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Abstract: 200

Title: Airway and systemic inflammation in obese children with asthma

Running head: Inflammation in paediatric obese asthma

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Conflict of Interest: Nil

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Abstract

Background: Obese asthma presents via altered airway and systemic inflammation in adults. This has not been comprehensively described in children.

Aim: To compare airway and systemic inflammation in obese and non-obese asthmatic children and controls.

Methods: In a cross-sectional study, children aged 8-17 yr were assigned to one of four groups: obese asthma (OA, n=74); non-obese asthma (NOA, n=249); obese control (OC, n=9); non-obese control (NOC, n=29). Lung function, and both sputum and systemic inflammatory biomarkers were measured.

Results: Non-eosinophilic asthma was more prevalent among OA females (60.0%) versus OA males (30.8%). However, there were no differences in %eosinophils or %neutrophils between OA and NOA. Leptin was higher in OC, but not OA, versus NOA and NOC, while adiponectin was reduced in OA versus NOC only. Expiratory reserve volume was reduced in OA, versus NOC. Residual volume (RV) and RV/total lung capacity were reduced in OC versus OA, and OC versus OA and NOA, respectively.

Conclusion: Obesity was associated with significant lung restriction in children with and without asthma. Obesity was not associated with significantly altered airway or systemic inflammation in asthmatic children. However, the higher prevalence of non-eosinophilic asthma in female obese asthmatics, compared to males, warrants further investigation.

Key words: body mass index; respiratory function tests.
Abbreviation list

ACQ, asthma control questionnaire; AHR, airway hyper-reactivity; ANOVA, analysis of variance; BMI, body
mass index; CRP, C-Reactive Protein; DRS, dose response slope; eNO, exhaled nitric oxide; ERV, expiratory
reserve volume; FEV₁, Forced expiratory volume in 1 sec; FVC, forced vital capacity; FRC, functional residual
capacity; GINA, global initiative for asthma; ICS, inhaled corticosteroid; IL-6, interleukin-6; IQR, inter-quartile
range; log-PD₁₅, log-transformed provocation dose; NOA, non-obese asthma; NOC, non-obese control; OA,
obese asthma; OC, obese control; PAQLQ(s), Paediatric Asthma Quality of Life Questionnaire (standardised);
RV, residual volume; SD, standard deviation; SDS, standard deviation score; TLC, total lung capacity.
Introduction

Overweight and obesity is highly prevalent among children with asthma, and exceeds the general population rate in Australia, Denmark and America[1-4], with a recent report that up to 45% of asthmatic children are carrying excess weight[4]. Obesity appears to complicate the management of childhood asthma, with previous studies indicating that obesity is associated with an increased risk of exacerbations, poorer asthma control and increased medication use, including steroids[5, 6]. Recently, significant reductions in lung volume indices have also been associated with overweight and obesity in asthmatic children[7, 8].

While the alarmingly high rates of obesity among asthmatic children warrant the development of specific interventions for this population, the first step is to better understand the presentation of obese asthma in order to inform clinical management. The mechanisms linking obesity and asthma remain poorly understood and multiple hypotheses have been proposed. Obesity may be associated with respiratory symptoms via cardio-respiratory de-conditioning, physiological restriction of the chest wall by excess mass, or co-morbidities, including gastro-oesophageal reflux and sleep disordered breathing[9]. Alternatively, chronic systemic inflammation, characteristic of obesity, has been hypothesised as an underlying factor in obese asthma that may contribute to altered airway inflammation and poorer clinical outcomes[10].

As a chronic inflammatory disease of the airways, asthma is traditionally characterised by eosinophilic airway inflammation. However, in adults, obese asthma has been described as a distinct clinical phenotype, involving non-eosinophilic airway inflammation and being unresponsive to current pharmacological treatment[11-13]. Levels of airway interleukin(IL)-8 have been shown to be raised in neutrophilic asthma[14]. Raised systemic inflammation is also characteristic of obese asthma in adults, including IL-6, and has been linked to airway inflammation and poorer clinical outcomes[13]. However, airway inflammation in obese asthmatic children has not been adequately described. Therefore, the aim of this study is to characterise obese asthma in children by describing airway inflammation, systemic inflammation and clinical asthma outcomes in obese and non-obese children, with and without asthma.
Methods

Subjects

Obese and non-obese children, with and without asthma, aged 8-17 years, were recruited from the general community and John Hunter Hospital (JHH) outpatient clinics, Newcastle NSW, Australia, from July 2004-2011. Asthma was defined by physician diagnosis and current respiratory symptoms. Obesity was defined as a body mass index (BMI) z-score ≥1.64 standard deviation score (SDS). Participants were assigned to one of four groups: obese asthma (OA, n=74); non-obese asthma (NOA, n=249); obese control (OC, n=9); or non-obese control (NOC, n=29). Exclusion criteria included unexplained weight change during the past three months, inflammatory/ endocrine disease, or respiratory disorder other than asthma. The Hunter New England and University of Newcastle Human Research Ethics Committees approved this study (09/05/20/5.08). Participant assent and guardian consent were obtained.

Clinical assessment

Participants attended JHH for clinical testing after an overnight fast and withholding antihistamines and asthma medications. Clinical asthma pattern, current asthma status, and quality of life was assessed using the Global Initiative for Asthma (GINA) guidelines[15], Juniper Asthma Control Questionnaire (ACQ)[16], and Paediatric Asthma Quality of Life Questionnaire (standardised) (PAQLQ(s))[17], respectively. Asthma stability was confirmed, defined as no exacerbation, respiratory tract infection or oral corticosteroid use in the past 4 weeks. The sequence of tests are shown in Fig. 1. Exhaled Nitric Oxide (eNO) was measured (NiOX chemiluminescent detector unit, Aerocrine, Zynergy Medical). Atopy was determined by positive skin prick test to common allergen(s) (Aspergillus fumigatus, Alternaria tenuis, Dust mite (Dermatophagoides Pteronyssinus), Cockroach mix, Grass mix). Tobacco exposure was measured by urinary cotinine (NicAlert, Nymox Pharmaceutical Corp, USA NJ). Weight and height were measured using 150 kg max scales (EB8271 NuWeigh, Newcastle Weighing Services NSW, Australia) and 2 m wall-suspended measuring tape with wall stop (Surgical and Medical Supplies Pty Ltd SA, Australia). BMI was calculated (weight (kg) / height (m)^2) and converted to BMI z-scores[18]. All participants performed spirometry (Windows KoKo PFT System Version 4.9 2005, PDS Inc Louisville USA). Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) are expressed as a % of the predicted value[19]. A subset of participants (OA=31, NOA=12, OC=9, NOC=15) performed lung plethysmography (MedGraphics Elite Series Plethysmograph, USA; Breeze Suite 6.4.1.14 Version 510 2008,
MedGraphics Corp., USA). Total lung capacity (TLC), functional residual capacity (FRC), expiratory reserve volume (ERV), and residual volume (RV) are presented as a percentage of their predicted value\[20\]. FEV\textsubscript{1}/TLC\% is also presented as an additional measure of obstruction\[21, 22\].

**Sputum inflammatory cells**

Participants underwent combined bronchial provocation testing and sputum induction with hypertonic saline (4.5\%) (ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000), as previously described\[23\]. Airway hyperresponsiveness (AHR) was defined as a fall in FEV\textsubscript{1} ≥15\% from baseline. The dose response slope (DRS) and log-transformed provocation dose (log-PD\textsubscript{15}) were calculated. Sputum was selected, dispersed with dithiothreitol, and total cell counts and viability determined. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained. Eosinophilic asthma was defined as sputum eosinophilia ≥2.0\%\[24\].

**Systemic inflammatory mediators**

Blood samples collected from a participant subset were centrifuged at 3000 rpm, 4°C for 10 minutes. High sensitivity C-Reactive Protein (CRP) was measured from serum mixed with monoclonal antibody-coated polystyrene particles, specific for human CRP (CRP Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA). Commercial ELISAs were used to measure plasma interleukin (IL)-6 (R&D Systems, Minneapolis MN USA), and serum leptin and adiponectin (Bio-Rad, Hercules CA USA). Assay sensitivity was 0.039pg/ml, 3.1pg/ml and 32.7pg/ml, respectively. All samples were tested in duplicate.

**Statistical analysis**

Data is presented as mean±SD, median[IQR], or proportion (n, (%)). Data variables were assessed for normality prior to analysis using one-way-ANOVA with post-hoc two-sample unpaired t-testing or Kruskal Wallis with post-hoc Wilcoxon rank sum testing for continuous data, and Pearson’s Chi-squared test for categorical data. Alpha was set at ≤0.05 for all tests, and ≤0.017 for post-hoc testing. Statistical analysis was performed using Intercooled Stata Version 11.0 for Windows (StataCorp, College Station, Texas, USA 1984-2005).
Results

Subject characteristics

Subject characteristics are presented in Table 1. The prevalence of atopy was higher in OA versus OC and higher in NOA versus OC and NOC. All participants were Caucasian, with the exception of n=1 Asian participant in the NOC. FEV₁ %predicted, FVC %predicted, and TLC %predicted did not differ by statistical significance across the four groups. However, FEV₁/TLC% was significantly lower in both asthma groups compared to NOC. ERV %predicted was lower in OA versus NOC, and RV %predicted was higher in OA versus OC. Likewise, RV/TLC% was significantly higher in OA and NOA versus OC.

Mean asthma duration was similar between OA and NOA (8.7(3.0) vs 8.5(4.1) years, p=0.815). The prevalence of AHR was similar in OA and NOA (42.5 vs 35.3%, p=0.346), as was the DRS (1.02[0.53, 2.9] vs 1.25[0.38, 3.22]% fall/ml, p=0.928). The OA had a significantly lower PAQLQ score (5.9[4.7, 6.4] vs 6.4[6.0, 6.6], p=0.035) and a smaller proportion used inhaled corticosteroids (ICS) (52.0 vs 75.8%, p=0.004), compared to NOA. However, there was no significant difference in the median ACQ score (0.6[0.3, 1.3] vs 0.7[0.4, 1.0], p=0.933) or steroid dose (400[400, 800] vs 400[200, 675] beclomethasone equivalents, p=0.721), between OA and NOA. A statistically significant difference (p=0.034) in the proportion of OA versus NOA classified as intermittent (35.7 vs 24.1%), mild (19.1 vs 44.8%), moderate (33.3 vs 27.6%) and severe (11.9 vs 3.5%) asthma was detected. Urinary cotinine levels were negligible in all participants and therefore not presented.

Airway inflammation

Compared to all groups, eNO was significantly lower in OC (Table 2). However, there was no difference between OA and NOA. Sputum induction was collected in a subset of participants (OA=52, NOA=185, OC=9, NOC=16). The number and proportion of airway eosinophils did not differ by statistical significance between the four groups. Conversely, airway %neutrophils were significantly different across the four groups, with a trend towards a higher proportion in the asthmatic groups versus the control groups. However, post-hoc analysis did not detect a statistically significant difference between the groups. Significantly lower airway %macrophages were detected in the NOA compared to both control groups, while absolute airway macrophage numbers were significantly lower compared to all groups. Among OA, females had significantly lower airway
%eosinophils versus males (Table 3), and a significantly higher proportion of females compared to males had non-eosinophilic asthma, based on % sputum inflammatory cells (Figure 2). This was not true for NOA. However, no statistically significant gender differences were evident in airway neutrophils (Table 3). Given the difference in airway %eosinophils in the OA, we compared medication usage between females and males. There was no difference in ICS or SABA usage, with a similar median steroid dose between female and male OA (400[400, 800] vs 400[200, 400] beclomethasone equivalents, p=0.199). Atopic status (53.3% vs 79.2%) and age (10.8(2.9) vs 11.5(2.8) years) were similar between female and male OA, while BMI z-score was not (2.0(0.3) vs 2.2(0.3) SDS, p=0.045).

Systemic inflammation

Serum leptin (p=0.032), serum adiponectin (p=0.010) and plasma IL-6 (p=0.025) levels differed significantly across the four groups, while serum CRP levels did not (p=0.197). In post-hoc analysis, serum leptin levels were significantly higher in OC compared to NOA and NOC, and serum adiponectin levels were lower in OA compared to NOC, while no statistically significant difference was detected for plasma IL-6 (Figure 3). Gender differences were not evident in systemic inflammatory biomarkers.
Discussion

Obese asthma was not associated with elevated airway inflammation or elevations in the systemic inflammatory markers, leptin, IL-6 and CRP, in this group of children. However, based on %sputum inflammatory cells, a higher proportion of obese females had non-eosinophilic asthma compared to obese males, suggesting gender differences in airway inflammation exist. Obesity was associated with lung restriction, which manifested as reduced ERV in asthma and reduced RV in controls. Furthermore, obesity was associated with a clinically significant reduction in quality of life in asthmatic children.

To our knowledge this is the first study to report airway inflammatory cell counts in obese children. In adults, obese asthma follows a non-eosinophilic steroid-resistant pattern of airway inflammation, characterised by neutrophilia and predominant among females[11, 13]. An association between airway %neutrophils and BMI has been reported in female, but not male, adults with asthma[13]. Our data did not detect altered airway neutrophils in obese female children with asthma. However, among obese asthmatics, airway %eosinophils were higher in males, and a significantly higher proportion of obese females had non-eosinophilic asthma, based on %sputum inflammatory cells. The obese female children also had a lower eNO, consistent with a non-eosinophilic pattern of airway inflammation. This requires further examination in a larger cohort.

Systemic inflammation occurs in adult obese asthma, with IL-6 almost 2-3 times higher and CRP approximately 7-8 times higher in obese versus non-obese adults[13, 25]. Previous studies have also reported adiponectin to be 50% lower in obese children and adults, compared to non-obese counterparts[26, 27]. In our study, serum adiponectin was reduced in asthmatic and non-asthmatic obese children, compared to non-obese controls. However, this reduction was statistically significant in obese asthmatics only, and may reflect the small sample of obese controls. Leptin has been suggested as a mediator between asthma and obesity, with systemic leptin levels reportedly 2-5 times higher in obese adults with asthma[13, 25, 27]. Studies in asthmatic and non-asthmatic children have found systemic leptin levels to be 2-3 times higher in the obese versus non-obese[10, 26]. In contrast, serum leptin levels in the current study were not elevated in obese asthmatics compared to non-obese asthmatics or controls, but were only significantly raised in obese children without asthma. This is a novel finding and requires further investigation. However, it should be noted that there was considerable variability in serum leptin levels in the OA, and a number of individuals had elevated serum leptin levels.
Plasma IL-6 and serum CRP were not significantly raised in the asthmatic or non-asthmatic obese children in our study population, which agrees with previous paediatric studies that did not detect elevated systemic CRP, IL-6 or tumour necrosis factor (TNF)-α levels in obese children[26, 28]. We hypothesise that the point at which adipose tissue becomes pathological may be an important distinction between children and adults when considering the effects of obesity on inflammatory and clinical outcomes. In contrast to adults[13], it is difficult to conclude from the present data that adipose tissue inflammation contributes to obese asthma in children.

Obesity exerts its greatest detriment on lung volume measurements, with reductions observed in ERV and FRC in adults, with and without asthma[29]. Similarly, reductions in ERV were detected in obese asthmatic children, compared to non-obese children without asthma. However, in contrast to recent reports in children[7, 8], the obese controls had a significantly reduced RV %predicted and RV/TLC%, which was not observed in the obese asthmatic children. Rastogi et al recently reported RV %predicted and RV/TLC% to be significantly lower in obese asthmatic children, compared to non-obese children without asthma[7]. Likewise, Mahut et al reported RV/TLC to be lower in overweight and obese children, compared to normal-weight children with asthma, identifying a negative relationship between BMI z-score and FRC and RV, expressed as both %predicted and relative to TLC[8]. Preserved RV in the asthmatic children in the presented study may be attributable to the obstructive effect of asthma, causing ‘air-trapping’ in the distal airways and an inflated RV[30]. Importantly, reductions in lung function associated with obesity were not detected through routine spirometry, and our data identifies lung volume assessment as an important clinical measure in this group of children.

Among asthmatic children, quality of life was both clinically and statistically significantly lower in the obese versus non-obese. Although some studies have found obesity is associated with poorer quality of life in asthmatic children, other studies have found no difference between obese and non-obese children, and it has been suggested that obesity may interfere with the perception of respiratory symptoms in children[5, 31]. Alternatively, obese asthmatic children may perceive worse symptoms related to a clinically significant reduction in ERV. Indeed, one could hypothesise that the poorer quality of life is due to poorer asthma control, in addition to obesity. However, despite fewer obese asthmatics being on controller medications, obesity was not associated with poorer asthma control, as measured by ACQ. Furthermore, airway reactivity to hypertonic saline did not differ between obese and non-obese asthmatics in our study. Previous studies examining obesity and AHR in children and adults provide conflicting results, with some reporting a higher prevalence or severity of AHR in obese compared to non-obese asthmatics, while others report no difference[5]. Previous studies also
report increased medication use in obese asthmatic children and adults[5, 6]. Our data didn’t detect a difference in steroid dose between obese and non-obese children with asthma. In fact, a smaller proportion of obese children were reportedly taking ICS medication. However, our data is limited by patient/guardian report of medication use, which may not reflect actual practice by this group. This study is limited by the small number of control subjects, particularly the obese, which may have impacted the power to detect differences between asthma and control subjects. The limited number of systemic inflammatory measures may also limit conclusions regarding the role of obesity-associated inflammation in childhood asthma.

In a large cohort of asthmatic children, stratified to mild-obesity or no obesity, obesity was associated with chest wall restriction and poorer quality of life, as seen in adult obese asthma. However, it was difficult to confirm that obesity-associated inflammation contributed to asthma in this group of children. Noting the absence of contribution from obesity-associated inflammation to asthma in this group of children, one may hypothesise that obesity is a co-morbidity, as opposed to a causative or precipitating factor in childhood asthma. Obese asthma in adults is associated with distinct airway and systemic inflammatory alterations which may become apparent in this group of children as they transition through adolescence into adulthood. Therefore, longitudinal studies, including repeated measures of sputum and systemic inflammatory markers, are needed to improve our understanding of obese asthma.

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Author contributions: MEJ contributed to study design, recruitment, data collection, statistical analysis, and manuscript preparation and review. PGG contributed to study design, statistical analysis, reporting of data, and manuscript preparation and review. CEC contributed to study design and manuscript preparation and review. LGW contributed to study design, statistical analysis, reporting of data, and manuscript preparation and review. LGW is guarantor of the manuscript. All authors approved the final version.
References


### Table 1: Subject characteristics & lung function summarised by obesity and asthma status

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Obese asthma (n=74)</th>
<th>Non-obese asthma (n=249)</th>
<th>Obese controls (n=9)</th>
<th>Non-obese controls (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years); mean (SD)</td>
<td>11.1(2.8)</td>
<td>11.1(3.0)</td>
<td>12.4(2.2)</td>
<td>11.8(2.9)</td>
<td>0.344</td>
</tr>
<tr>
<td>Gender (% females); %</td>
<td>47.3</td>
<td>38.6a</td>
<td>44.4</td>
<td>69.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Height (cm); mean (SD)</td>
<td>150.5(15.3)b</td>
<td>143.8(16.1)</td>
<td>154.0(12.0)</td>
<td>151.5(15.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg); mean (SD)</td>
<td>65.4(24.7)b</td>
<td>39.1(13.4)</td>
<td>70.9(18.8)b</td>
<td>44.1(15.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI z-score (SDS); mean (SD)</td>
<td>2.1(0.3)abc</td>
<td>0.2(0.9)</td>
<td>2.1(0.4)ab</td>
<td>0.1(0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic (Y/N); n (%Y)†</td>
<td>27/12 (69.2)</td>
<td>42/88 (84.0)</td>
<td>2/7 (22.2)</td>
<td>14/14 (50.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV₁, % predicted (%); mean (SD)</td>
<td>96.1(14.1)</td>
<td>96.3(14.0)</td>
<td>94.3(12.7)</td>
<td>97.8(13.1)</td>
<td>0.908</td>
</tr>
<tr>
<td>FVC, % predicted (%); mean (SD)</td>
<td>100.5(11.2)</td>
<td>97.4(11.9)</td>
<td>97.4(12.5)</td>
<td>97.0(11.8)</td>
<td>0.581</td>
</tr>
<tr>
<td>FEV₁/FVC % predicted (%); mean (SD)</td>
<td>79.2(6.6)</td>
<td>80.7(8.2)b</td>
<td>84.1(4.4)</td>
<td>88.0(7.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TLC % predicted (%); mean (SD)†</td>
<td>106.8(13.3)</td>
<td>100.3(10.7)</td>
<td>97.3(9.5)</td>
<td>99.1(9.3)</td>
<td>0.568</td>
</tr>
<tr>
<td>FEV₁/TLC (%); mean (SD) †</td>
<td>88.5(12.9)</td>
<td>86.5(9.4)b</td>
<td>96.7(5.3)</td>
<td>99.1(8.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>FRC % predicted (%); mean (SD)†</td>
<td>102.2(23.6)</td>
<td>116.7(18.7)</td>
<td>93.5(22.0)</td>
<td>112.0(16.2)</td>
<td>0.046</td>
</tr>
<tr>
<td>ERV % predicted (%); mean (SD)†</td>
<td>86.1(37.1)</td>
<td>112.3(23.7)</td>
<td>117.8(24.5)</td>
<td>121.3(27.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>RV % predicted (%); mean (SD)†</td>
<td>121.4(50.1)</td>
<td>120.9(29.6)d</td>
<td>64.9(36.0)</td>
<td>101.1(30.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>RV/TLC (%); mean (SD) †</td>
<td>24.0(8.0)</td>
<td>26.0(7.0)d</td>
<td>14.0(7.0)</td>
<td>22.0(6.6)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

BMI, body mass index; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; SDS, standard deviation score; TLC, total lung capacity; a vs non-obese asthma; b vs non-obese controls; c vs obese controls; p<0.017 for post-hoc analyses; †data available on a subset of participants only (OA=31, NOA=12, OC=9, NOC=15)
<table>
<thead>
<tr>
<th>Airway Inflammatory Markers</th>
<th>Obese asthma (n=52)</th>
<th>Non-obese asthma (n=185)</th>
<th>Obese controls (n=9)</th>
<th>Non-obese controls (n=16)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled nitric oxide (eNO) (ppb); median [IQR]</td>
<td>27.9[10.5, 46.7]</td>
<td>27.5[16.8, 55.7]</td>
<td>6.1[2.4, 10.4]</td>
<td>15.0[9.3, 28.9]</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml); median [IQR]</td>
<td>1.7[0.4, 6.4]</td>
<td>1.2[0.3, 3.1]</td>
<td>n/a</td>
<td>n/a</td>
<td>0.242</td>
</tr>
<tr>
<td>Total cell count (x 10⁶/ml); median [IQR]</td>
<td>2.5[1.3, 4.6]</td>
<td>1.7[0.7, 3.7]*</td>
<td>3.7[2.9, 6.5]</td>
<td>3.4[2.3, 6.0]</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Eosinophils (%); median [IQR]</td>
<td>0.8[0, 5.9]</td>
<td>1.1[0, 7.0]</td>
<td>0.0[0, 0.3]</td>
<td>0.5[0.0, 1.5]</td>
<td>0.136</td>
</tr>
<tr>
<td>Eosinophils (x 10⁶/ml); median [IQR]</td>
<td>0.02[0, 0.1]</td>
<td>0.02[0, 0.1]</td>
<td>0.00[0, 0.02]</td>
<td>0.02[0, 0.04]</td>
<td>0.508</td>
</tr>
<tr>
<td>Neutrophils (x 10⁶/ml); median [IQR]</td>
<td>0.5[0.2, 1.5]</td>
<td>0.4[0.2, 1.2]</td>
<td>0.5[0.1, 1.2]</td>
<td>0.3[0.1, 1.0]</td>
<td>0.794</td>
</tr>
<tr>
<td>Macrophages (%); mean (SD)</td>
<td>61.8(22.6)</td>
<td>51.9(25.9)**</td>
<td>79.6(20.6)</td>
<td>75.7(22.8)</td>
<td>&lt;<strong>0.001</strong></td>
</tr>
<tr>
<td>Macrophages (x 10⁶/ml); median [IQR]</td>
<td>1.8[1.1, 2.5]*</td>
<td>1.0[0.5, 1.6]**</td>
<td>2.7[1.8, 5.4]</td>
<td>2.6[1.7, 3.7]</td>
<td>&lt;<strong>0.001</strong></td>
</tr>
<tr>
<td>Lymphocytes (%); median [IQR]</td>
<td>0.9[0.2, 2.4]</td>
<td>0.5[0, 1.5]</td>
<td>1.1[0.0, 1.3]</td>
<td>1.3[0.5, 3.1]</td>
<td>0.139</td>
</tr>
<tr>
<td>Lymphocytes (x 10⁶/ml); median [IQR]</td>
<td>0.02[0, 0.1]</td>
<td>0.01[0, 0.04]*</td>
<td>0.04[0.0, 0.1]</td>
<td>0.1[0.02, 0.1]</td>
<td><strong>0.047</strong></td>
</tr>
<tr>
<td>Epithelial (%); median [IQR]</td>
<td>2.3[0.5, 5.5]*</td>
<td>3.0[0.5, 7.0]**</td>
<td>0.3[0.0, 2.3]</td>
<td>0.4[0.0, 2.4]</td>
<td><strong>0.005</strong></td>
</tr>
</tbody>
</table>

* vs non-obese asthma; ** vs non-obese controls; * vs obese controls; p=0.017 for post-hoc analyses
Table 3: Exhaled nitric oxide and sputum inflammatory cell counts in asthmatic children, summarised by gender and obesity status

<table>
<thead>
<tr>
<th>Airway inflammatory markers</th>
<th>Obese asthma</th>
<th>Non-obese asthma</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled nitric oxide (eNO) (ppb); median [IQR]</td>
<td>33.5[12.8, 49.8]</td>
<td>11.2[8.6, 28.2]</td>
<td>28.0[20.3, 73.7]</td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml); median [IQR]</td>
<td>2.27[0.5, 5.56]</td>
<td>1.67[0.03, 6.84]</td>
<td>1.19[0.15, 3.29]</td>
</tr>
<tr>
<td>Eosinophils (%); median [IQR]</td>
<td>3.8[0.5, 9.8]</td>
<td>0.0[0, 1.0]</td>
<td>1.4[0.0, 7.5]</td>
</tr>
<tr>
<td>Eosinophils (x 10^6/ml); median [IQR]</td>
<td>0.11[0.01, 0.12]</td>
<td>0.0[0, 0.04]</td>
<td>0.03[0.0, 0.14]</td>
</tr>
<tr>
<td>Neutrophils (x 10^6/ml); median [IQR]</td>
<td>0.5[0.2, 2.4]</td>
<td>0.5[0.1, 1.3]</td>
<td>0.4[0.2, 1.2]</td>
</tr>
<tr>
<td>Macrophages (%); mean (SD)</td>
<td>60.0(22.4)</td>
<td>63.7(23.1)</td>
<td>51.9(25.6)</td>
</tr>
<tr>
<td>Macrophages (x 10^6/ml); median [IQR]</td>
<td>1.8[1.1, 3.0]</td>
<td>1.7[1.1, 2.5]</td>
<td>1.0[0.5, 1.8]</td>
</tr>
</tbody>
</table>

*vs obese asthma females; **vs non-obese asthma males; ***vs non-obese asthma females; p<0.017 for post-hoc analyses
Figure Legends

Fig. 1 Sequence of testing for participants during clinic visit. All patients fasted ≥12 hours and attended clinic to begin first test between 7:30-9:30 am. Lung function and/or eNO conducted prior to sputum combined bronchial provocation and sputum induction. Blood collected at any time point between 7:30-10:00 am. Questionnaires and skin prick testing completed at any time point during clinic visit.

Fig. 2 The proportion of eosinophilic asthma (≥2% sputum eosinophils) and non-eosinophilic asthma among obese and non-obese, male and female children. OA, obese asthma; NOA, non-obese asthma.

Fig. 3 Systemic inflammatory biomarkers, a) serum leptin (ng/ml), b) serum adiponectin (μg/ml), c) plasma interleukin (IL)-6 (pg/ml), d) serum C-Reactive Protein (mg/ml). OA, obese asthma (n=32); NOA, non-obese asthma (n=14); OC, obese control (n=9); NOC, non-obese control (n=16).