Respiratory Medicine (2010) **104**, 1138–1144

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Mild asthma in overweight women: A new phenotype?

Marissa Engbers [a,b], Isabelle Vachier [a], Peter Sterk [b], Arnaud Bourdin [a], Delphine Gras [c], Philippe Godard [a], Pascal Chanez [a,c,*]

[a] *CHU-Montpellier, Respiratory Department, Hôpital Arnaud de Villeneuve, 371 av doyen Gaston Giraud, 34295 Montpellier Cx 5, France*
[b] *Amsterdam University Hospital, Amsterdam, The Netherlands*
[c] *Département des Maladies Respiratoires, AP-HM, Laboratoire d'immunologie INSERM CNRS U 600, UMR6212, Université de la Méditerranée, Marseille, France*

Received 7 April 2009; received in revised form 18 February 2010; accepted 19 February 2010
Available online 2 April 2010

KEYWORDS
Asthma;
Overweight;
Bronchial hyper responsiveness;
Inflammation

Summary
Background: Epidemics of asthma and overweight have been linked recently. They might be associated with systemic inflammation. In asthma hyperresponsiveness to adenosine (AMP) is more closely related to inflammation than to methacholine (MCh). The aim of the study was to determine responsiveness to AMP and MCh in overweight compared with normal weight asthmatics.
Methods: Thirty women were enrolled (19 overweight) with mild controlled asthma according to GINA. A Body Mass Index (BMI) less than 25 kg/m^2 was considered as normal and a BMI above 25 kg/m^2 as overweight. We assessed the recent control of asthma (ACQ), pulmonary function tests, bronchial responsiveness to MCh and AMP (PC_{20} and O'Connor two-point dose–response slope), perception of symptoms (Borg scale), and blood inflammatory markers (leptin and hs-CRP by ELISA).
Results: Overweight had a significant lower dose-response slope of the MCh challenge ($p = 0.009$) as compared to normal weight patients, whereas no significant difference was observed for AMP challenge ($p = 0.27$). Overweight patients had higher intercepts of the Borg scale measured before the MCh and AMP challenge tests ($p = 0.01$ and $p = 0.03$). Plasma leptin ($p = 0.001$) and hs-CRP ($p = 0.05$) concentrations were higher in overweight than normal weight patients. There was no correlation between challenges and inflammatory markers.

* Corresponding author. Université de la Méditerranée, AP-HM, 270 boulevard de Sainte-Marguerite, F-13009 Marseille, France. Tel.: +33 49174 46 30; fax: +33 49174 16 06.
E-mail address: pascal.chanez@univmed.fr (P. Chanez).

0954-6111/$ - see front matter
doi:10.1016/j.rmed.2010.02.016

Conclusions: Overweight asthmatic women have more pronounced systemic inflammation, but are less responsive to MCh. AMP responsiveness appeared to be comparable between both groups. Our findings suggest that overweight asthmatic women do not feature increased airway inflammation, but do represent a distinct phenotype as compared to normal weight patients.

Introduction

During the past two decades a simultaneous rise in prevalence of asthma and overweight has been reported in the developed countries.[1,2] This pattern has led to several correlation-studies in which the epidemiological association between obesity (Body mass index (BMI) over 30 kg/m^2) and the likelihood ratio of asthma has been confirmed. However, the relation between a higher BMI and bronchial hyperresponsiveness (BHR), an important feature of asthma, remains controversial in these studies.[3–8] The hypothesis that a rise in BMI is a state of chronic low-grade systemic inflammation is being supported by several studies. First, macrophage infiltration into the expanding adipose tissue, which participates to the inflammatory cascade, has been described.[9] Second, subjects with excess body fat demonstrate elevated levels of inflammatory mediators as C-reactive protein (CRP).[10–14] Moreover, leptin, a protein that plays a key role in adipocyte lipid storage, seems to fulfil a pro-inflammatory function.[15–17] More recently, it has been shown that obese with asthma had poorer asthma control than nonobeses,[11,18] despite similar symptom perception and expiratory flows.[14] Most research studies concerning asthma and a higher BMI include obese subjects (BMI $>$ 30 kg/m^2) and exclude the overweight group (BMI $>$ 25 kg/m^2), while the prevalence of overweight seems to be of more importance in the western countries.[19,20]

Two major pathophysiologic features of asthma are BHR and airway inflammation.[21] Nowadays, the most widely used method to assess the degree of BHR is the methacholine (MCh) challenge testing. MCh is a pharmacological analogue of acetylcholine that causes airflow limitation predominantly via a direct effect on the airway smooth muscle without intervening pathways. Unlike the MCh, Adenosine monophosphate (AMP) is an indirect pharmacological stimulant that binds to ''primed'' mast cells leading to degranulation and release of pro-inflammatory mediators, such as histamine and leukotrienes. Therefore, AMP challenges are considered to be more closely related to airway inflammation.[22,23] In most of the epidemiological studies, it has been reported that asthma was more significantly associated with obesity in women than in men. In one study, BMI was even related to asthma severity,[24] this finding was confirmed by the European cohort study of severe asthma (ENFUMOSA).[25] These data were sufficient to focus the present study on women with asthma with a variable BMI.

The primary hypothesis of this study was that overweight asthmatic women demonstrate more pronounced BHR when exposed to AMP as compared with normal weight asthma women. Secondarily, we hypothesized that hyperresponsiveness to AMP in overweight asthmatic women is related to plasma inflammatory markers.

Materials and methods

Subjects

Female subjects with asthma were recruited from out patient clinic of the Respiratory department of Montpellier hospital from June to December 2006. All subjects diagnosed with mild asthma according the Global Initiative for Asthma (GINA) classification had to fulfil the following inclusion criteria: aged between 30 and 75 years, non-smoking not pregnant, and baseline with FEV_1 over 65% of the predicted value (% pred).[26] Patients were using short-acting $ß_2$-agonist as needed only, and they were all steroid naïve. They were excluded when they reported an exacerbation during the last 6 months and when clinically significant co-morbidity was present. To avoid the possible confounder effect of gender differences only female subjects were included. Patients were divided into two groups according to their BMI: one with normal weight (BMI $\leq$ 25 kg/m^2) and one with overweight and obese subjects (BMI $>$ 25 kg/m^2). The study was approved by the local Ethic Review Committee and written informed consent was obtained from participating subjects.

Design

The study was conducted over two visits. When not menopausal, the first visit was planned in the first week after the menstruation. The Asthma Control Questionnaire (ACQ French version for France) was filled in by all the patients, and a general medical as well as asthma history was noted. A basic physical examination was performed, then after spirometry and lung volumes were measured. After blood samples were collected, the MCh provocation test was done. The second visit was perform 1 week after and consisted of the AMP provocation test. Before each visit, patients refrained from the asthma treatment for 48 h and caffeine containing beverages for 4 h.

Questionnaire

For each patient the score for the validated French version of the Juniper Asthma Control Questionnaire was calculated.[27] Subjects recalled their experiences during the previous 7 days and responded to each question using a 7-point scale (0–6). The items were equally weighed; the mean score for the seven questions was then calculated. A mean score of 0 indicated a very good control of asthma, whereas a score of 6 meant a poor control.

Pulmonary function tests

Preceding the first provocation test, spirometric measurements, including FEV_1 and forced vital capacity (FVC) were

recorded.[28] Total lung capacity (TLC), residual volume (RV) and functional residual capacity (FRC) were measured by body plethysmography (Sensor Medic Vmax series 229). The pulmonary function results were expressed as percentage of predicted normal values (% pred).

Inhalation challenge tests

MCh and AMP provocation tests were performed according to a standardized two minutes tidal breathing method.[29] MCh (chlorure MCh, Allerbio, France) and AMP (Sigma-Aldrich, France) solutions were administered to the patients in serial doubling concentrations ranging respectively from 0.03 to 8 mg/ml and 3.125 to 400 mg/ml, respectively. FEV_1 was measured 60 and 180 s after each dose. The provocation test was discontinued when FEV_1 felt more than 20% from baseline (PC_{20}), or if the highest concentration had been given. When PC_{20} was attained, 400 μg salbutamol was given to ensure that the FEV_1 returned to within 90% of the baseline value.

Borg scale

The severity of dyspnoea, during the challenge test, was assessed by the Borg scale at steady state and after inhalation of each dose of MCh or AMP.[30] All subjects were asked to rate the magnitude of their dyspnoea on a scale from 1 to 10. They were told that 1 represented no breathlessness at all while 10 represented the strongest feeling of breathlessness they had ever experienced.

Biochemical assay

Before starting with the first challenge test, blood was taken from each of the subjects by venipuncture. After centrifugation, serum was recovered and hs-CRP (high sensitivity) and leptin levels were measured by ELISA test. Briefly, serum samples were aliquoted and frozen at −80 °C. Leptin ELISA kit obtained from R&D system used a mouse monoclonal antibody and gave a sensitivity of 7.8 pg/ml. hs-CRP ELISA kit obtained from Immunobiological Laboratories used a mouse monoclonal antibody and gave a sensitivity of 10ng/ml.

Data analysis

BMI was considered as a categorical variable ($\leq$25 and $>$25 kg/m^2). The highest percentage decline of FEV_1 of the two measurements after each dose of stimulant (MCh and AMP) was used for analysis. The calibrated nebulizer output of 0.130 ml/min was used to calculate the cumulative dose for the 2 minutes tidal breathing method at each level of challenges. The two-point dose—response slope was calculated as previously described by O'Connor et al.[31] The index of dyspnoea was calculated according to the Borg scale value when 20% fall FEV_1 was reached as previously described.[32] Dyspnoea was assessed by Borg and VAS (Visual Analogic Scale), plotted against the percent fall in FEV1, and expressed as the slope of the regression line. Non-parametric statistical tests were used because of the non normal distribution. Difference between groups was analyzed by Mann—Whitney U-test. Correlation between data was assessed by non-parametric Spearman correlations test. Values are presented as median and interquartiles and significant differences were considered for $p < 0.05$. All data were analyzed using SPSS Statistics system.

Results

Subjects

A total of 30 individuals, 11 normal weight (BMI $\leq$ 25 kg/m^2) and 19 overweight and obese (BMI $>$ 25 kg/m^2) subjects completed the study protocol (median and 27—75 interquartile 22 (21—22) kg/m^2 and 32 (27—35) kg/m^2, respectively). Table 1 shows general characteristics of the study population. The mean period between the two provocation tests was 5.9 ± 9.4 days. No relation has been found between BMI and clinical outcomes at baseline.

Pulmonary function tests

The mean FEV_1 of the two groups was 96.7 ± 16.8% pred at baseline level, and did not differ significantly between the two groups (Table 1). Furthermore, no significant difference was found in FVC and TLC comparing the groups (Table 1). However, normal weight subjects had a significantly higher FRC (108% (85—122) pred) than the high BMI subjects (81% (39—80) pred, $p = 0.02$), and a lower FEV_1/FVC ratio (76% (72—79)% versus 81% (78—99), $p = 0.02$) (Table 1).

Inhalation challenge tests

All subjects reached PC20 for the MCh test but not for the AMP test. Accordingly we calculated the PC10, which tend to be significant for AMP challenge with $p = 0.07$ (80 (6—65.9) versus 4.9 (1.2—45.3) mg/ml) and PC10 for methacholine was not significant (0.6 (0.06—1.6) mg/ml vs 0.1 (0.08—0.7) mg/ml). Subjects with high BMI had a significantly lower dose—response slope for the MCh challenge (0.98 ± 0.7% fall/μmol and 0.42 ± 0.3% fall/μmol, respectively with $p = 0.009$) (Table 2). No significant difference of dose-response curves for the AMP challenge was seen between these two groups ($p = 0.27$) (Table 2).

Borg scale

Baseline perception, as measured by the Borg/FEV_1 intercepts, differed significantly between the two different groups (MCh challenge: 2.1 ± 0.4% fall FEV_1; AMP challenge: 2.4 ± 0.6% fall FEV_1 for normal weight versus MCh challenge: 4.0 ± 0.5% fall FEV_1; AMP challenge: 4.3 ± 0.6% fall FEV_1, for high BMI, respectively, $p = 0.008$ and $p = 0.03$).

The slopes of Borg/FEV_1 for MCh challenge differed between normal weighted subjects and overweight subjects. The slopes were significantly steeper for the subjects with a BMI $\leq$ 25 kg/m^2 (0.7 ± 0.3% fall FEV_1 versus0.3 ± 0.3% fall FEV_1, $p = 0.005$). Borg/FEV_1 slopes for AMP challenge were not significantly different ($p = 0.08$).

Questionnaire

The Asthma Control Questionnaire score was significantly higher in the group with a BMI $>$ 25 kg/m^2 (1.4 ± 1.0) as

Table 1 General characteristics of two BMI groups.

	BMI $\leq$ 25 kg/m^2	BMI $>$ 25 kg/m^2	*p*-Value
	n = 11	*n* = 19	
Age (year)	45 (40–56)	55 (48–65)	0.06
BMI	22 (20–23)	32 (25–45)	NA
Cyclus period (y/n)	6/5	6/13	0.22
Anti-contraception (y/n)	3/8	2/17	0.24
Hormonal Treatment (y/n)	0/11	2/17	0.27
SABA Treatment (y/n)	5/6	10/9	0.71
Rhinitis (y/n)	1/10	8/11	0.06
Atopy (y/n)	6/5	8/11	0.51
GERD (y/n)	1/10	8/11	0.06
FEV_1 (% Pred)	94 (87–97)	106 (88–113)	0.15
FVC (% Pred)	102 (92–113)	104 (89–118)	0.68
RV (% Pred)	122 (105–126)	94 (84–117)	0.27
TLC (% Pred)	105 (92–113)	94 (84–112)	0.49
FEV_1/FVC (%)	76 (72–79)	81 (78–99)	**0.02**[a]
FRC (% Pred)	108 (85–122)	81 (39–80)	**0.02**[a]
ACQ	1.21 (0.75–1.96)	0.28 (0.07–0.79)	**0.05**[a]
Leptin (μg/l)	13.48 (10.44–24.94)	35.25 (23.20–63.49)	**0.001**[a]
hs-CRP (mg/ml)	5.15 (1.55–7.00)	6.54 (4.40–8.15)	**0.05**[a]

Cyclus period: yes, not menopausal; no, menopausal; SABA: short-acting ß2-agonist; GER, gastro eosophagal reflux; FEV_1, forced expired volume in 1 s; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity; FRC, forced residual capacity; ACQ, asthma control questionnaire; hs-CRP, C-reactive protein; y, yes; n, no; % Pred, percentage of predicted normal values.
[a] Significant difference between the two groups using Mann–Whitney U-test.

compared with the group with a normal BMI (0.6 ± 0.8) with $p = 0.05$ (Table 1).

Inflammatory markers

Blood leptin and HS-CRP were significantly higher in overweight women (Table 1). A positive correlation was found between BMI and blood leptin (Rho = 0.800, $p = 0.0005$), and HS-CRP (rho = 0.425, $p = 0.02$) (Fig 1). A higher BMI was associated with a higher concentration of leptin and HS-CRP in the blood. An inverse correlation was present between the leptin concentration in blood and the dose-response slopes of the MCh challenge (rho = 0.484, $p = 0.03$). No correlation was found concerning the leptin concentration and the dose-response slope of the AMP challenges (p = 0.7), neither with the FEV_1/FVC ($p = 0.09$). No relation was found for HS-CRP concentration.

Discussion

The results of this study indicate respectively a reduced BHR to MCh in the overweight group compared to the normal weight group, whereas BHR to adenosine, as a marker of airway inflammation is present similarly in both groups. Interestingly, we found that baseline perception of dyspnoea was higher in the overweight group. In addition during the MCh challenge the perception of dyspnoea was lower in this group, assuming that bronchoconstriction was less well perceived by the overweight patients. The overweight BMI

Table 2 Inhalation Challenge tests to Methacholine and Adenosine and Borgscale results in the two populations.

	BMI $\leq$ 25 kg/m^2	BMI $>$ 25 kg/m^2	*p-value*
	n=11	n=19	
MCh slope			
PC10	0.1 (0.08-0.7)	0.6 (0.06-1.6)	0.37
FEV_1/cum dose	0.8 (0.2-1.5)	0.3 (0.1-0.7)	**0.009**[a]
Borg/FEV_1 intercept	1 (1-3)	4 (1-6)	**0.008**[a]
Borg/FEV_1 slopes	0.8 (0.45-0.9)	0.2 (0.05-0.6)	**0.005**[a]
AMP slope			
PC10	4.9 (1.2-45.3)	80 (6-65.9)	0.07
FEV_1/cum dose	0.02 (0.001-0.004)	0.002 (0.001-0.003)	0.27
Borg/FEV_1 intercept	2 (1-5)	3 (1-7)	**0.03**[a]
Borg/FEV_1 slopes	0.6 ± 0.4	0.3 ± 0.4	0.08

MCh, methacholine; AMP, adenosine; cum, cumulative; FEV1, Forced Expired Volume in one second.
[a] Significant difference between the two groups using Mann-Whitney U-test.

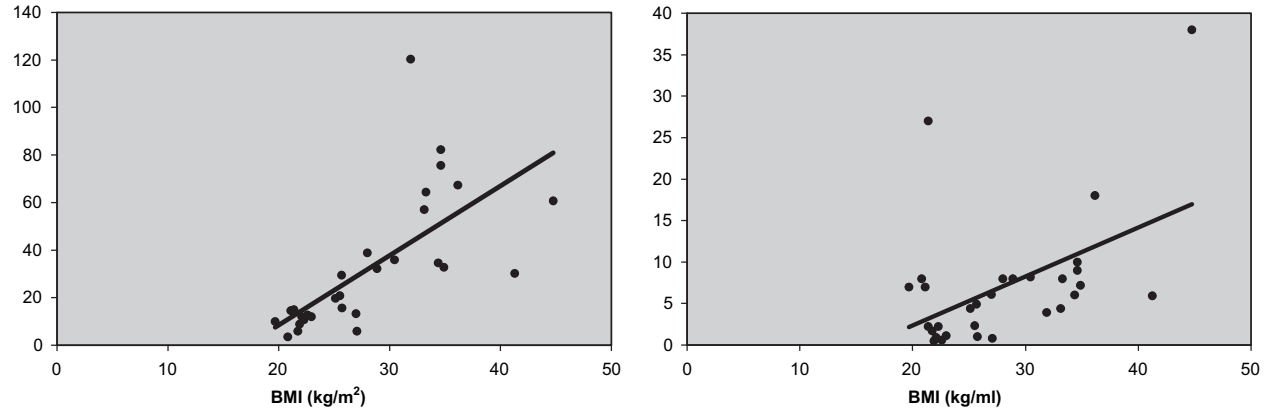

Figure 1 (A) Correlation between BMI and blood leptin concentration ($p = 0.0005$, using non-parametric Spearman correlations test). (B) Correlation between BMI and blood hs-CRP concentration ($p = 0.02$, using non-parametric Spearman correlations test).

group had higher blood concentrations of hs-CRP and leptin, suggesting the presence of a systemic inflammatory state. Our findings suggest that overweight asthmatics may represent a separate asthma phenotype that was not associated with more pronounced airways inflammation.

To the best of our knowledge it is the first study, which explores a direct, and indirect provocation test in asthmatics with BMI as the discriminative factor. Our results extend findings of other studies in which the correlation between airway responsiveness and airway inflammation in asthma has always been largely inconsistent.[33,34] Moreover, Brightling et al. has described the eosinophilic bronchitis, characterised by a corticosteroid responsive cough and sputum eosinophilia, although they found a normal test of airway responsiveness.[35] It is conceivable that airway inflammation in obese subjects is caused by other mechanisms, like inflammatory mechanisms that are independent to the existence of BHR but and leading to the same clinically diagnosis, namely asthma. Furthermore, in overweight subjects, we found a significant diminution of the FRC, which is compatible with the results of the study of Li and colleagues concerning the effect of obesity on pulmonary function.[36] It can be explained by changes in mechanical forces between the lung and the thorax on one hand and the abdomen on the other. The finding that the FEV_1/FVC ratio was lower in the normal weight group reveals that the subjects with a BMI less than 25 kg/m^2 were relatively more obstructive at the beginning of the study. This is in contrast to the study of Tantisira et al. who found an inverse association between BMI and FEV_1/FVC ratio.[37] This may be explained by differences in the selected subjects in the two groups.

The finding of a higher baseline perception of dyspnoea in the high BMI group together with a lower degree of airflow obstruction suggest that bronchoconstriction is not the only factor that account for perception of dyspnoea. Other studies found that perception of dyspnoea was mediated by several mechanisms including hyperinflation[38] and increase in the work of breathing.[12]

In addition to these mechanical pathways, eosinophilic airway inflammation had also been proposed as a determinant of breathlessness.[32] As we suggested, if airway inflammation plays a key role in overweight asthmatics, this may explain, together with mechanical pathways, the dyspnoeic feeling at the beginning of the challenges by this group.

The reduced perception of airflow constriction in overweight group compared with the normal group during the MCh challenge is in accordance to previous studies, which are suggesting that asthmatics do not appropriately perceive their acute bronchoconstriction.[39] Theoretically, adaptation of a dyspnoeic feeling by the high BMI group could explain the higher threshold for experiencing dyspnoea during the test. No difference in perception of dyspnoea was found between the two groups during the AMP challenge even if PC10 tended to be significant.

This study shows a higher ACQ-score for the group with a BMI more than 25 kg/m^2. The adequacy of treatment by the overweight asthmatics seems to be less compared to the normal BMI group. In agreement with our results Lessard et al. found that obese subjects reported poorer asthma control than nonobese subjects, despite a similar perception during the methacholine challenge.[14] In addition we have already reported that compared with nonobese subjects, overweight and obese patients were more likely to have poorly controlled asthma despite pharmacologic treatment.[18]

An inverse correlation existed between BHR and leptin concentration in the blood, whereas no correlation was found between the leptin concentration and the airway inflammation, measured by the AMP challenge. This inverse correlation argue in favour of a relation between inflammatory role of Leptin and the non specific BHR. Finally, no correlation was found between hs-CRP concentration in blood and (i) both challenges or (ii) the degree of airflow obstruction. One of the potential limitations of the present study is that we did not measure directly airway inflammation using FeNO nor sputum eosionophilia. But obesity per se was not found to be associated with increase FeNO.[40,41] In the same way induced sputum eosinophil and neutrophil counts were found similar in obese and nonobeses asthmatic patients.[14]

Taken together, we found that the overweight women had significantly less BHR to MCh, whereas airway inflammation, measured with the AMP challenge as marker, seemed to be present in both groups. Moreover, the high BMI group had significant higher pro-inflammatory mediators in the blood, which did not have a direct correlation with the airway inflammation.

In conclusion, the limited BHR to MCh in obese but the present BHR to AMP with a tend to a significant difference

in PC10, suggest that this group represent a distinct phenotype to regular mild asthmatics. These findings could be relevant for a tailored asthma management, the first step being a weight reduction, even in the milder form of the disease. It is important to further investigate the airway inflammation in overweight asthmatics peoples.

Conflict of interest statement

The authors have no conflict of interest to disclose.

Acknowledgements

This study has been funded by the Jean Baptiste Desbrest Association (Montpellier, France). The authors acknowledge the patients who agreed participating in the study and the study nurses (Catherine Devautour, Marie Bernadette Croze and Pascale Robert) and technicians (Martine Farce and Brigitte Mainprice) from Med Bio Med clinical research centre and Cécile Bouquet, CHU Montpellier, for their technical assistance.

References

1. Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, et al. Surveillance for asthma—United States, 1960—1995. *MMWR CDC Surveill Summ* 1998;**47**(1):1—27.
2. Vastag B. Obesity is now on everyone's plate. *JAMA* 2004; **291**(10):1186—8.
3. Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am J Respir Crit Care Med* 2007;**175**(7):661—6.
4. Castro-Rodriguez JA, Holberg CJ, Morgan WJ, Wright AL, Martinez FD. Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years. *Am J Respir Crit Care Med* 2001;**163**(6):1344—9.
5. Celedon JC, Palmer LJ, Litonjua AA, Weiss ST, Wang B, Fang Z, et al. Body mass index and asthma in adults in families of subjects with asthma in Anqing, China. *Am J Respir Crit Care Med* 2001;**164**(10 Pt 1):1835—40.
6. Chinn S, Jarvis D, Burney P. Relation of bronchial responsiveness to body mass index in the ECRHS. European Community Respiratory Health Survey. *Thorax* 2002;**57**(12):1028—33.
7. Schachter LM, Salome CM, Peat JK, Woolcock AJ. Obesity is a risk for asthma and wheeze but not airway hyperresponsiveness. *Thorax* 2001;**56**(1):4—8.
8. Taylor B, Mannino D, Brown C, Crocker D, Twum-Baah N, Holguin F. Body mass index and asthma severity in the National Asthma Survey. *Thorax* 2008;**63**(1):14—20.
9. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;**112**(12):1796—808.
10. Butland BK, Strachan DP, Rudnicka AR. C-reactive protein, obesity, atopy and asthma symptoms in middle-aged adults. *Eur Respir J* 2008;**32**(1):77—84.
11. Komakula S, Khatri S, Mermis J, Savill S, Haque S, Rojas M, et al. Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in asthmatics. *Respir Res* 2007;**8**:32.
12. Tobin MJ. Dyspnea. Pathophysiologic basis, clinical presentation, and management. *Arch Intern Med* 1990;**150**(8):1604—13.
13. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *Jama* 1999;**282**(22):2131—5.
14. Lessard A, Turcotte H, Cormier Y, Boulet LP. Obesity and asthma: a specific phenotype? *Chest* 2008;**134**(2):317—23.
15. Guerre-Millo M. Adipose tissue and adipokines: for better or worse. *Diabetes Metab* 2004;**30**(1):13—9.
16. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. *Faseb J* 1998;**12**(1):57—65.
17. Sood A, Cui X, Qualls C, Beckett WS, Gross MD, Steffes MW, et al. Association between asthma and serum adiponectin concentration in women. *Thorax*; 2008.
18. Saint-Pierre P, Bourdin A, Chanez P, Daures JP, Godard P. Are overweight asthmatics more difficult to control? *Allergy* 2006; **61**(1):79—84.
19. Thyagarajan B, Jacobs Jr DR, Apostol GG, Smith LJ, Jensen RL, Crapo RO, et al. Longitudinal association of body mass index with lung function: the CARDIA study. *Respir Res* 2008;**9**:31.
20. Weiss ST, Shore S. Obesity and asthma: directions for research. *Am J Respir Crit Care Med* 2004;**169**(8):963—8.
21. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; **31**(1):143—78.
22. Polosa R, Holgate ST. Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma? *Thorax* 1997; **52**(10):919—23.
23. Van Den Berge M, Meijer RJ, Kerstjens HA, de Reus DM, Koeter GH, Kauffman HF, et al. PC(20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. *Am J Respir Crit Care Med* 2001;**163**(7):1546—50.
24. Varraso R, Siroux V, Maccario J, Pin I, Kauffmann F. Asthma severity is associated with body mass index and early menarche in women. *Am J Respir Crit Care Med* 2005;**171**(4):334—9.
25. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. *Eur Respir J* 2003;**22**(3):470—7.
26. Asthma Management and Prevention. Global initiative for asthma. *Ir Med J* 2000;**Suppl. i—vi**:1—39.
27. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;**14**(4):902—7.
28. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 1981;**123**(6):659—64.
29. Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, et al. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;**16**:53—83.
30. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;**14**(5):377—81.
31. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;**136**(6):1412—7.
32. Veen JC, Smits HH, Ravensberg AJ, Hiemstra PS, Sterk PJ, Bel EH. Impaired perception of dyspnea in patients with severe asthma. Relation to sputum eosinophils. *Am J Respir Crit Care Med* 1998;**158**(4):1134—41.
33. Brusasco V, Crimi E, Pellegrino R. Airway hyperresponsiveness in asthma: not just a matter of airway inflammation. *Thorax* 1998;**53**(11):992—8.
34. Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;**157**(1):4—9.

35. Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax* 2003;**58**(6): 528—32.
36. Li AM, Chan D, Wong E, Yin J, Nelson EA, Fok TF. The effects of obesity on pulmonary function. *Arch Dis Child* 2003;**88**(4): 361—3.
37. Tantisira KG, Litonjua AA, Weiss ST, Fuhlbrigge AL. Association of body mass with pulmonary function in the Childhood Asthma Management Program (CAMP). *Thorax* 2003;**58**(12): 1036—41.
38. Lougheed MD, Lam M, Forkert L, Webb KA, O'Donnell DE. Breathlessness during acute bronchoconstriction in asthma. Pathophysiologic mechanisms. *Am Rev Respir Dis* 1993;**148**(6 Pt 1):1452—9.
39. Lavietes MH, Matta J, Tiersky LA, Natelson BH, Bielory L, Cherniack NS. The perception of dyspnea in patients with mild asthma. *Chest* 2001;**120**(2):409—15.
40. Kazaks A, Uriu-Adams JY, Stern JS, Albertson TE. No significant relationship between exhaled nitric oxide and body mass index in people with asthma. *J Allergy Clin Immunol* 2005;**116**(4): 929—30.
41. Sutherland TJ, Cowan JO, Young S, Goulding A, Grant AM, Williamson A, et al. The association between obesity and asthma: interactions between systemic and airway inflammation. *Am J Respir Crit Care Med* 2008;**178**:469—75.