of normal saline was instilled after wedging in the right middle lobe bronchus. BAL aliquots were immediately placed on ice and transported to the laboratory for immediate analysis.

BAL and plasma measures

BAL fluid was processed within 1 hour of collection. BAL was centrifuged at 1200 rpm for 7 minutes at 4° C to separate the supernatant and cellular fractions. The supernatant was divided into 250 μl aliquots and frozen at −80°C prior to analysis. Total cell counts were performed manually with a hemocytometer and cellular differentials were determined from 300 consecutive cells after Diff Quick staining (Sigma Aldrich, St. Louis, MO).

Leptin and adiponectin levels were determined in the plasma and the BAL supernatant using commercially available ELISA kits (Invitrogen, Camarillo, CA) with sensitivities of 3.5 pg/mL for leptin and 100 pg/mL for adiponectin. 8-Isoprostane concentrations in the BAL supernatant were obtained by ELISA (Cayman Chemical, Ann Arbor, MI) with a detection limit of 2.7 pg/mL. H_2O_2 concentrations in the BAL supernatant were determined spectrophotometrically from a microplate assay kit (Amplex® Red, Molecular Probes, Eugene, OR) with a sensitivity of 50 nM.

Nitrite and total nitrite + nitrate concentrations were determined from the BAL supernatant using the Griess method (Cayman Chemical, Ann Arbor, MI) with a lower detection limit of 0.1 uM. Nitrate concentrations were determined by subtracting the concentration of nitrite from total nitrite + nitrate. The pH was also measured in the BAL supernatant using a standard Orion pH probe and meter (Thermo Scientific, Waltham, MA). All samples were measured in duplicate with a coefficient of variation of less than 10%.

Statistical analyses

For non-parametrically distributed data, the Kruskall Wallis test was used to compare variables between BMI categories in asthmatics and controls. Distribution of proportion between groups where compared using Fisher's exact test or the Chi squared test. Non-parametric trend analysis was done to determine trends for continuous data across BMI strata. Plasma and BAL adipokines were log-transformed to perform ANOVA tests and multivariable regression analysis. Regression models were adjusted for age, gender, ethnicity, smoking, pre-bronchodilator FEV_1 and case vs. control status to evaluate the association between BMI categories and BMI as a continuous variable. All linear regressions were checked for collinearity, influential points and model fitness. The

exponential of the log-transformed coefficients from the regression models was used to present the results in their original units. The Spearman and Pearson's tests were used to determine the correlation coefficients of non-parametric and normally distributed data, respectively. Non parametric results are presented as median and interquartile range (IQR), whereas normally distributed data is displayed as mean and 95% confidence interval (CI). A p value of < 0.05 was considered statistically significant. All statistics were performed with Stata 11.0, College Tx.

Results

Study population

A total of 48 adult subjects participated in the study. Of them, 44% had asthma and 56% were healthy controls. The characteristics of the study population are shown in Table 1. The majority of subjects in both the asthmatics and the controls were obese. In both groups obese subjects were older and predominantly Caucasian, when compared to their lean or overweight counterparts. There was a marginal significant difference in the distribution of ACQ scores across BMI categories in asthmatics, and no significant differences in lung function or BAL cell counts between the BMI categories in either subjects with asthma or controls.

BAL and plasma adipokine levels in asthmatics and controls by BMI categories and in relation to BMI as a continuous variable

The levels of plasma leptin increased in relation to the BMI categories in both asthmatics (p trend=0.002) and controls (p trend=0.007); in contrast, BAL leptin only increased significantly in relation with the BMI categories in asthmatics (p trend=0.01). In the multiple group comparison analysis using ANOVA, there were significant differences between lean and obese asthmatics and controls. For BAL leptin levels, there were significant differences between lean and obese asthmatics only. There were no differences in the distribution of plasma or BAL adiponectin in relation to BMI categories in either group (See Table 2). Within each BMI category, comparisons of plasma and BAL levels across asthmatics and controls were largely not significant with the exception of plasma leptin levels which were higher in obese asthmatics vs. obese controls. (See figure 1).

In multivariable linear regression analysis, obesity and overweight (marginally) categories where associated with reduced plasma and BAL leptin, after adjusting for age, gender, race, and previous smoking history. Models were also adjusted by case status (asthma vs. control), which showed no evidence of confounding, suggesting that the association between body weight categories and adipokine levels, after adjusting for these covariates, was not different across these two groups (See Table 3). No significant associations were observed with adiponectin and BMI categories in the multivariable analysis (Table 3).

Linear models were also fitted to determine the association between adipokines and BMI as a continuous variable (Table 3 lower end). In univariate analysis leptin was associated with BMI in both subjects with asthma and controls. These associations remained significant after adjusting for the previously described confounders. BAL adiponectin was marginally and inversely associated with BMI in the univariate and multivariable analysis.

Among subjects with asthma only, the addition of average Juniper ACQ scores did not significantly change any of the model estimates (Results not shown).

BAL and plasma adipokines

To determine the association between BAL and plasma adipokine levels, linear models were fitted and adjusted using the previously described covariates. BAL leptin levels were significantly associated with plasma levels; adjustment for case status did not confound the model estimate, suggesting that the association between BAL and plasma leptin, after adjusting for covariates, is similar in asthmatics and in controls (See Figure 2). No significant associations were found between BAL and plasma levels of adiponectin (See Figure 3).

Among subjects with asthma only, the addition of the Juniper average ACQ scores did not significantly change these associations (Results not shown).

BAL adipokines and BAL biomarkers of oxidation and inflammation

As shown in Table 4, there were no significant differences in the concentration of BAL biomarkers across lean and obese asthmatics and controls (In table 4E in the online supplement, results are presented corrected for dilution using urea). BAL or plasma leptin and adiponectin were not correlated with any of the oxidative or inflammatory biomarkers after Bonferroni adjustment for multiple comparisons.

Discussion

This study describes the distribution of BAL leptin and adiponectin levels in obese and non-obese asthmatics and controls and their association with BMI, plasma levels, and biomarkers of airway inflammation and oxidation. Our results show that obese and overweight asthmatics and controls have significantly higher BAL leptin and that the concentration of plasma leptin is higher in obese asthmatics vs. obese controls. BAL leptin was significantly correlated with BMI and its corresponding plasma levels in both asthmatics and controls. In contrast, BAL adiponectin was marginally and inversely related to BMI but showed no correlation with its corresponding plasma level. There were no associations between BAL adipokines or plasma adipokines with biomarkers of oxidation or inflammation.

Animal studies have found that body weight strongly influences the concentration of adipokines in the airways. Obese leptin receptor deficient mice (db^{-}/db^{-}) have been shown to have significantly larger concentration of leptin in the BAL and reduced BAL adiponectin when compared to lean wild-type mice[15]. Changes in the airway concentration of these adipokines in relation to body weight may be relevant to our understanding of the mechanisms by which obesity affects asthma incidence and asthma severity. There are potentially several mechanisms by which leptin could lead to greater BHR and allergic inflammation[10]. Leptin is a pleiotropic hormone which can promote activation and production of oxidative burst of inflammatory cells[19]. Also, in murine alveolar macrophages, leptin has been shown to increase the production of arachidonic acid, PGE_2 and leukotrienes in a dose dependent manner via activation of phospholipase A_2[20]. Further, activation of leptin receptors found in airway smooth muscle (ASM) have been shown to increase release of vascular endothelial growth factor (VEGF), which is implicated in asthma severity[11, 21, 22]. Epidemiologically, cross sectional studies in adults and children have shown that asthmatics have higher plasma leptin levels; however, airway leptin levels not been previously reported in subjects with asthma[23, 24]. There are several explanations for the lack of associations between leptin and airway biomarkers in our study, including lack of power and potentially due to the fact that increased airway leptin levels may potentially be relevant only during asthma exacerbations. The lack of significant differences in BAL 8-isoprostanes, contrasts our own previous results, which had shown that 8-isoprostanes increased in relation to BMI[25]. This inconsistency may be potentially explained by the differences in sampling methods (exhaled breath condensate vs. BAL). This study is the first

one to show that in humans there is a significant association between plasma and airway leptin levels, which is highly suggestive of passive leptin diffusion between these compartments.

In contrast to leptin, adiponectin is a pleitrophic hormone with many anti-inflammatory properties, which may have a protective role in asthma. In a murine model, pre treatment with adiponectin reduced OVA-induced BHR and airway inflammation by decreasing the amount of inflammatory cells in the airway and Th-2 derived inflammatory cytokines[14]. Further, when compared to wild-type mice, adiponectin deficient mice (APN −/−) exposed to OVA had increased airway accumulation of eosinophils and monocyte/macrophages and increased Chemokine levels (CCL 2, 7, 11 & 12)[26]. Adiponectin receptors Adipo R1 and Adipo R2 are found in ASM and respiratory epithelium and are up-regulated by tobacco exposure and in the respiratory epithelium of smokers[12], however, it remains unclear what physiological role do Adipo R1 and R2 have in lung biology. Epidemiologically, higher plasma adiponectin levels are associated with lower asthma prevalence rates in women[16]; however any protective effects from increased plasma adiponectin is likely to be independent from airway levels, given the lack of correlation found in our study. Lack of correlations between adiponectin and airway biomarkers of inflammation and oxidative stress may be subject to the same factors outlined for leptin. In contrast to leptin, we did not observe any correlation between airway and plasma levels of adiponectin, which may be explained by adiponectin's higher molecular weight. There is evidence that high molecular weight adiponectin is actively transported into the airways, which appears to be mediated by T-Cadherin, a cell-cell adhesion molecule and a known receptor for HMW adiponectin[27].

Several limitations need to be considered in interpreting these results. This is a small convenience sample of subjects with asthma and healthy controls; it is therefore possible that several comparisons were not statistically significant given the high probability of a type II error. Also, given that all the patients had persistent asthma and were taking inhaled corticosteroids, it is impossible to determine whether this fact explained the lack of differences in 8-isoprostanes or exhaled NO between asthmatics and controls.

In summary, this study shows that overweight and obesity are associated with greater BAL leptin than lean asthmatics and controls, although plasma leptin levels in obese asthmatics are higher than in the obese controls. Also, increased BMI was marginally and inversely associated with BAL adiponectin. BAL and plasma leptin levels were significantly associated whereas the opposite was true for adiponectin. This result may imply that leptin and adiponectin have different transit mechanisms into the airways. Although no associations between BAL or plasma adipokines with biomarkers of oxidation and inflammation were found, this relationship may be more complex and could vary substantially depending on the degree of airway inflammation, asthma control or with exposure to triggering factors.

Acknowledgments

Funding: NIH/NCRR K12 RR017643, Emory Carlyle Fraser Heart Center & Emory University Research Committee.

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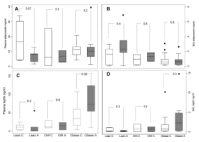

Figure 1.

Comparison of the BAL and plasma leptin and adiponectin distribution between asthmatics and controls within each weight category

Footnote: A: Plasma adiponectin, B: BAL adiponectin, C: Plasma leptin, d: BAL leptin. C: Control; A: asthma; OW: Overweight.

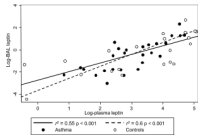

Figure 2.
Association between BAL and plasma leptin

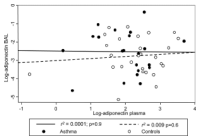

Figure 3.
Association between BAL and plasma adiponectin

Table 1

Demographic and clinical characteristics of the study population

Variables	Asthma				Controls			
	Lean	Overweight	Obese	p	Lean	Overweight	Obese	p
N (%)	5 (24%)	2 (10%)	14 (66%)		7 (26%)	3 (11%)	17 (63%)	
Gender (% female)	60	0	79	0.3	35	12	53	0.3
Age (median, range) years	28 (18 – 60)	25 (20 – 30)	42 (32 – 58)	.09	30 (22 – 39)	49 (42 – 50)	42 (21 – 63)	0.02
BMI	22 (21 – 23)	27 (26 – 28)	37 (34 – 42)		22 (23 – 24)	28 (27 – 29)	33 (32 – 37)	
Ever smoked (%)	40	0	35	0.3	0	33	23	0.5
Caucasian n (%)	20	0	78		28	100	71	
African American n (%)	80	100	22	0.01	42	0	29	0.07
Other n(%)	0	0	0		28	0	0	
Inhaled steroids n (%)	100	100	100					
Long acting beta agonists (%)	0	0	7	0.7				
Leukotriene antagonists (%)	0	50	35	0.3				
ACQ	1.3 (0.6 – 1.4)	0.9 (0.6 – 1.2)	2.1 (1.3 – 2.6)	0.06				
FEV_1%	81 (80 – 97)	86 (85 – 87)	84 (79 – 92)	0.8	102 (86 n– 109)	98 (90 – 101)	98 (93 – 105)	0.7
FEV_1 (L)	2.9 (1.8 – 3.7)	4 (4.1 – 4.2)	2.2 (1.9 – 2.6)	0.1	3.3 (2.4 – 3.9)	2.3 (2 – 3.2)	3.2 (2.4 – 3.5)	0.1
FVC%	88 (85 – 105)	90 (87 – 94)	86 (74 – 91)	0.4	102 (87 – 107)	89 (76 – 95)	96 (83 – 101)	0.2
FVC (L)	3.4 (2.3 – 4.8)	5.1 (4.9 – 5.4)	2.3 (2 – 3.3)	0.09	4.3 (4 – 6)	2.3 (2.2 – 3.7)	3.6 (3.2 – 3.9)	0.1
FEV_1/FVC	0.83 (0.8 – 0.87)	0.8 (0.76 – 0.86)	0.83 (0.78 – 0.86)	0.6	0.87 (0.85 – 0.89)	0.88 (0.87 – 0.97)	0.86 (0.83 – 0.91)	0.5
BAL Eosinophils%	0.3 (0.2 – 2.3)	0.8 (0.67 – 0.97)	1.1 (0.3 – 1.6)	0.9	0.6 (0 – 1)	0 (0 – 2)	1 (0 – 1.6)	0.6
BAL Macrophages%	91 (91 – 93)	90 (89 – 91)	92 (89 – 93)	0.6	90 (86 – 93)	94 (80 – 95)	92 (87 – 92)	0.6
BAL Neutrophils%	2.6 (2.3 – 3.3)	2.4 (1.6 – 3.3)	1.7 (1.3 – 2.2)	0.2	2 (1.9 – 3.2)	1.3 (1 – 14)	2 (1.8 – 4.5)	0.6
BAL Lymphocytes%	4 (2.6 – 4.2)	5.9 (5.8 – 6)	4.4 (3 – 7.3)	0.2	6 (3 – 7.6)	3 (2.5 – 4.2)	4.8 (3.2 – 6.2)	0.2

BMI: Body mass index; ACQ: Asthma control questionnaire; FEV1: Forced exhalation volume in one second; FVC: Forced vital capacity; BAL: Broncheoalveolar lavage

p= Kruskall Wallis.

Table 2

Plasma and BAL leptin and adiponectin by weight categories in subjects with asthma and healthy controls.

	Asthma				Controls			
	Lean	Overweight	Obese		Lean	Overweight	Obese	
n	5	2	14	Anova	7	3	17	Anova
Plasma leptin* ng/ml	2 (.6 – 11)	14 (4 – 23)	72 (55 – 124)	<0.01	11 (4 – 17)	12 (9 – 29)	34 (16 – 60)	0.01
BAL Leptin* ng/ml	0.3 (0.15 – 0.27)	0.7 (0.3 – 1.3)	2 (0.3 – 5)	0.02	0.2 (.1 – 1)	0.6 (0.05 – 2)	0.8 (0.3 – 1.8)	0.1
Plasma adiponectin mg/ml	8 (3 – 9)	7 (3 –11)	10 (7 –13)	0.5	16 (4 – 30)	6 (0.5 – 26)	11 (8 – 13)	0.2
BAL adiponectin mg/ml	0.2 (0.2 –0.3)	0.1 (0.07 – 0.2)	0.06 (0.03 – 0.2)	0.3	0.16 (0.02 – 0.2)	0.09 (0.02 – 0.15)	0.06 (0.03 – 0.1)	0.8

Results are presented as median and inter quartile range.

BAL: Broncheoalveolar lavage

* = In all the significant p ANOVA tests, the between group comparisons were different ($p < 0.05$) between lean vs. obese categories. Values were log-transformed to achieve normal distribution for the ANOVA analysis

Table 3

Multivariable analysis of the association between obesity and plasma and BAL levels of leptin and adiponectin with BMI categories.

	Plasma leptin (ng/ml)	BAL leptin (ng/ml)	Plasma Adiponectin (mg/ml)	BAL adipoenctin (mg/ml)
	β (95% C.I.) n=48	**β (95% C.I.) n=48**	**β (95% C.I.) n=48**	**β (95% C.I.) n=48**
Overweight (univariate)	2 (0.7 – 6.6)	2 (0.4 – 7.4)	0.53 (0.22 – 1.3)	0.22 (0.3 – 2.7)
Obese (Univariate)	6.7 (3 – 13.4)*	5.4 (1.08 – 13.4)*	1.06 (0.95 – 1.9)	0.57 (0.3 – 1.8)
Overweight (adjusted)	4.5 (1.7 – 11)*	3.7 (0.94 – 14.8)§	1.05 (0.22 – 1.5)	0.90 (0.3 – 2.7)
Obese (Adjusted)	11 (5.4 – 20)*	7.4 (2.7 – 20)*	0.12 (−0.5 – .82)	0.52 (0.22 – 1.2)
BMI (univariate)	1.9 (1.05 – 1.12)*	1.08 (1.04 – 1.12)*	1 (0.8 – 1.3)	0.98 (0.94 – 1)†
BMI (Adjusted)	1.08 (1.05 – 1.11)*	1.08 (1.04 – 1.12)*	1 (0.8 – 1.3)	0.97 (0.94 – 1)±

Models adjusted for: age, gender, ethnicity, case status (asthma vs. control), previous smoking (yes/no), and pre-bronchodilator FEV_1. Lean category is the reference comparison

* $p < 0.05$

§ $p=0.06$

† $p=0.09$

± $p=0.058$

Table 4

Airway inflammatory and oxidative biomarkers in subjects with asthma and controls by BMI categories.

	Asthma				Controls				p*
	Lean	Overweight	Obese		Lean	Overweight	Obese		
				p				p	
FeNO ppb	10 (8.7 – 14)	26 (8 – 45)	13 (9 – 27)		17 (10 – 20)	14 (7.5 – 17)	11 (8 – 14)		0.8
8-Isoprostanes (pg/ml)	4.2 (3 – 4.5)	3.5 (1.4 – 6)	6 (3 – 6)	.1	9.4 (3.2 – 11)	1.2 (0 – 1.6)	6.2 (4.2 – 7)	.05	0.06
NOx (μM/L)	0.3 (0.22 – 0.24)	0.10 (0 – 0.21)	0.36 (0.24 – 0.52)	.1	0.46 (0.21 – 0.51)	0.27 (0.21 – 0.28)	0.26 (0.21 – 0.56)	.7	0.4
Nitrites (μM/L)	0.10 (0.07 – 0.13)	0.13 (0.13 – 0.14)	0.12 (0.07 – 0.14)	.7	0.12 (0.08 – 0.18)	0.12 (0.12 – 0.13)	0.13 (0.11 – 0.18)	.9	0.9
Nitrates (μM/L)	0.27 (0.1 – 0.28)	0.04 (0 – 0.08)	0.3 (0.12 – 0.46)	.1	0.3 (0.09 – 0.51)	0.11 (0.09 – 0.14)	0.27 (0.1 – 0.43)	.7	0.6
H_2O_2 (μM/L)	0.05 (0.04 – 0.06)	0.09 (0.04 – 0.14)	0.07 (0.02 – 0.1)	.9	0.09 (0.04 – 0.13)	0.11 (0.08 – 0.17)	0.10 (0.04 – 0.16)	.7	0.5
pH	6.5 (6.4 – 6.9)	6.2 (5.8 – 6.6)	6.6 (6.5 – 6.8)	.6	6.8 (6.6 – 7)	6.2 (4.4 – 6.2)	6.6 (6.2 – 6.8)	.07	0.1

FENO: Fractional exhaled nitric oxide; NOx: Nitrogen oxide products. MDA: Malondialdehyde

Results are shown as median and interquartile range values

P= Kruskal Wallis for between BMI categories for asthmatics and controls

*p= Kruskal Wallis for overall difference between groups.

Table 4E

Urea corrected levels of airway inflammatory and oxidative biomarkers in subjects with asthma and controls by BMI categories, corrected for dilution using urea.

	Asthma				Controls				p*
	Lean	Overweight	Obese		Lean	Overweight	Obese		
8-Isoprostanes (pg/ml)	261 (62 – 348)	302 (119 – 485)	194 (111 – 273)	.9	242 (63 – 349)	159 (58 – 261)	264 (136 – 443)	.3	.7
NOx (μM/L)	29 (28.8 – 37.6)	36 (.07 – 72)	19 (7 – 41.9)	.9	8.17 (6 – 22)	12.1 (9.3 – 16.2)	16.6 (9.7 – 33.6)	.4	.7
Nitrites (μM/L)	15.6 (3.8 – 18)	23.4 (2.87 – 44.08)	5.4 (2 – 18)	.6	3 (2.4 – 4.88)	5.7 (5.5 – 8.5)	6.9 (3.2 – 9.1)	.2	.6
Nitrates (μM/L)	13.2 (11 – 23.6)	14 (0 – 28)	10 (5.6 – 24)	.9	5.2 (3.2 – 13)	6.4 (3.8 – 7.7)	11.8 (4.5 – 24.8)	.4	.6
H_2O_2 (μM/L)	5.7 (2.7 – 7)	8.5 (3 – 13)	2.4 (1.3 – 5)	.2	1.7 (0.6 – 5.3)	4.9 (3.8 – 12.8)	4.9 (1.3 – 4.9)	.4	.3
pH	6.49 (6.44 – 6.96)	6.25 (5.88 – 6.63)	6.63 (6.46 – 6.88)	.6	6.76 (6.58 – 6.99)	6.21 (4.36 – 6.44)	6.48 (6.34 – 6.44)	.07	.6

FENO: Fractional exhaled nitric oxide; NOx: Nitrogen oxide products. MDA: Malondialdehyde

Results are shown as median and interquartile range values

p= Kruskal Wallis for overall difference between groups.