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Title: Morbid obesity and asthma: co-morbidity or causal relationship?
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Bronchial and systemic inflammation in morbidly obese asthmatic subjects: a biopsy study


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Inflammation in morbidly obese asthmatic subjects

To the editor

Asthma in the obese has been described as a specific phenotype, with a late onset and high symptom expression\(^{(1)}\). However, the nature of bronchial inflammation in obese subjects with asthma is not fully understood. In contrast to eosinophilic airway inflammation in lean asthmatics, a predominance of airway neutrophilia has been reported in obese asthmatic women\(^{(2)}\). Some groups reported increased sputum neutrophil counts\(^{(2, 3)}\), whereas others found no increase of neutrophilic inflammation in obese asthma patients\(^{(4, 5)}\).

All aforementioned studies in obese asthmatics investigated induced sputum or brush biopsies\(^{(6)}\) which may not fully reflect tissue inflammation. Thus far, only one study has described the analysis of bronchial biopsies in obese asthmatics, showing increased eosinophil counts in patients with severe asthma and obesity as compared to lean asthmatics\(^{(7)}\). However, that study did not include an obese control group, nor were other cell populations investigated such as airway neutrophils. To our knowledge, there are no data on bronchial biopsies from morbidly obese patients with mild-to-moderate asthma compared to morbidly obese control subjects without asthma.

We performed a cross-sectional study in 27 morbidly obese patients with asthma, defined according to GINA guidelines\(^{(8)}\) and 43 morbidly obese control subjects undergoing bariatric surgery. Some of the results of this study have been previously reported in the form of an abstract\(^{(9)}\).

This study is part of a larger study, the results of which have been reported elsewhere\(^{(10)}\). Subjects were between 18 and 50 years of age, with a BMI above 35 kg/m\(^2\), and were excluded if they smoked more than 10 cigarettes per day or had smoked more than 10 pack years. The study was approved by the local ethics committee (Netherlands Trial Register 3204), and all subjects gave written informed consent. Lung function tests, asthma control, asthma quality of life, comorbidities, parameters of systemic inflammation and cell counts of bronchial biopsies were compared between obese patients with asthma and obese control patients.

A total of 86 patients were included\(^{(10)}\), of whom 70 patients (table 1) had bronchial biopsies with acceptable quality for further analysis. When comparing the obese patients with asthma and obese control groups, no differences were detectable in several parameters assessed in peripheral blood, except for the neutrophil count and levels of serum IL-6 which were both slightly, but significantly, increased in the morbidly obese asthma group compared to the morbidly obese control group (table 1). We found no other significant differences between these groups concerning markers of systemic inflammation (IL-8, hs-CRP, TNFα, GM-CSF, leptin or adiponectin).

When assessing bronchial biopsies, there were no significant differences in submucosal cell counts of eosinophils, neutrophils, mast cells, macrophages, B-cells, CD4\(^+\) or CD3\(^+\) T-cells (figure 1 A – G). CD8\(^+\) T-cells were significantly lower in the asthma group.
## Table 1 Demographics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>No asthma</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%female)</td>
<td>77.8%</td>
<td>81.4%</td>
<td>0.764</td>
</tr>
<tr>
<td>Ethnicity (%non-Caucasian)</td>
<td>18.5%</td>
<td>11.6%</td>
<td>0.493</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33 (19-48)</td>
<td>38 (19-50)</td>
<td>0.623</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>127.1 (101-202)</td>
<td>126.0 (94-199)</td>
<td>0.850</td>
</tr>
<tr>
<td>Body Mass index (kg/m²)</td>
<td>44.59 (38.4-63.8)</td>
<td>43.35 (36.9-60.0)</td>
<td>0.326</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>130 (112-165)</td>
<td>128 (98-200)</td>
<td>0.985</td>
</tr>
<tr>
<td>Bio-impedance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat free Mass</td>
<td>58.2 (50.1-100.5)</td>
<td>62.3 (48.0-83.9)</td>
<td>0.248</td>
</tr>
<tr>
<td>Fat weight (%)</td>
<td>50.9 (37.6-70.4)</td>
<td>51.1 (31.1-59.7)</td>
<td>0.878</td>
</tr>
<tr>
<td>Fat weight (kg)</td>
<td>65.4 (44.5-134.4)</td>
<td>63.0 (33.9-100)</td>
<td>0.396</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>% never smoked</td>
<td>55.6%</td>
<td>72.1%</td>
<td></td>
</tr>
<tr>
<td>% stopped smoking</td>
<td>14.8%</td>
<td>18.6%</td>
<td></td>
</tr>
<tr>
<td>% current smoker</td>
<td>29.6%</td>
<td>9.3%</td>
<td></td>
</tr>
<tr>
<td>Pack years</td>
<td>0 (0-10)</td>
<td>0 (0-10)</td>
<td>0.246</td>
</tr>
<tr>
<td>Asthma Control Questionnaire¹</td>
<td>1.1 (0.4-2.9)</td>
<td>0.3 (0-2.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Asthma Quality of Life Questionnaire²</td>
<td>5.8 (3.7-6.8)</td>
<td>6.6 (3.7-7.0)</td>
<td>0.007</td>
</tr>
<tr>
<td>Medication use at inclusion study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short acting bronchodilator</td>
<td>48.1%</td>
<td>23.3%</td>
<td>0.039</td>
</tr>
<tr>
<td>Long acting bronchodilator</td>
<td>3.7%</td>
<td>2.3%</td>
<td>1.000</td>
</tr>
<tr>
<td>Antileukotrienes</td>
<td>0%</td>
<td>2.3%</td>
<td>1.000</td>
</tr>
<tr>
<td>B₂-sympaticomimetica/ICS</td>
<td>22.2%</td>
<td>7.0%</td>
<td>0.070</td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
<td>22.2%</td>
<td>7.0%</td>
<td>0.079</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>25.9%</td>
<td>11.6%</td>
<td>0.192</td>
</tr>
<tr>
<td>Nasal corticosteroids</td>
<td>14.8%</td>
<td>11.6%</td>
<td>0.726</td>
</tr>
<tr>
<td>Atopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopy</td>
<td>70.4%</td>
<td>41.9%</td>
<td>0.027</td>
</tr>
<tr>
<td>IgE (kU/L) **</td>
<td>205 (5-1838)</td>
<td>59 (1.4-761)</td>
<td>0.049</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epworth Sleepiness Scale</td>
<td>2 (0-8)</td>
<td>2 (0-15)</td>
<td>0.670</td>
</tr>
<tr>
<td>GERD-questionnaire</td>
<td>6 (4-12)</td>
<td>7 (2-14)</td>
<td>0.524</td>
</tr>
<tr>
<td>Steps a day</td>
<td>5107 (2156-12176)</td>
<td>5158 (2061-11705)</td>
<td>0.766</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>51.9%</td>
<td>51.2%</td>
<td>1.000</td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirometry</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>FEV₁, pre(% predicted)</td>
<td>88 (66-119)</td>
<td>97 (73-125)</td>
<td>0.023</td>
</tr>
<tr>
<td>FEV₁, post (% predicted)</td>
<td>95 (74-118)</td>
<td>101 (75-129)</td>
<td>0.141</td>
</tr>
<tr>
<td>FVC, pre (% predicted)</td>
<td>97 (75-126)</td>
<td>102 (79-144)</td>
<td>0.623</td>
</tr>
<tr>
<td>FEV₁/FVC, pre (%)</td>
<td>76 (63-86)</td>
<td>81 (66-93)</td>
<td>0.010</td>
</tr>
<tr>
<td>RV, post (% predicted) *</td>
<td>68 (39-126)</td>
<td>75 (33-118)</td>
<td>0.757</td>
</tr>
<tr>
<td>ERV, post (% predicted) *</td>
<td>45 (24-78)</td>
<td>47 (11-66)</td>
<td>0.682</td>
</tr>
<tr>
<td>TLC, post (% predicted) *</td>
<td>95 (80-106)</td>
<td>94 (76-114)</td>
<td>0.822</td>
</tr>
<tr>
<td>FRC, post (% predicted) *</td>
<td>60 (47-95)</td>
<td>61 (41-88)</td>
<td>0.925</td>
</tr>
</tbody>
</table>
### Table 1 Demographics of the study population (continued)

<table>
<thead>
<tr>
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<th>Asthma N=27</th>
<th>No asthma N=43</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV/TLC, post (%) *</td>
<td>22 (10-41)</td>
<td>25 (12-86)</td>
<td>0.249</td>
</tr>
<tr>
<td>Reversibility FEV₁</td>
<td>10 (-6-20)</td>
<td>3 (-7-11)</td>
<td>0.001</td>
</tr>
<tr>
<td>FeNO (ppb) **</td>
<td>16 (5-45)</td>
<td>16 (7-53)</td>
<td>0.934</td>
</tr>
<tr>
<td>Diffusion capacity (% predicted)</td>
<td>95 (69-130)</td>
<td>96 (69-134)</td>
<td>1.000</td>
</tr>
<tr>
<td>PD₂₀ (mg)</td>
<td>0.33 (0.04-1.8)</td>
<td>&gt; 1.8</td>
<td></td>
</tr>
<tr>
<td>IOS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₅ (kPa/sec)</td>
<td>0.70 (0.42-1.39)</td>
<td>0.56 (0.32-0.85)</td>
<td>0.003</td>
</tr>
<tr>
<td>R₂₀ (kPa/sec)</td>
<td>0.45 (0.27-1.03)</td>
<td>0.42 (0.19-0.68)</td>
<td>0.623</td>
</tr>
<tr>
<td>R₅ - R₂₀</td>
<td>0.26 (0.06-0.66)</td>
<td>0.15 (0.03-0.28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>X₅ (kPa/sec)</td>
<td>-0.26 (-0.87 -0.12)</td>
<td>-0.20 (-0.41 -0.08)</td>
<td>0.061</td>
</tr>
<tr>
<td>F_res (Hz)</td>
<td>22.67 (10.5-29.0)</td>
<td>17.95 (8.4-23.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

#### Laboratory

<table>
<thead>
<tr>
<th></th>
<th>Asthma</th>
<th>No asthma</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7 (3.4-7.4)</td>
<td>5.0 (2.3-6.9)</td>
<td>0.972</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.1 (0.7-2.3)</td>
<td>1.1 (0.7-2.1)</td>
<td>0.910</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.9 (1.6-5.1)</td>
<td>3.0 (0.7-4.8)</td>
<td>0.920</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.7 (0.6-3.3)</td>
<td>1.4 (0.4-5.1)</td>
<td>0.326</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 (4.6-9.5)</td>
<td>5.6 (4.0-27.1)</td>
<td>0.910</td>
</tr>
<tr>
<td>Vitamin D (nmol/L)</td>
<td>40.5 (11-127)</td>
<td>40.0 (10-62)</td>
<td>0.877</td>
</tr>
<tr>
<td>Peripheral blood count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes (10⁹/L)</td>
<td>8.7 (5.3-13.1)</td>
<td>7.2 (5.2-11.9)</td>
<td>0.141</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>61 (45-72)</td>
<td>59 (46-70)</td>
<td>0.049</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>28 (17-45)</td>
<td>32 (20-47)</td>
<td>0.141</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.5 (0.4-1.5)</td>
<td>0.5 (0.3-0.9)</td>
<td>0.141</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2 (0-6)</td>
<td>2 (0-8)</td>
<td>0.942</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0 (0-1)</td>
<td>0 (0-2)</td>
<td>0.850</td>
</tr>
<tr>
<td>HS-CRP (pg/ml)</td>
<td>36.0 (4.3-142.0)</td>
<td>31.5 (3.7-120.4)</td>
<td>0.732</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.72 (0.61-3.78)</td>
<td>0.70 (0.7-13.3)</td>
<td>0.020</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>3.86 (1.79-13.46)</td>
<td>3.84 (1.86-9.12)</td>
<td>0.877</td>
</tr>
<tr>
<td>TNF-alfa (pg/ml)</td>
<td>0.8 (0.8-1.3)</td>
<td>0.8 (0.8-1.0)</td>
<td>0.910</td>
</tr>
<tr>
<td>GM-CSF (pg/ml)</td>
<td>0.61 (0.61-3.78)</td>
<td>0.61 (0.61-8.44)</td>
<td>0.275</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>68 (18-100)</td>
<td>70 (11-100)</td>
<td>0.877</td>
</tr>
<tr>
<td>Adiponectin (pg/ml)</td>
<td>12.0 (4.5-22.0)</td>
<td>14.1 (0.06-28.9)</td>
<td>0.251</td>
</tr>
</tbody>
</table>

Data are presented as median (min-max)

* Because of weight limitations (<150 kg) of body box different numbers; asthma = 18, control = 30
** Log transformed for statistical purposes
1 Scores of the asthma control questionnaire range from 0 to 6, with lower scores indicating better asthma control
2 Scores of the AQLQ range from 1 to 7, with higher scores indicating better asthma-specific quality of life
3 Defined as either one positive reaction to the skin-prick test or one positive reaction to specific IgE inhalation screen
4 Non-fasting blood sample

Diffusion capacity, kCO; ERV, expiratory reserve volume; FeNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; ICS, inhaled corticosteroid; PD₂₀, provocative dose of methacholine inducing a 20% fall in FEV₁; RV, residual volume; TLC, total lung capacity
compared to the controls (median 682 versus 1017, p=0.014; figure 1H). There was no difference in CD4/CD8 ratio or in reticular basement membrane (RBM) thickness between the two groups (figure 1I-J).

In contrast, a subgroup analysis showed that asthmatics with uncontrolled asthma (Asthma Control Questionnaire score >1.5) compared to obese control had significantly higher levels of IL-6 (median 0.89 vs. 0.70 pg/ml, p<0.001) and lower adiponectin (median 9.2 vs. 14.1 ng/ml, p=0.014), whereas we found no differences in cell counts in bronchial biopsies. Further subgroup analysis (ICS use at time of biopsy, females, high IgE, large abdominal circumference, childhood-onset versus adult onset asthma, smoking) showed no differences between obese control patients and obese patients with asthma regarding all parameters representing bronchial or systemic inflammation.

We found no significant correlations between clinical parameters (lung function parameters \(\text{FEV}_1\), \(\text{FEV}_1/\text{FVC}\), provocative dose of methacholine inducing a 20% fall in \(\text{FEV}_1\), and fractional exhaled nitric oxide) or symptoms (Asthma Control Questionnaire or Asthma Quality of Life Questionnaire) and bronchial or systemic inflammation.

In contrast to previous reports\(^2,7\), the present results show neither eosinophilic nor neutrophilic inflammation in the group of morbidly obese asthmatics. In contrast to Desai and colleagues\(^7\), who analyzed exclusively patients with severe asthma, we included only patients with mild-to-moderate asthma. Furthermore, the median body mass index was significantly higher in our study (44 kg/m\(^2\)) compared to Desai's study (36 kg/m\(^2\)).

In addition, we also investigated other cell types in the bronchial submucosa, such as mast cells, macrophages, B-cells, CD3\(^+\), and CD4\(^+\) and positive T-cells, and again found no differences between the asthma and the control group. Moreover, we also measured the reticular basement membrane thickness, as a marker of airway remodeling\(^11\), and found no difference between obese asthmatics and obese control patients. These data suggest that asthma in this cohort is not driven by a classical Th2-mediated mechanism and probably needs to be regarded as a distinct phenotype of the disease that is not related to significant inflammatory responses in the airways. It needs to be noted that we did not assess innate immune cells such as innate lymphoid cells, natural killer cells and dendritic cells in our analysis, and therefore cannot exclude the possibility that differences existed in these cells between the asthma and control groups.

Sutherland and colleagues\(^12\) previously demonstrated that asthma phenotypes are not homogenous in obese individuals. However, in the present study, we found no differences in lung function, symptoms, or inflammation parameters when analyzing different subgroups such as subjects with a larger abdominal circumference, age of onset of asthma, high IgE or use of inhaled corticosteroids. We cannot exclude the possibility that morbidly obese mild to moderate asthma patients constitute an obese asthma phenotype that is characterized by the absence of bronchial inflammation.
**Figure 1** Bronchial submucosal cell counts and reticular basement membrane thickness

Bronchial submucosal cell count in obese subjects with and without asthma (a) eosinophils (p=1.000) (asthma n=27, control n=43), (b) neutrophils (p=0.141), (c) mast cells, (d) macrophages (p=0.326), (e) B-cells (p=0.141), (f) CD4⁺ T cells (p=1.000), (g) CD3⁺ T cells (p=0.326), (h) CD8⁺ T cells (p=0.014), (i) reticular basement membrane thickness (p=0.874) (asthma n=15, control n=18), (j) CD4 / CD8 ratio (k) Photomicrograph of a bronchial biopsy from an obese subject with asthma showing stained eosinophils.

The horizontal bar is the median.
Several hypotheses may explain the relationship between obesity and asthma, such as co-morbidities (Gastroesophageal reflux disease, obstructive sleep apnea syndrome) or the metabolic syndrome\textsuperscript{(13)} for which we found no differences between obese patients with asthma and obese control patients. Body composition in the obese, however, might cause asthmatic symptoms and lung function impairment\textsuperscript{(14)}, and in particular, the significant difference in impulse oscillometry ($R_5$-$R_{20}$) suggests an abnormality in the lung periphery, which is in line with recently reported data\textsuperscript{(15)}.

The strength of our study is the inclusion of an obese control group of subjects without asthma. Furthermore, the diagnosis of asthma was performed strictly according to the Global Initiative for Asthma guidelines, and was not based on a doctor diagnosis in which symptoms play a major role, which has been shown to be incorrect\textsuperscript{(16)}. Nonetheless, there are some limitations to our study. First, we did not include a lean asthma or lean control group. Furthermore, we used a heterogeneous asthma group with a majority of subjects with mild to moderate disease and relatively few symptoms, in contrast to other studies that investigated more severe obese patients with asthma.

In summary, despite evidence for systemic inflammation, which seemed to be related to the level of asthma control, there was no evidence for bronchial inflammation, characterized by increased numbers of eosinophils or neutrophils. Further research aiming at the effects of weight reduction on inflammation, symptoms, lung function and quality of life in the morbidly obese may provide valuable further insights in the pathogenesis and treatment of asthma in that population.

**ACKNOWLEDGEMENTS**

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REFERENCES


