

ORIGINAL ARTICLE

AIRWAY DISEASES

Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response

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To cite this article: Telenga ED, Tideman SW, Kerstjens HAM, ten Hacken NHT, Timens W, Postma DS, van den Berge M. Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response. *Allergy* 2012; DOI:10.1111/j.1398-9995.2012.02855.x.

Keywords

asthma; bronchial hyper-responsiveness; corticosteroids; lung function; obesity.

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Accepted for publication 05 May 2012

DOI:10.1111/j.1398-9995.2012.02855.x

Edited by: Hans-Uwe Simon

Abstract

Background: The incidence of asthma and obesity is increasing worldwide, and reports suggest that obese patients have more severe asthma. We investigated whether obese asthma patients have more severe airway obstruction and airway hyper-responsiveness and a different type of airway inflammation than lean asthmatics. Furthermore, we assessed the effect of obesity on corticosteroid treatment response.

Methods: Patient data from four well-documented asthma cohorts were pooled ($n = 423$). We evaluated FEV₁, bronchial hyper-responsiveness (PC₂₀) to either methacholine/histamine or adenosine 5'-monophosphate (AMP) (differential) cell counts in induced sputum and blood and corticosteroid treatment response in 118 patients.

Results: At baseline, FEV₁, PC₂₀ methacholine or histamine, and PC₂₀ AMP values were comparable in 63 obese (BMI ≥ 30 kg/m²) and 213 lean patients (BMI < 25 kg/m²). Obese patients had significantly higher blood neutrophils. These higher blood neutrophils were only seen in obese women and not in obese men. After a two-week treatment with corticosteroids, we observed less corticosteroid-induced improvement in FEV₁ predicted in obese patients than in lean patients (median 1.7% vs 6.3% respectively, $P = 0.04$). The percentage of sputum eosinophils improved significantly less with higher BMI ($P = 0.03$), and the number of blood neutrophils increased less in obese than in lean patients ($0.32 \times 10^3/\mu\text{L}$ vs $0.57 \times 10^3/\mu\text{L}$, $P = 0.046$).

Conclusions: We found no differences in asthma severity between obese and non-obese asthmatics. Interestingly, obese patients demonstrated more neutrophils in sputum and blood than nonobese patients. The smaller improvement in FEV₁ and sputum eosinophils suggests a worse corticosteroid treatment response in obese asthmatics.

Obesity is a worldwide epidemic, and the prevalence of obesity has tripled in the last two decades (1). More than 1.5 billion of adults are overweight [body mass index (BMI)

> 25 kg/m²], and more than 500 million are obese (BMI > 30 kg/m²). More recently, the presence of overweight and obesity has also been associated with asthma (2–4). Prevalence of asthma is higher in obese than in nonobese adults (9% vs 5%), and the presence of overweight and obesity increases the relative risk of having asthma by 2.0- and 2.7-fold, respectively (2, 3). Further, there exists a dose-response effect of BMI, in that overweight increases the odds to develop asthma by 38% and obesity by 92% (4).

An increased body weight has also important consequences in patients with established asthma. It has been suggested

Abbreviations

BMI, body mass index (kg/m²); FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; TNF α , tumor necrosis factor- α ; IL-6, interleukin 6; AMP, adenosine 5'-monophosphate; PC₂₀, provocative concentration of an inhaled stimulus inducing a 20% fall of FEV₁; ICS, inhaled corticosteroids.

that obese asthma patients respond less to treatment with inhaled corticosteroids, which may be due to a different type of inflammation (5–9). In this context, studies on the systemic effects of obesity are of interest. Obesity is associated with a chronic low-grade inflammatory state that may contribute to inflammation at sites distant from the adipose tissue such as the lungs (10–16). In agreement with this, Haldar et al. identified a phenotype of asthma consisting of predominantly female, obese asthma patients (17). Patients with this phenotype had high percentages of sputum neutrophils and more asthma symptoms. Finally, the presence of obesity may be associated with more severe airway obstruction in asthma. However, there is controversy about this in the literature (5, 7–9, 18–21).

The aim of this study was to investigate whether obese patients have more severe asthma than lean patients, that is, patients without obesity or overweight ($\text{BMI} < 25 \text{ kg/m}^2$), and whether they have a different type of airway inflammation. Furthermore, we investigated whether obesity affects corticosteroid responsiveness in asthma. To this end, we combined individual patient data from four studies previously performed in the Groningen Research Institute for Asthma and COPD (GRIAC).

Materials and methods

We pooled the data from four clinical studies that were conducted in the University Medical Center Groningen. Patient characteristics and measurements in the studies are summarized in Table 1 (22–26). All studies were approved by the Medical Ethics Committee of the University Medical Center Groningen, and all patients provided written informed consent.

Patients

All patients had a doctor's diagnosis of asthma and bronchial hyper-responsiveness, defined as a provocative concentration causing a 20% fall in FEV_1 (PC_{20}) methacholine $< 8 \text{ mg/ml}$ or PC_{20} histamine $< 8 \text{ mg/ml}$. Further inclusion criteria were dependent on the different study protocols. From study 3, only the subpopulation of patients who were 18–60 years, with episodic respiratory symptoms, PC_{20} histamine $< 8 \text{ mg/ml}$ and < 10 pack-years smoking, was used for analysis (25). In studies 1, 3 and 4, inhaled corticosteroids were tapered before the baseline visit (22, 25, 26). In studies 1 and 4, inhaled corticosteroids were tapered and when possible discontinued completely, at least 3 weeks before the measurements. When patients experienced a worsening of their asthma before complete discontinuation for 3 weeks, they were asked to visit the hospital earlier. Patients experiencing an exacerbation for which an oral prednisolone course was necessary were not included. In study 3, inhaled corticosteroids were discontinued for 4 weeks before the measurements. In study 2, patients were not required to stop inhaled corticosteroids. In Table 2, the number of patients who still used inhaled corticosteroids at the time of the measurements is presented.

Table 1 Baseline characteristics and measurements in the studies included in the analyses

Baseline measurements									
	Number	BMI	BMI ≥ 30	Age	FEV ₁ % predicted	BHR		Sputum	Blood
						Mch/Hist	AMP		
Study 1 (22)	118	24 (22–28)	19 (16.1)	27 (22–38)	81 (68–92)	+	+	+	+
Study 2 (23, 24)	144	27 (25–29)	30 (20.8)	49 (38–57)	91 (79–102)	–	+	+	+
Study 3 (25)	140	24 (22–26)	13 (9.3)	31 (25–46)	66 (53–76)	+	–	–	–
Study 4 (26)	21	25 (22–26)	1 (4.8)	31 (26–39)	107 (88–112)	+	+	+	+

Values are presented as numbers (with percentage) or medians (with interquartile range). BMI, body mass index; FEV_1 , forced expiratory volume in 1 s; BHR, bronchial hyper-responsiveness; Mch/Hist, methacholine/histamine; AMP, adenosine 5'-monophosphate; +, measurement performed; –, measurement not performed.

Table 2 Baseline characteristics of obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients*

	BMI < 25 (N = 213)	BMI ≥ 30 (N = 63)	P-value
Age (years)	29 (24–42)	45 (36–52)	<0.001[‡]
Males n (%)	105 (49)	22 (35)	0.03[§]
Use of ICS at baseline n (%)	19 (9)	12 (19)	0.04[§]
Current smoking n (%)	42 (20)	10 (16)	0.3 [§]
	N = 207		
FEV ₁ prebronchodilator (L)	2.9 (2.2–3.5)	2.5 (2.0–3.0)	0.9
	N = 212		
FEV ₁ %predicted prebronchodilator	77 (61–92)	84 (67–98)	0.7
	N = 212		
PC ₂₀ methacholine/histamine (mg/ml) [†]	0.26 (0.02–7.31)	0.35 (0.02–5.74)	0.5
	N = 166	N = 33	
PC ₂₀ AMP (mg/ml) [†]	9.8 (0.02–640)	14.5 (0.02–640)	0.5
	N = 124	N = 50	
AMP dose–response slope (%/mg/ml)	2.27 (0.27–8.33)	1.02 (0.04–9.77)	0.98
	N = 124	N = 49	
Blood eosinophils (10 ³ /μl)	0.27 (0.18–0.43)	0.21 (0.13–0.33)	0.6
	N = 122	N = 49	
Sputum eosinophils (%)	3.3 (1.0–8.5)	0.5 (0.2–4.9)	0.3
	N = 115	N = 40	
Blood neutrophils (10 ³ /μl)	3.4 (2.5–4.4)	4.2 (3.3–5.0)	0.001
	N = 122	N = 49	
Sputum neutrophils (%)	36 (22–60)	52 (39–70)	0.095
	N = 115	N = 40	

*All values are presented as median with interquartile range unless stated otherwise.

[†]Geometric mean with range.

[‡]Tested by Mann–Whitney *U*-test.

[§]Tested by Fisher's exact test; all other variables tested by linear regression analysis with the presence of obesity as an independent variable and age and gender as covariates (except for FEV₁ %predicted); N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI, body mass index; ICS, inhaled corticosteroids; FEV₁, forced expiratory volume in 1 s; AMP, adenosine 5'-monophosphate.

Bold values are significantly different between obese and lean patients.

Data pooling

We collected the following available data: BMI, FEV₁, PC₂₀ methacholine/histamine, PC₂₀ adenosine 5'-monophosphate (AMP) and inflammatory cell differential counts in sputum and blood. We pooled individual patient data from all four studies, and duplicates were removed. Five patients were excluded because either their weight or height could not be retrieved. The corticosteroid treatment response in obese and nonobese patients was analyzed in study 1. In this study, all 118 patients were treated with either 500 μg/day fluticasone, 2000 μg/day fluticasone or 30 mg/day prednisolone for 2 weeks. More information about the design of this study can be found in the original publication or in the Appendix S1 and Figure S1 (22).

Measurement of lung function and bronchial hyper-responsiveness

In all four studies, spirometry was performed according to international guidelines, and reference values were obtained from the study of Quanjer et al (27). Provocation tests were performed with a 2-min tidal-breathing method. Patients inhaled increasing concentrations of a direct stimulus, either

histamine (0.03–32 mg/ml) or methacholine (0.03–16 mg/ml), or the indirect stimulus AMP (0.04–320 mg/ml). The challenge was discontinued when FEV₁ had fallen by 20% or more from the prechallenge level or when the highest concentration had been administered. PC₂₀ was calculated by linear interpolation between the last two data points of the logarithmic concentration–response curve. If a patient responded to an initial saline challenge or the lowest concentration, half of the lowest concentration was used as PC₂₀ value. If a patient did not respond at the highest concentration, twice the highest concentration was used as the PC₂₀ value (25). The results from the provocation tests with histamine and methacholine were analyzed together, because PC₂₀ values for histamine and methacholine are equivalent on a mg-for-mg basis (28). All calculations of PC₂₀ were performed with a base-2 logarithm, as this reflects doubling concentrations. We also calculated the dose–response slope. This was calculated as the percentage fall in FEV₁ per concentration of either methacholine or histamine or AMP.

Sputum induction and processing

Sputum induction was performed following a standard protocol. Fifteen minutes after inhalation of 200 μg salbutamol,

hypertonic saline (3%, 4%, and 5%) was nebulized with an ultrasonic nebulizer (Ultraneb 2000, DeVilbiss, Somerset, PA, USA) and inhaled for 7 min. The output of the nebulizer was calibrated at 1.5 ml/min. After each concentration, patients were encouraged to cough and expectorate sputum. All sputum inductions and sputum processings were performed in the same laboratory according to standard operating procedures that were the same in all studies. Whole samples were processed according to the method of Fahy et al. with some modifications (29). An equal volume of dithiothreitol 0.1% (Sputalysin 10%, Behring Diagnostics Inc., Sommerville, NY, USA) was added to the weight of the sputum and after 15 min filtered through a nylon (48 µm) gauze. A haematocytometer was used to count the total cell number, viability, and squamous epithelial contamination of the cell suspension. The sputum sample was centrifuged (10 min, 450 g, 4°C). The cell pellet was resuspended in phosphate-buffered saline and cytopins were stained with May–Grünwald–Giemsa. At least 200 nonsquamous cells were separately counted by an investigator blinded to any personal data. Samples with a contamination of >80% squamous cells were excluded from analyses.

Statistical analysis

Inflammatory cell counts in sputum and blood were log-transformed to normalize their distribution. We investigated the effect of BMI as a discrete variable, that is, BMI ≥ 30 kg/m² (obese) vs BMI < 25 kg/m² (lean), and as a continuous variable. To test for differences in age, gender, smoking status and inhaled corticosteroid use at baseline between obese and nonobese patients, we used Fisher's exact tests for categorical variables and Mann–Whitney *U*-tests for continuous variables. To test for baseline differences in lung function, bronchial hyper-responsiveness and inflammatory cells, we performed a linear regression analysis with the presence of obesity as an independent variable and age and gender as covariates. Effects of increasing BMI on baseline values of lung function, bronchial hyper-responsiveness and inflammatory cells were performed by similar regression analyses with BMI (continuous) as an independent variable. To test for differences in a variable after treatment, we performed a linear regression analysis on the change in this variable with the presence of obesity or continuous BMI as an independent variable and age, gender, and type of treatment (inhaled or oral corticosteroids) as covariates. In all analyses of treatment response, we also included the baseline value of the variable as a covariate, to correct for baseline differences. To assess whether our results would be different when using other cutoff points for BMI, we repeated our analyses with overweight patients, thus obese (BMI ≥ 30 kg/m²) and nonobese patients (BMI < 30 kg/m²). Also, we repeated our analyses only in men or women and without current smokers, to investigate whether gender or smoking status would affect our results. We corrected for age, gender, and height in all analysis in which absolute values of FEV₁ were used. We did not correct for age and gender in regression analyses in which FEV₁ %predicted was

used, because the predicted value already includes these covariates. A *P*-value of ≤ 0.05 was considered statistically significant.

Results

Baseline differences in obese and lean asthma patients

We included 423 asthma patients in our study. Median BMI was 24.9 kg/m² (range 16.9–44.5 kg/m²). A total of 63 patients were obese, 147 patients were overweight and 213 patients were lean. Obese patients were older than lean patients (45 vs 29 years respectively, *P* < 0.001, Table 2), and obese patients were more frequently female (65%) than lean patients (51%). The proportion of patients using inhaled corticosteroids at baseline was similar in the obese and lean patients. Smoking status was also similar in the two groups.

Clinical variables such as FEV₁, PC₂₀ methacholine/histamine and PC₂₀ AMP did not differ significantly between obese and lean patients at baseline. We found a positive association between BMI and FEV₁ %predicted, with an increase of 0.5% in FEV₁ for every kg/m² increase in BMI (*P* = 0.02, Table 3). Obese asthma patients had higher blood neutrophil cell counts (4.2 vs 3.4 $\times 10^3/\mu\text{l}$, *P* < 0.001) than lean asthma patients. There was also a trend for higher percentages of sputum neutrophils in obese patients (52% vs 36%, *P* = 0.095), whereas values for eosinophils were similar between obese and lean

Table 3 Associations between baseline characteristics and BMI

	<i>b</i>	<i>P</i> -value
FEV ₁ prebronchodilator (L)	0.012 <i>N</i> = 359	0.2
FEV ₁ %predicted pre bronchodilator (%)	0.487 <i>N</i> = 359	0.02
PC ₂₀ methacholine/histamine (doubling concentrations)	0.034 <i>N</i> = 278	0.3
PC ₂₀ AMP (doubling concentrations)	0.006 <i>N</i> = 278	0.9
AMP dose–response slope (%/mg/ml)*	−0.022 <i>N</i> = 276	0.5
Blood eosinophils (10 ³ /µl)*	0.001 <i>N</i> = 273	0.6
Sputum eosinophils (%)*	−0.011 <i>N</i> = 239	0.5
Blood neutrophils (10 ³ /µl)*	0.015 <i>N</i> = 273	<0.001
Sputum neutrophils (%)*	0.006 <i>N</i> = 239	0.4

Linear regression with outcome parameter as dependent variable and BMI as independent variable and age and gender as covariates (except for FEV₁ % predicted); *N* (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI, body mass index; *b*, regression coefficients; FEV₁, forced expiratory volume in 1 s; AMP, adenosine 5'-monophosphate.

*Variable log-transformed.

Bold values are significantly associated with BMI.

patients. BMI was positively correlated with blood neutrophils ($b = 0.015$, $P < 0.001$). The increased number of blood neutrophils was only present in female, but not in male obese patients (4.3 vs $3.2 \times 10^3/\mu\text{l}$ in female obese vs female lean patients and 4.0 vs $3.5 \times 10^3/\mu\text{l}$ in male obese vs male lean patients, Fig. 1 and Table S1). Furthermore, there was a trend for an interaction between obesity and gender on blood neutrophil counts ($P = 0.09$).

The results were similar when repeating our analyses also including overweight patients (Table S2). Because smoking is known to affect neutrophilia, we also performed these analyses without the current smokers and the results remained similar (Table S3).

Response to corticosteroids

Forced expiratory volume in 1 s %predicted improved less in obese than in lean asthma patients (median 1.7% vs 6.3%, $P = 0.04$, Table 4). Absolute FEV₁ appeared to also improve less in obese patients (50 ml vs 220 ml); however, this was not statistically significant. When BMI was analyzed as a continuous variable, FEV₁%predicted also tended to improve less with increasing BMI (0.4% less improvement for each kg/m² increase in BMI, $P = 0.08$, Table S4).

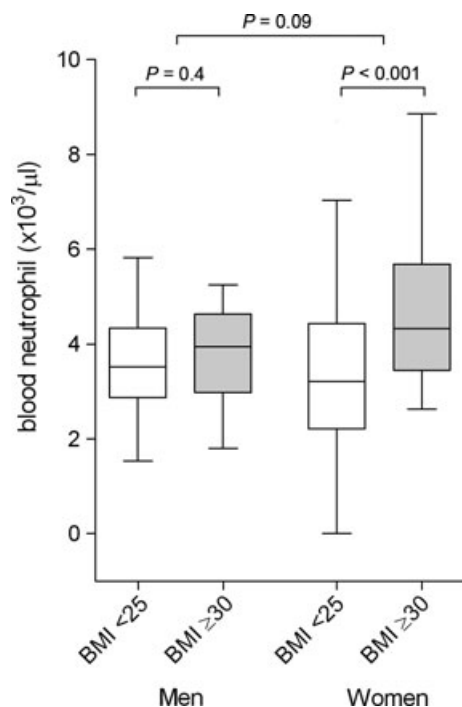


Figure 1 Blood neutrophils in obese and lean patients by gender. Differences between obese and nonobese patients tested with linear regression with blood neutrophils (log-transformed) as dependent variable and BMI as independent variable and age and gender as covariates. Difference between men and women tested with linear regression with an interaction term for gender and obesity; BMI, body mass index.

Although no significant differences in corticosteroid-induced improvement in PC₂₀ methacholine or PC₂₀ AMP were found between obese and lean patients, there was a trend for less improvement in the methacholine dose-response slope in obese patients ($P = 0.06$, Table 4). Blood neutrophil numbers increased significantly less with corticosteroids in obese than in lean patients. Furthermore, we found a smaller corticosteroid-induced reduction in the percentage of sputum eosinophils in obese than in lean asthmatics (-0.7% vs -4.3% , respectively), but the difference was not statistically significant after correction for baseline values. When analyzing BMI as a continuous variable, the percentage sputum eosinophils improved less with increasing BMI ($b = 0.04$, $P = 0.03$, Fig. 2). In the analyses also including overweight patients, the results were similar (Table S5). In the analyses without current smokers, the smaller improvement in FEV₁%predicted in obese patients was no longer present (Table S6). Our sample size was not large enough to test for gender differences in corticosteroid treatment response.

Discussion

The results of our study show that the severity of airway obstruction and bronchial hyper-responsiveness is similar in obese and lean asthma patients. Interestingly, obese asthma patients have a higher level of neutrophilic inflammation, as reflected by both a higher percentage of sputum neutrophils and increased blood neutrophil counts. This increase in neutrophils is only seen in female, obese asthmatics and not in male, obese asthmatics. Finally, obese asthma patients have a blunted corticosteroid treatment response compared to lean patients.

Our finding of a higher level of neutrophilic inflammation in sputum and blood in obese asthma patients may help to explain the reduced corticosteroid treatment response in obese asthma patients (30–32). In our study, a higher percentage of sputum neutrophils at baseline was also correlated with a lower improvement in FEV₁%predicted ($r = -0.22$, $P = 0.007$, Figure S2).

In agreement with our findings, Scott et al. also found that obese asthma patients have a higher percentage of sputum neutrophils than nonobese patients and that BMI and sputum neutrophils are positively correlated in females (33). The findings by Halder and colleagues further strengthen the notion that obesity is associated with neutrophilic inflammation and gender (17). They identified an asthma phenotype that was characterized by obesity and noneosinophilic inflammation. These patients were mostly female and had high percentages of sputum neutrophils, low percentages of sputum eosinophils, and a high level of symptoms. A possible explanation for the increased neutrophilic inflammation in obese asthma patients may be that adipose tissue produces several pro-inflammatory mediators such as leptin, TNF α , and IL-6, also called adipokines (13, 34). This chronic low-grade systemic inflammation, as reflected by leukocytosis (10, 16) and increased serum levels of C-reactive protein (11, 12), may theoretically affect local inflammation in asthmatic airways,

Table 4 Changes with corticosteroid treatment in obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients* (option 2)

	BMI < 25 (N = 70)			BMI ≥ 30 (N = 19)			P-value
	Before	After	Difference	Before	After	Difference	
Age (years)	25 (21–29)			38 (35–45)			
Males n (%)	20 (29)			7 (37)			
Use of ICS at baseline n (%)	1 (1)			2 (11)			
Current smoking n (%)	19 (29)			6 (32)			
Treatment (FP500/FP2000/P30)	21/21/28			8/7/4			
FEV ₁ (ml)	2965 (2543–3420)	3180 (2850–3615)	220 (0–515)	2550 (2050–2750)	2600 (1930–3080)	50 (–80–300)	0.3
FEV ₁ (%predicted)	82.1 (68.8–95.2)	88.7 (78.1–98.0)	6.3 (0.1–13.0)	77.9 (64.1–87.5)	77.5 (64.7–93.0)	1.7 (–2.8–9.0)	0.04
PC ₂₀ methacholine (doubling concentrations)	–1.7 (–2.9–0.3)	0.3 (–0.6–1.7)	4.2 (0.7–6.0)	–1.6 (–2.8–0.5)	–0.7 (–2.2–1.5)	1.4 (–0.2–3.0)	0.1
Methacholine dose–response slope (%/mg/ml)†	3.83 (2.58–4.71)	2.72 (1.74–3.34)	–0.99 (–1.84 to –0.40)	3.83 (2.39–4.91)	3.17 (2.41–4.49)	–0.42 (–2.16 to –0.02)	0.06
PC ₂₀ AMP (doubling concentrations)	1.9 (0.0–3.7)	6.4 (3.5–9.3)	4.2 (0.7–6.0)	1.6 (0.0–6.1)	6.7 (0.4–9.3)	1.3 (0.0–6.6)	0.5
AMP dose–response slope (%/mg/ml)†	1.47 (0.23–2.72)	–1.65 (–2.47–0.70)	–2.76 (–3.63 to –0.97)	1.18 (–1.30 to 2.77)	–1.46 (–2.62–2.61)	–0.66 (–3.87–0.79)	0.1
Blood eosinophils (10 ³ /μl)	0.34 (0.25–0.51)	0.20 (0.12–0.35)	–0.12 (–0.28 to –0.02)	0.27 (0.13–0.50)	0.20 (0.14–0.34)	–0.03 (–0.24 to –0.01)	0.6
Sputum eosinophils (%)	6.0 (2.2–12.6)	0.5 (0.0–2.3)	–4.3 (–11.3–1.0)	2.5 (0.5–7.5)	0.8 (0.2–2.2)	–0.7 (–4.3–0.0)	0.2
Blood neutrophils (10 ³ /μl)	3.42 (2.84–4.55)	4.04 (2.89–5.45)	0.57 (–0.28–1.38)	4.48 (3.77–6.34)	4.89 (4.03–5.62)	0.32 (–0.36–1.29)	0.02
Sputum neutrophils (%)	29.2 (21.0–45.1)	34.4 (18.5–48.4)	0.8 (–10.9–12.0)	52.2 (42.5–71.1)	45.8 (34.3–70.5)	–3.2 (–18.9–2.9)	0.8

*All values are presented as medians with interquartile range, unless stated otherwise.

†Variable log-transformed. Treatment induced differences between the two groups (BMI < 25 kg/m² and BMI ≥ 30 kg/m²) tested by linear regression with change in outcome parameter as dependent variable and BMI as independent variable and age, gender (except FEV₁ % predicted), and type of treatment as covariates; BMI, body mass index; FP500, fluticasone propionate 500 μg/day; FP2000, fluticasone propionate 2000 μg/day; P30, prednisolone 30 mg/day; FEV₁, forced expiratory volume in 1 s; AMP, adenosine 5'-monophosphate. Bold values are significantly associated with BMI.

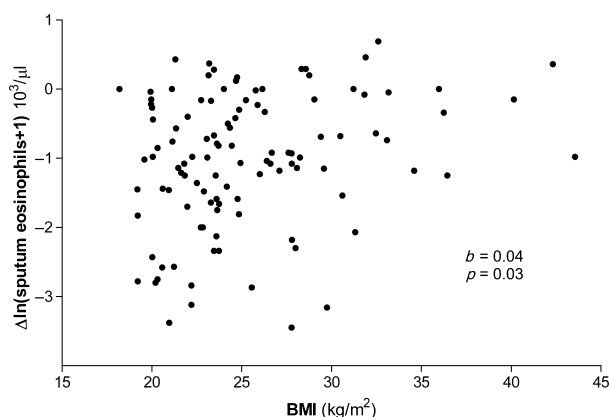


Figure 2 Association between change in sputum eosinophils with corticosteroids treatment and BMI. Linear regression with change in sputum eosinophils with corticosteroid treatment (log-transformed) as dependent variable and BMI as independent variable and age, gender, and type of treatment as covariates; BMI, body mass index.

leading to more neutrophilic instead of eosinophilic inflammation. The question then is how do these increased levels of adipokines induce neutrophilia in asthma. Monocytes release $\text{TNF}\alpha$ after stimulation with leptin, which then activates human neutrophils (35). $\text{TNF}\alpha$ could also be responsible for the recruitment of neutrophils to the airways, because increased expression of genes from the $\text{TNF}\alpha$ pathway is associated with increased neutrophilic inflammation in induced sputum of asthma patients (36). We observed that the neutrophilic inflammation is increased primarily in women. This may be explained by the difference in body composition between men and women, women having mostly subcutaneous adipose tissue, which is more metabolic active than intra-abdominal adipose tissue (37). For instance, it secretes two to three times more leptin than intra-abdominal adipose tissue (38). This leads to higher levels of plasma leptin in obese women than in men, which in turn may lead to the increased neutrophilic inflammation (39).

We found a smaller $\text{FEV}_1\%$ predicted improvement in obese than in lean asthma patients after 2 weeks of treatment with corticosteroids. A reduced corticosteroid treatment response in obese asthmatics has been found previously. Peters-Golden et al. showed that obese asthma patients have a lower number of days with total- or well-controlled asthma than nonobese patients after treatment with beclomethasone (5). Furthermore, obese patients are less likely to achieve well-controlled asthma after a 12-week treatment with fluticasone, or fluticasone and salmeterol (6). Importantly, all abovementioned studies observed less asthma control in obese patients, yet not less improvement in FEV_1 . Two other studies showed a lower improvement in FEV_1 in obese than in lean patients after treatment with inhaled corticosteroids and a long-acting β -agonist (7, 9). Together, the balance of evidence suggests that obese asthma patients respond less well to treatment with corticosteroids with regard to improvement in asthma control and lung function.

Obese and lean asthmatics had comparable $\text{FEV}_1\%$ predicted levels at baseline in our study. The effect of obesity on lung function in asthma has been a matter of controversy. An association between obesity and lower FEV_1 has been observed in some studies (9, 20, 21, 40) but not in others (5, 7, 8, 18, 19). A possible explanation for these discrepant observations could be that inhaled corticosteroids were discontinued prior to inclusion in most studies that did not show a difference in FEV_1 in obese vs nonobese asthmatics (5, 7, 19), whereas this was not the case in those studies that did find a lower FEV_1 in obese asthma patients (9, 20, 21, 40). Based on our findings, we hypothesize that the lower FEV_1 in obese asthma patients in those studies that allowed continuation of corticosteroids actually reflects a lack of corticosteroid treatment response in obese compared to nonobese asthmatics.

The strength of our study is that we were able to investigate obese and lean asthma patients who were extensively characterized before and after corticosteroid treatment, including lung function, bronchial hyper-responsiveness to both a direct and indirect stimulus, and sputum induction. However, there are also some limitations. Our study was a pooled, *post hoc* analysis of clinical studies, and patients were treated with different types of corticosteroids, that is, oral and inhaled. These studies were not designed to investigate differences between obese and lean patients, and obese asthma patients were somewhat older and more often females. For this reason, we corrected for age, gender, and corticosteroid type (i.e., oral or inhaled corticosteroids) in all our analyses. Finally, we did not include healthy controls in our study. It remains an open question whether the increased neutrophilic inflammation in blood and sputum is also present in healthy obese subjects or whether this is specific for obese asthma patients.

In conclusion, the results of our study show that obese asthma patients have a distinct phenotype of asthma that is characterized by a higher level of neutrophilic inflammation in sputum and blood. Especially, obese female asthma patients show this increased neutrophilic inflammation. The increased neutrophilic inflammation may help to explain why obese asthma patients respond less to corticosteroid treatment.

Acknowledgments

Author's contributions: Eef Telenga performed the analysis and wrote the manuscript. Saskia Tideman collected the data, performed the analyses, and wrote the manuscript. Huib Kerstjens performed study 1, coordinated study 2, debated data analyses, edited the manuscript, and approved the final version. Nick ten Hacken coordinated study 3, debated data analyses, edited the manuscript, and approved the final version. Wim Timens coordinated study 3, debated data analyses, edited the manuscript, and approved the final version. Dirkje Postma coordinated studies 1, 2, 3, and 4, debated data analyses, edited the manuscript, and approved the final version. Maarten van den Berge performed study 4, debated data analyses, and wrote and approved the manuscript.

Study 1 was supported by a government grant from the Netherlands Health Research Promotion Program. Studies 2 and 4 were financially supported by GlaxoSmithKline. Study 3 was supported by the Netherlands Asthma Foundation (Astmafonds). Study 1 was a multicenter study of the Dutch CNSLD Study Group consisting of the following people and institutions: Departments of Pulmonology: Academic Medical Centre, Amsterdam (D.F.M.E. Schoonbrood, E.M. Pouw, C.M. Roos, and H.M. Jansen); University Medical Center Groningen, Groningen (H.A.M. Kerstjens, P.L.P. Brand, D.S. Postma, H.J. Sluiter[†], H.J. A. de Gooyer, Th.W. van der Mark, and G.H. Koëter); Leiden University Medical Center (P.M. de Jong, P.J. Sterk, A.M.J. Wever, and J.H. Dijkman[†]); University Medical Center St. Radboud, Nijmegen (P.N.R. Dekhuijzen, H. Folgering, and C.L.A. van Herwaarden); Erasmus Medical Center, Rotterdam (S.E. Overbeek, J.M. Bogaard, and C. Hilvering); and University Medical Center Utrecht, Utrecht (H.J.J. Mengelers[†], S.J. Gans, B. v.d. Bruggen, and J. Kreukniet[†]); Departments of Pediatric Pulmonology: Sophia Children's Hospital, Rotterdam (E.E.M. van Essen-Zandvliet, and K.F. Kerrebijn[†]); Juliana Children's Hospital, The Hague (E.J. Duiverman, J. M. Kouwenberg, and J.E. Prinsen); and Beatrix Children's Hospital, Groningen (H.J. Waalkens, J. Gerritsen, and K. Knol[†]); Department of Allergology: University Medical Center Groningen, Groningen (J.G.R. de Monchy); Department of General Practice: University of Leiden (A.A. Kaptein and F.W. Dekker); Department of Physiology: University of Leiden (P.J.F.M. Merkus and Ph.H. Quanjer); Scientific counsel: Department of Epidemiology and Population Sciences, Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London (M.D. Hughes, N.J. Robinson, S.J. Pocock); and Department of Medicine, Johns Hopkins.

Conflicts of interest

HK has received funding from the following manufacturers of inhaled corticosteroids: GlaxoSmithKline, the manufacturer of beclomethasone and fluticasone; AstraZeneca, the manufacturer of budesonide; and Nycomed, the manufacturer of ciclesonide.

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NtH received funding for research from Boehringer Ingelheim, GSK, AstraZeneca, Nycomed, and Chiesi. He has been a consultant to Chiesi.

DP received funding for research from AstraZeneca, GSK, and Nycomed. Travel to ERS or ATS has been partially funded by AstraZeneca, GSK, Chiesi, and Nycomed. She has been a consultant to AstraZeneca, Boehringer Ingelheim, Chiesi, GSK, Nycomed, and TEVA.

MB has received a research grant from GlaxoSmithKline in 2009.

All other authors have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Flow chart of study 1.

Figure S2. Monovariate correlation between baseline sputum neutrophils and change in FEV₁ %predicted with corticosteroid treatment.

Table S1. Baseline characteristics of obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients in men and women.

Table S2. Baseline characteristics for obese and nonobese patients.

Table S3. Baseline characteristics of obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients without current-smokers.

Table S4. Associations between corticosteroid treatment response and BMI.

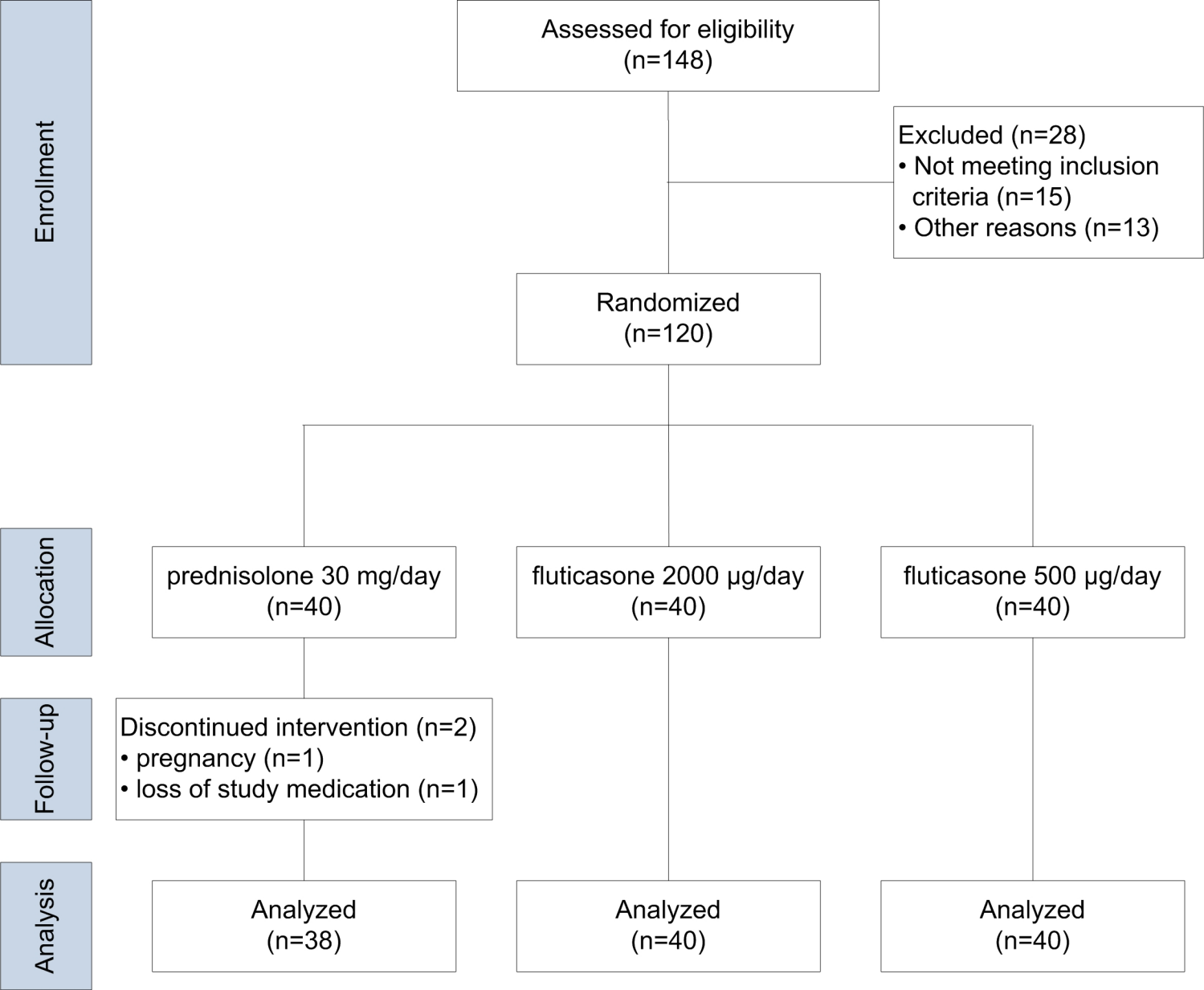
Table S5. Changes with corticosteroid treatment in obese and nonobese patients.

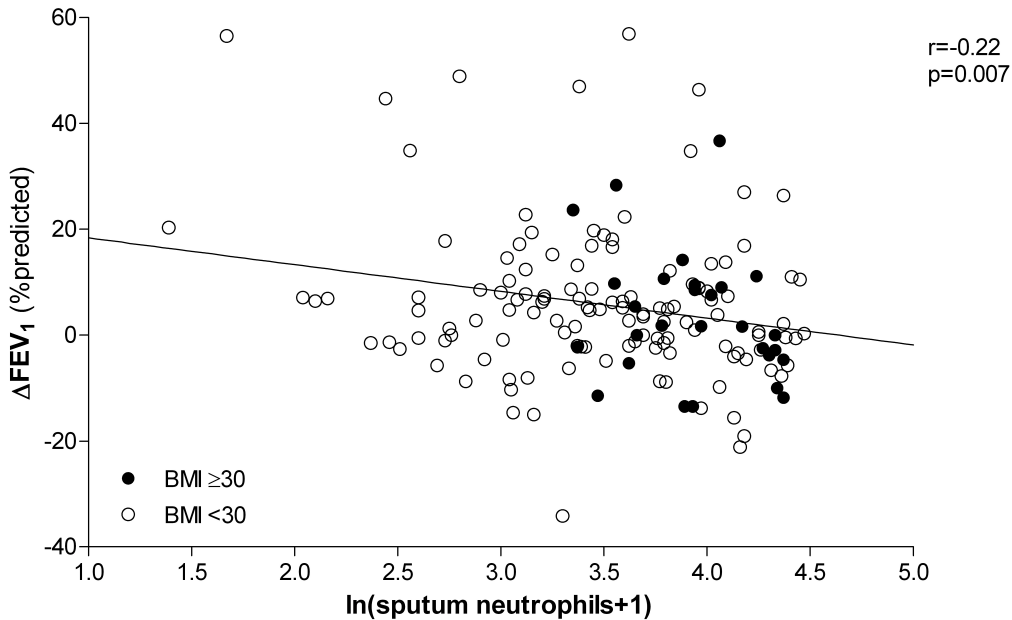
Table S6. Changes with corticosteroid treatment in obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients without smokers.

Appendix S1: Study design of study 1.

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Appendix 1: Study design of study 1

Study 1 was a 2-week, double-blind, 3 arm parallel group, double dummy study.

Patients, 18 to 56 years old, with a diagnosis of asthma were included if they met the following criteria: a PC₂₀ methacholine ≤ 8 mg/ml, at least one positive skin test out of 17 aero-allergens, reversibility to β_2 -agonist $\geq 9\%$ of the predicted FEV₁ and ability to expectorate sputum after hypertonic saline inhalation. The study took place at the out-patient clinic of the University Medical Center Groningen. Inhaled corticosteroids (ICS) were tapered and when possible discontinued completely at least 3 weeks prior to the randomization visit. When patients experienced a worsening of their asthma before complete discontinuation of ICS for 3 weeks, they were asked to visit the hospital to be earlier randomized for inclusion. Patients experiencing an exacerbations of their asthma necessitating a prednisolone course were not randomized. Randomization was performed by a computerized minimization method with stratification according to age, previous dose of ICS, FEV₁ %predicted, reversibility after β_2 -agonist, smoking status, serum IgE and PC₂₀ methacholine. Patients were treated with either oral prednisolone (30 mg/day), fluticasone propionate 2000 μ g/day or fluticasone propionate 500 μ g/day, both via Diskhaler dry powder inhalation.

Online supplementary figure 1: Flow chart of study 1

Online supplementary figure 2: Monovariate correlation between baseline sputum neutrophils and change in FEV₁ %predicted with corticosteroid treatment

Overall Pearson correlation = -0.22, p=0.007; BMI <30 kg/m² Pearson correlation = -0.17, p=0.06; BMI ≥30 kg/m² Pearson correlation = -0.28, p=0.14; BMI = body mass index.

Online supplementary table 1: Baseline characteristics of obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients in men and women*

	Men			Women		
	BMI < 25 (N=105)	BMI ≥ 30 (N=22)	p-value	BMI < 25 (N=108)	BMI ≥ 30 (N=41)	p-value
Age (years)	35 (25 – 48)	47 (35 – 52)	0.01	26 (22 – 34)	45 (37 – 53)	< 0.001
Use of ICS at baseline n (%)	11 (11)	5 (23)	0.2	8 (7)	7 (17)	0.1
Current-smoking n (%)	20 (20)	3 (14)	0.8	22 (21)	7 (17)	0.7
	N=102					
FEV₁ pre bronchodilator (L)	2.9 (2.2 – 3.7)	2.6 (2.0 – 3.5)	0.4	2.7 (2.1 – 3.3)	2.4 (2.0)	0.2
	N=104					
FEV₁ %predicted pre bronchodilator	70 (56 – 81)	67 (49 – 86)	0.5	85 (67 – 97)	88 (72 – 100)	0.2
	N=104					
PC₂₀ methacholine/histamine (mg/ml) [£]	0.24 (0.02 – 7.20)	0.31 (0.02 – 5.43)	0.6	0.27 (0.02 – 7.31)	0.38 (0.03 – 5.74)	0.9
	N=78	N=13		N=88	N=20	
PC₂₀ AMP (mg/ml) [£]	10.1 (0.02 – 640)	13.2 (0.02 – 640)	0.8	9.6 (0.02 – 640)	15.2 (0.02 – 640)	0.7
	N=49	N=16		N=75	N=34	
AMP dose response slope (%/mg/ml)	1.44 (0.25 – 7.97)	1.02 (0.03 – 10.83)	0.7	2.72 (0.28 – 8.42)	0.90 (0.06 – 9.37)	0.8
	N=49	N=15		N=75	N=34	
Blood eosinophils (10³/μL)	0.27 (0.22 – 0.46)	0.25 (0.18 – 0.36)	0.5	0.29 (0.15 – 0.43)	0.18 (0.12 – 0.30)	0.6
	N=49	N=16		N=73	N=33	
Sputum eosinophils (%)	3.4 (1.1 – 13.7)	1.4 (0.2 – 6.7)	0.4	3.3 (0.7 – 7.3)	0.5 (0.2 – 3.1)	0.4
	N=46	N=12		N=69	N=33	
Blood neutrophils (10³/μL)	3.5 (2.9 – 4.3)	4.0 (3.0 – 4.6)	0.4	3.2 (2.2 – 4.4)	4.3 (3.4 – 5.7)	< 0.001
	N=49	N=16		N=73	N=33	
Sputum neutrophils (%)	46 (25 – 65)	50 (38 – 68)	0.6	32 (21 – 55)	53 (40 – 71)	0.02
	N=46	N=12		N=69	N=28	

Online supplementary table 2: Baseline characteristics for obese and non-obese patients*

	BMI <30 (N=360)	BMI ≥30 (N=63)	p-value
Age (years)	35 (25 – 48)	45 (36 – 52)	<0.001 [#]
Males n (%)	181 (50)	22 (35)	0.03 [‡]
Use of ICS at baseline n (%)	43 (12)	12 (19)	0.2 [‡]
Current-smoking n (%)	71 (20)	10 (16)	0.5 [‡]
	N=350		
FEV₁ pre bronchodilator (L)	2.8 (2.2 – 3.5)	2.5 (2.0 – 3.0)	0.5
	N=359		
FEV₁ %predicted pre bronchodilator	79 (65 – 93)	84 (67 – 98)	0.7
	N=359		
PC₂₀ methacholine/ histamine (mg/ml) [£]	0.27 (0.01 – 16)	0.35 (0.02 – 5.74)	0.5
	N=245	N=33	
PC₂₀ AMP (mg/ml) [£]	16.9 (0.02 – 640)	14.5 (0.02 – 640)	0.2
	N=228	N=50	
AMP dose response slope (%/mg/ml)	1.20 (0.13 – 5.34)	1.02 (0.04 – 9.77)	0.5
	N=227	N=49	
Blood eosinophils (10³/μL)	0.22 (0.14 – 0.39)	0.21 (0.13 – 0.33)	0.6
	N=223	N=49	
Sputum eosinophils (%)	2.5 (0.7 – 6.8)	0.5 (0.2 – 4.9)	0.2
	N=199	N=40	
Blood neutrophils (10³/μL)	3.3 (2.7 – 4.3)	4.2 (3.3 – 5.0)	<0.001
	N=224	N=49	
Sputum neutrophils (%)	40 (24 – 63)	52 (39 – 70)	0.04
	N=199	N=40	

*All values are presented as median with interquartile range unless stated otherwise; £ geometric mean with range; # tested by Mann-Whitney U test; ‡ tested by Fisher's exact test; all other variables tested by linear regression analysis with the presence of obesity as an independent variable and age and gender as covariates (except for FEV₁ % predicted); N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI = body mass index; ICS = inhaled corticosteroids; FEV₁ = forced expiratory volume in one second; AMP = adenosine 5'-monophosphate.

Online supplementary table 3: Baseline characteristics of obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients without current-smokers*

	BMI <25 (N=165)	BMI ≥ 30 (N=53)	p-value
Age (years)	30 (24 – 44)	45 (35 – 53)	<0.001 [#]
Males n (%)	82 (50)	19 (36)	0.08 [‡]
Use of ICS at baseline n (%)	18 (11)	12 (23)	0.04 [‡]
FEV₁ pre bronchodilator (L)	2.8 (2.1 – 3.5) N=164	2.4 (2.0 – 3.0)	0.9
FEV₁ %predicted pre bronchodilator	76 (61 – 92) N=164	84 (66 – 98)	0.3
PC₂₀ methacholine/ histamine (mg/ml) [£]	0.25 (0.02 – 7.31) N=130	0.31 (0.02 – 5.74) N=26	0.7
PC₂₀ AMP (mg/ml) [£]	10.9 (0.02 – 640) N=89	11.70 (0.02 – 640) N=41	0.5
AMP dose response slope (%/mg/ml)	2.19 (0.25 – 6.56) N=89	1.01 (0.04 – 11.31) N=40	0.8
Blood eosinophils (10 ³ /μL)	0.26 (0.15 – 0.42) N=88	0.24 (0.12 – 0.36) N=40	0.7
Sputum eosinophils (%)	2.8 (1.0 – 7.7) N=84	0.5 (0.2 – 5.2) N=32	0.7
Blood neutrophils (10 ³ /μL)	3.2 (2.4 – 4.1) N=88	4.0 (3.3 – 4.8) N=40	0.001
Sputum neutrophils (%)	34 (21 – 57) N=84	52 (38 – 68) N=32	0.1

*All values are presented as median with interquartile range unless stated otherwise; £ geometric mean with range; # tested by Mann-Whitney U test; ‡ tested by Fisher's exact test; all other variables tested by linear regression analysis with the presence of obesity as an independent variable and age and gender as covariates (except for FEV₁ % predicted); N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI = body mass index; ICS = inhaled corticosteroids; FEV₁ = forced expiratory volume in one second; AMP = adenosine 5'-monophosphate.

Online supplementary table 4: Associations between corticosteroid treatment response and BMI

	b	p-value
ΔFEV₁ (mL)	-5.121	0.6
	N=115	
ΔFEV₁ (% predicted)	-0.405	0.08
	N=114	
ΔPC₂₀ methacholine/histamine (doubling concentrations)	-0.036	0.3
	N=110	
ΔMethacholine dose response slope (%/mg/ml) *	0.033	0.2
	N=105	
ΔPC₂₀ AMP (doubling concentrations)	-0.045	0.5
	N=107	
ΔAMP dose response slope (%/mg/ml)	0.044	0.2
	N=104	
ΔBlood eosinophils (10³/μL) *	0.005	0.99
	N=106	
ΔSputum eosinophils (%) *	0.042	0.03
	N=110	
ΔBlood neutrophils (10³/μL) *	0.011	0.08
	N=108	
ΔSputum neutrophils (%) *	0.011	0.4
	N=111	

Linear regression with change in outcome parameter as dependent variable and BMI as independent variable and age, gender (except FEV₁ % predicted) and type of treatment as covariates; N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI = body mass index; b = regression coefficients; FEV₁ = forced expiratory volume in one second; AMP = adenosine 5'-monophosphate; * variable log transformed.

Online supplementary table 5: Changes with corticosteroid treatment in obese and non-obese patients *

	BMI <30 (N=99)	BMI ≥30 (N=19)	p-value
Type of treatment (n)			
fluticasone 500 µg/day (DPI), n(%)	32 (32)	8 (42)	
fluticasone 2000 µg/day (DPI), n(%)	33 (33)	7 (37)	
prednisolone 30 mg/day, n(%)	34 (35)	4 (21)	
ΔFEV₁ (mL)	210 (-15 – 560) N=96	50 (-80 – 300)	0.3
ΔFEV₁ (% predicted)	6.2 (-0.5 – 13.2) N=95	1.7 (-2.8 – 9.0)	0.052
ΔPC₂₀ methacholine/ histamine (doubling concentrations)	1.5 (0.6 – 2.4) N=93	1.4 (-0.2 – 3.0) N=17	0.2
ΔMethacholine dose response slope (%/mg/ml) #	-0.99 (-1.61 – -0.42) N=90	-0.42 (-0.216 – -0.02) N=15	0.07
ΔPC₂₀ AMP (doubling concentrations)	3.3 (0.6 – 5.6) N=90	1.3 (0.0 – 6.6) N=17	0.6
ΔAMP dose response slope (%/mg/ml) #	-2.22 (-3.47 – -0.49) N=87	-0.66 (-3.87 – 0.79) N=16	0.1
ΔBlood eosinophils (10³/µL)	-0.11 (-0.27 – 0.00) N=87	-0.03 (-0.24 – -0.01) N=19	0.5
ΔSputum eosinophils (%)	-3.8 (-11.3 – 1.3) N=93	-0.7 (-4.3 – 0.0) N=17	0.1
ΔBlood neutrophils (10³/µL)	0.58 (-0.26 – 1.18) N=89	0.32 (-0.36 – 1.29) N=19	0.046
ΔSputum neutrophils (%)	0.8 (-11.2 – 13.4) N=94	-3.2 (-18.9 – 2.9) N=17	0.8

*All values are presented as median with interquartile range unless stated otherwise; # Variable log transformed. Differences tested by linear regression with change in outcome parameter as dependent variable and BMI as independent variable and age, gender (except FEV₁ % predicted) and type of treatment as covariates; N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI = body mass index; ICS = inhaled corticosteroids; FEV₁ = forced expiratory volume in one second; AMP = adenosine 5'-monophosphate.

Online supplementary table 6: Changes with corticosteroid treatment in obese (BMI ≥ 30 kg/m²) and lean (BMI < 25 kg/m²) patients without smokers *

	BMI <25 (N=47)	BMI ≥ 30 (N=13)	p-value
Type of treatment (n)			
fluticasone 500 µg/day (DPI), n(%)	17 (36)	6 (46)	
fluticasone 2000 µg/day (DPI), n(%)	13 (28)	4 (31)	
prednisolone 30 mg/day, n(%)	17 (36)	3 (23)	
ΔFEV₁ (mL)	220 (0 – 450)	220 (-25 – 495)	0.5
ΔFEV₁ (% predicted)	5.9 (0.4 – 16.6)	5.9 (0.8 – 12.7)	0.3
	N=46		
ΔPC₂₀ methacholine/ histamine (doubling concentrations)	1.5 (0.6 – 2.4)	1.5 (0.2 – 3.5)	0.3
	N=46	N=12	
ΔMethacholine dose response slope (%/mg/ml) #	-0.99 (-1.83 – -0.41)	-0.80 (-2.53 – -0.02)	0.3
	N=46	N=11	
ΔPC₂₀ AMP (doubling concentrations)	4.6 (0.7 – 6.0)	3.2 (0.0 – 7.7)	0.9
	N=45	N=12	
ΔAMP dose response slope (%/mg/ml) #	-3.16 (-3.67 – -1.19)	-1.38 (-4.37 – 2.24)	0.3
	N=44	N=11	
ΔBlood eosinophils (10 ³ /µL)	-0.12 (-0.33 – 0.00)	-0.08 (-0.28 – 0.00)	0.5
	N=42		
ΔSputum eosinophils (%)	-3.8 (-11.3 – 1.1)	-1.2 (-5.7 – 0.2)	0.5
	N=45	N=11	
ΔBlood neutrophils (10 ³ /µL)	0.56 (-0.27 – 1.10)	0.32 (-0.71 – 1.10)	0.03
	N=42	N=113	
ΔSputum neutrophils (%)	0.9 (-5.8 – 10.8)	-4.7 (-23.7 – 2.0)	0.5
	N=46	N=11	

*All values are presented as median with interquartile range unless stated otherwise;
Variable log transformed. Differences testes by linear regression with change in outcome parameter as dependent variable and BMI as independent variable and age, gender (except FEV₁ % predicted) and type of treatment as covariates; N (number of patients) is only given for variables that were not available in all individuals in the specific BMI group; BMI = body mass index; ICS = inhaled corticosteroids; FEV₁ = forced expiratory volume in one second; AMP = adenosine 5'-monophosphate.