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Nicola Pietro Consilvio, Sabrina Di Pillo, Marcello Verini, Tommaso de Giorgis, Anna Cingolani, Valentina Chiavaroli, Francesco Chiarelli, Angelika Mohn

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3 **The reciprocal influences of asthma and obesity on lung function**
4 **testing, AHR and airway inflammation in prepubertal children.**
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10 *Nicola P. Consilvio, MD, Sabrina Di Pillo, MD, Marcello Verini, MD, Tommaso de Giorgis, MD,*
11 *Anna Cingolani, MD; Valentina Chiavaroli, MD, Francesco Chiarelli, PhD; Angelika Mohn, MD.*
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16
17 Department of Pediatrics, University of Chieti, Chieti, Italy.
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50 **Correspondence:**

51 Angelika Mohn
52 Department of Pediatrics
53 University of Chieti
54 Via Dei Vestini 5
55 66100 Chieti
56 Tel +39 0871 358827
57 Fax +39 0871 584731
58 Email amohn@unich.it
59
60

SUMMARY

Although asthma and obesity are among the major chronic disorders their reciprocal or independent influences on lung function testing, airways hyperresponsiveness (AHR) and bronchial inflammation has not been completely elucidated.

In 118 pre-pubertal Caucasian children anthropometric measurements functional respiratory parameters (flow/volume curves at baseline and after 6-Minute Walk Test [6MWT]) together with bronchial inflammatory index (FeNO) were assessed. The study population was divided into four groups according to BMI and the presence or absence of asthma: Obese asthmatic (*ObA*) Normal-weight asthmatic (*NwA*), Obese non asthmatic (*Ob*), non-asthmatic Normal-weight children (*Nw*).

Baseline PEF and MEF₇₅ (%-expected) were significantly different across the four groups with significantly lower values of MEF₇₅ in ObA and Ob children when compared to Nw children ($p=0.004$ and $p=0.0001$, respectively) and this independent role of obesity on upper respiratory flows was confirmed by multiple analysis of covariance.

After 6 MWT respiratory parameters decreased only in ObA and NwA children and 12 children presented a positive fall in FEV₁, in contrast no changes of respiratory function testing were detected in Ob and Nw children, and only 2 Ob children presented a significant fall in FEV₁.

FeNO analysis demonstrated significantly higher values in ObA and NwA children when compared to Ob ($p=0.008$ and $p=0.0002$, respectively) and Nw children ($p=0.0001$ and $p=0.0003$, respectively), although a significant difference was found between Ob and Nw children ($p=0.0004$).

Multiple analysis of covariance confirmed an independent role of asthma on this parameter.

In conclusion while AHR and airway inflammation are clearly associated with an asthmatic status, obesity seems to induce reduction of upper airways flows associated with a certain degree of pro-inflammatory changes.

INTRODUCTION

Over several decades the prevalence of obesity has steadily increased reaching epidemic proportions. Current estimates of childhood obesity coming from the National Health and Nutrition Examination Survey (NHANES) revealed an increase from 13.8% in 1999-2000 to 16.0% in 2003-2004 for American females and from 14.0% to 18.2% in American males subjects.¹ In Europe, Italy together with other Mediterranean countries explicates the highest rate of obesity in children aged between 7 and 11 years, reaching almost 17%.² This has been accompanied by a similar rise in the rates of asthma. In fact, the CDC National Surveillance for Asthma revealed that its prevalence in children has risen from 3.5% to 7.5% over a period ranging from 2001-2003.³ Similarly in developing countries undergoing their epidemiological transition phase comparable significant rises have been observed.⁴ Accordingly, the World Health Organization (WHO) includes both obesity and asthma among the major chronic disorders representing world-wide public health priorities.⁵

Given this dramatic rise in the prevalence of obesity and asthma, it is not surprising that there has been an increasing body of literature on the association between Body Mass Index (BMI) and asthma.⁶

Obesity may have effects on both the upper and lower airway,⁷ and this hypothesis is supported to a certain extent by the observation that BMI, the best single measure of body adiposity,⁸ seems to be a reliable predictor of allergic respiratory symptoms and airways hyperresponsiveness (AHR).⁹ In fact, recent literature emphasises the role of obesity in inducing a pro-inflammatory state due to increased levels of different pro-inflammatory cytokines. This might be implicated in the genesis of chronic inflammation of the airway system¹⁰ similar to those observed in asthma which is characterized by persistent infiltration of eosinophils, T-lymphocytes and mast cells combined with different cytokines. In this respect exhaled Nitric Oxide (eNO) has been shown to tightly reflect this airway inflammation¹¹ and its fractional concentration in exhaled air (FeNO) has been recently accepted by the PRACTALL Consensus Report for the Diagnosis, Management and Treatment of Childhood Asthma as a logical complementary item in the follow-up of bronchial inflammation.¹²

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3 The reported association between increased BMI and onset of AHR has been elegantly
4 demonstrated by Litonjua et al.¹³ in a longitudinal study of aging and has been further confirmed by
5 two large cross-sectional studies performed in adults.^{14,15} This is in contrast with other
6 epidemiological studies performed in childhood where this association has not been universally
7 observed.^{16,17,18} This represents an important discordance of the recent literature as it might indicate
8 that different factors such as duration of obesity and subsequent physiological changes of airway
9 structures might play an important role in the development of obesity induced AHR.
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19 Pre-pubertal children are a special study population as the shorter duration combined with better
20 control of both diseases eliminates confounding factors commonly present in older subjects. In fact,
21 this kind of population offers the exclusive opportunity to tangle whether obesity and asthma
22 explicate an independent effect on lung function testing or influence themselves in a reciprocal
23 manner.
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31 Therefore, with the primary aim to establish the possible dependent or independent role of asthma
32 and obesity on airway system function we evaluated AHR and bronchial inflammation expressed by
33 FeNO in a group of obese and non obese asthmatic children in comparison to non-asthmatic obese
34 and normal-weight pre-pubertal children.
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MATERIALS AND METHODS

Study subjects

Clinical, anthropometric measurements and respiratory parameters of a group of 118 Caucasian pre-pubertal children referred to the Children Obesity Clinic, to the Paediatric Allergy and Respiratory Clinic and to the Department of Paediatrics, University of Chieti, Italy, were sequentially evaluated between May 2008 and August 2008.

Asthma was classified according to Global Initiative for Asthma (GINA) guidelines¹⁹ as intermittent asthma, mild persistent, moderate persistent and severe persistent asthma. The diagnosis was established by a single paediatric respiratory physician on the basis of a clinical history of repeated episodes of coughing, dyspnoea and wheezing, according to American Thoracic Society - European Respiratory Society (ATS-ERS) criteria.²⁰ Only subjects who fulfilled the definition of intermittent/mild persistent asthma were recruited. Low dose inhaled corticosteroids (ICSs) therapy was withdrawn for not more than weeks before the study evaluation. Over this period all children were closely monitored by the medical staff of the Paediatric Allergy and Respiratory Clinic, none experienced any adverse event and at the time of the study evaluation all subjects were symptom-free.

Obesity was defined as Body Mass Index [BMI] above 2 Standard Deviations (SD) for the mean of age and sex.²¹ In all subjects endocrine diseases as cause for obesity were excluded prior to study entry. None of the non-asthmatic obese children had a personal history for asthma and allergic diseases.

We recruited as controls a group of healthy children, comparable for age, gender, and pubertal stage, who were admitted to the Department of Paediatrics for minor diseases. None of the children had a personal history for asthma and allergic diseases, and all were otherwise in a good health. Anthropometric measurement and respiratory function were assessed only after complete recovery of the disease.

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3 Exclusion criteria for all children were: presence of upper or lower respiratory infections including
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5 asthma during the previous 4 weeks, congenital abnormalities of cardio-respiratory system, chest or
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7 skeletal deformities, or neuromuscular system disease, chronic and autoimmune disease, gastro-
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9 oesophageal reflux, presence of medical condition in which exercise was forbidden. None of the
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11 children was taking any medication.
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15 Based on the presence or absence of asthma and according to BMI, the study population was
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17 divided into four groups:
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- 19 1. *Obese Asthmatic Children [ObA]* (sixteen boys and 14 girls);
 - 20 2. *Normal-weight Asthmatic Children [NwA]* (sixteen boys and 14 girls);
 - 21 3. *Non-Asthmatic Obese Children [Ob]* (fourteen boys and 16 girls);
 - 22 4. *Non-Asthmatic Normal-weight Children [Nw]* (fourteen boys and 14 girls).
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29 All children underwent a complete physical examination including anthropometric parameters
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31 [height, weight]. Pubertal stage was defined on the basis of breast development in girls and genital
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33 development in boys according to the criteria of Tanner (all subjects had pre-adolescent
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35 characteristics corresponding to stage 1). Respiratory function was assessed by standard
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37 Flow/Volume curves at baseline and after Six-Minute Walk Test (6MWT), and respiratory
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39 inflammatory status was assessed by FeNO. Only asthmatic children performed allergy evaluation
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41 by Skin Prick Test (SPT), and blood sample was taken for specific IgE measurement.
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46 Written informed consent was obtained from all parents and oral consent from all children.
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48 The study was approved by Ethical Committee of University of Chieti.
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53 **Methods**

54 *Anthropometric measurements*

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57 Body weight was determined to the nearest 0.1 Kg, and height was measured in triplicate with
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59 Harpenden stadiometer to the nearest 0.1 cm. As fatness indices, we used BMI (the weight in
60 kilograms divided by the square of the height in meters). SDS-height and BMI-SDS were calculated

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3 as difference between the individual observed value and the reference mean for age and sex, divided
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5 by the corresponding standard deviation.²²
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8 *Spirometric and Flowmetric Measurements:*

9 *Baseline Flow/Volume curves*

10 Lung function evaluation was performed by Flow/Volume curves (KitMicro Spirometer 7.3a –
11 COSMED) in the standing position with nose-clip until two consecutive technically acceptable
12 curves were achieved, according to ATS/ERS Guidelines.²³ The main spirometric parameters
13 included: Forced Expiratory Volume in 1 sec (FEV₁), Forced Vital Capacity (FVC), FEV₁/FVC,
14 and Forced Expiratory Flows between 25% and 75% of the FVC (FEF₂₅₋₇₅) and the results are
15 expressed as Z-scores according to Stanojevic S et al.²⁴ Peak Expiratory Flow (PEF) and Maximal
16 Expiratory Flows at 75% of the FVC (MEF₇₅) were expressed as a percentage of theoretical values
17 for age, gender, and height according to Quanjer PH et al.²⁵
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31 *Six-Minute Walk Test (6MWT)*

32 In order to estimate the bronchial hyperresponsiveness, after baseline lung function testing, a
33 6MWT was performed according to standard protocol.²⁶ The target of the test was to achieve 6
34 minutes of free walk until the hearth-rate was 80-90% of the predicted maximum (calculated as 220
35 – age in years), according to ATS/ERS recommendations for exercise challenge test in older
36 children.²⁷ Spirometry was performed in duplicate sets at 1, 3, 5, 10 and 20 minutes post-exercise.
37 The better of the two curves was selected as the representative value at each interval, but
38 differences between the two values of FEV₁ had to be <5%. Exercise response was defined as the
39 greatest decrease in FEV₁ expressed as a percentage of the baseline values. A bronchial obstruction
40 response to exercise was considered positive when the FEV₁ reduction from baseline was greater
41 than 12%.²⁸
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57 *Allergic sensitization*

58 Skin Prick Test (SPT) were performed and serum specific IgE were measured for the most common
59 respiratory allergens: Dust Mite (*Dermatophagoides Pteronyssinus* and *Farinae*), Grass, Parietaria,
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3 Artemisia Vulgaris, Olive, Cypress, Lime, Stone, Elm, Plane, Cat and Dog dander, Alternaria
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5 Alternata and Aspergillus Fumigatus (moulds). Determination of Allergen-Specific IgE was made
6
7 by an Immunoenzymatic Allergo-sorbent Test (Cap test Pharmacia).^{29,30}
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10 *Respiratory inflammation marker*

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12 FeNO was determined with an on-line method using a single breath exhalation and a sensitive
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14 chemiluminescence assay (Ecomedics CLD 88), according to ATS-ERS.³¹ Patients made an
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16 inspiration of eNO-free air via a mouthpiece immediately followed by full exhalation at a constant
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18 rate (50 ml/sec) for at least 5 seconds. The mean of three readings at the end of the expiration
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20 (plateau phase) was taken as the representative value for each measurements. 12 ppb or more were
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22 considered positive, according to ATS-ERS criteria.³²
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29 **Statistical analysis**

30 All values were expressed as Median and Interquartile Range.

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33 Kruskal Wallis Test was performed for evaluating differences between the four groups (ObA, NwA,
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35 Ob, Nw). Statistical significance level was $p < 0.05$. Subsequently, differences within the single
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37 groups were tested by Mann-Whitney Test, adjusting the significance level by $p < 0.008$. Differences
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39 in functional respiratory baseline parameters and after 6MWT within each group were analyzed
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41 using Wilcoxon Signed Ranks Test. Categorical variables were tested by Pearson's chi-square (X^2)
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43 test.
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48 A multiple analysis of covariance (MANCOVA) was performed to investigate the independent
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50 effect of obesity and asthma on respiratory indices (PEF, MEF₇₅ and FEV₁/FVC) and bronchial
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52 inflammation (FeNO), after adjusting for gender, age and height.
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54 Statistical significance level was $p < 0.05$.

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56 All calculations were made with the computer program SPSS (Statistical Package for the Social
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58 Science), version 16.0 software for Windows.
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RESULTS

Anthropometric measurements and respiratory functional data of the whole study population are reported in Table 1. The four groups were similar for age, gender, and SDS-height.

As expected BMI-SDS was similar between ObA and Ob children, but was significantly different between obese children and normal-weight children (ObA vs NwA $p=0.0004$; ObA vs Nw: $p=0.0008$; Ob vs NwA $p=0.0001$; Ob vs Nw: $p=0.0002$).

All the asthmatic children were sensitized to *Dermatophagoides Pteronyssinus*.

Baseline Respiratory Functional Parameters

No significant differences were found for FVC, FEV₁, and FEF₂₅₋₇₅ Z-scores between the four groups, whereas FEV₁/FVC Z-score resulted significantly different (Tab 1). In fact, this parameter was significantly lower in the ObA group compared to the Nw group ($p=0.005$). Moreover a significant difference was detected between the four groups in terms of PEF ($p=0.03$) and MEF₇₅ ($p=0.001$) (Fig 1). By comparing the four groups directly no further significances could be detected for PEF, whereas MEF₇₅ was significantly lower in the ObA group and the Ob group compared to the Nw group ($p=0.004$ and $p=0.0001$, respectively).

Respiratory Functional Parameters after 6MWT

After 6MWT no significant differences were found in post-FVC and post-FEF₂₅₋₇₅ Z-scores among the four groups (Tab 1). Although post-FEV₁/FVC Z-scores resulted significant different between the four groups, no further differences were detected by comparing the four groups directly. In contrast, post-FEV₁ Z-score resulted significantly lower in NwA children when compared to Ob children and Nw children ($p=0.006$ and $p=0.006$, respectively). Post-PEF and post-MEF₇₅ resulted significantly different between the four groups ($p=0.01$, both). In fact, post-PEF was significantly lower in ObA children when compared to Nw children ($p=0.002$). This was associated with significantly lower values of post-MEF₇₅ in the ObA group and the Ob group when compared to the Nw group ($p=0.003$ and $p=0.007$, respectively).

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3 Changes in respiratory parameters after 6MWT within each group are shown in Table 2. Post-
4 FEV₁/FVC Z-score was significantly lower in the ObA group, while in NwA children a significant
5 reduction was detected for post-FEV₁, post-FVC and post-FEF₂₅₋₇₅ Z-scores. In contrast no changes
6 were detected for the Ob and Nw groups.
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11 A positive response to exercise challenge was found in 5 ObA children and 7 NwA children
12 compared to only 2 Ob children. As expected none of the healthy control children experienced a fall
13 in FEV₁ greater than 12%.
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18 19 *Respiratory inflammation marker*

20 FeNO values were significantly different across all four groups (p=0.0002) (Tab. 1).

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22 In fact FeNO values were significantly higher in the ObA group and the NwA group compared to
23 the Ob group (p=0.008 and p=0.0002, respectively) and to the Nw group (p=0.0001 and p=0.0003,
24 respectively).
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31 Furthermore FeNO was significantly higher in the Ob group when compared to the Nw group
32 (p=0.0004). In contrast no differences were detected between the two asthmatic groups.
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35 36 *Effect of asthma and obesity on respiratory functional parameters and bronchial inflammation*

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38 In order to investigate the role of asthma and obesity on resulted significant different baseline
39 respiratory variables and FeNO, a multiple analysis of covariance was performed. Asthma was
40 significantly and independently related to FeNO values, whereas no association was found for
41 obesity. In contrast obesity was significantly and independently related to PEF and MEF₇₅. In the
42 same model no relationship was found for asthma and obesity on FEV₁/FVC Z-score (Table 3).
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DISCUSSION

Although the effect of obesity on pulmonary function tests has been extensively investigated in adults, the number of similar studies in children is limited and confirm the controversial results observed in adults between obesity and AHR. In this study we compare for the first time obese asthmatic with obese non-asthmatic pre-pubertal children and healthy controls with the aim to analyse the reciprocal influences of obesity and asthma on lung function and bronchial inflammation. Our findings clearly demonstrate that while an asthmatic status is tightly and directly involved in AHR and bronchial inflammation, obesity mainly affects the upper airways flows.

As obese children often experience dyspnoea and breathlessness during physical activities it is a crucial clinical question as to whether these symptoms are effectively related to AHR such as in asthmatic children. In order to explore this possibility a 6MWT was performed. While basal values of spirometric indices were normal in all examined groups, in ObA and NwA children exercise challenge induced AHR documented by the reduction of functional respiratory parameters. In contrast in Ob and Nw children no changes of any of the obtained variables were detected. This is confirmed by the number of children exhibiting a positive response to exercise defined as fall in FEV1 greater than 12%. In fact within the asthmatic children 12 subjects met this definition compared to only 2 obese children. In agreement with our data, epidemiological studies do not uniformly support the conclusions that obesity leads to airflow limitation, AHR or asthma. In this respect Schachter and co-workers showed in a group of 1971 adults, that although BMI was associated with a clinical diagnosis of asthma and subjective symptoms of dyspnoea and wheeze, airflow obstruction or AHR subsequent to histamine challenge could not be detected in the sole obese subjects.¹⁶ Similar findings were obtained by Bibi et al.¹⁷ who demonstrated in a large population of pre-pubertal children that bronchodilation could not be induced by salbutamol inhalation in non-asthmatic obese subjects.³³ These data demonstrate that obesity should not be considered as being a risk factor for AHR in pre-pubertal children. Furthermore asthma like symptoms especially during physical activity should not be overestimated by parents and medical

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3 staff. This is of paramount relevance as it might induce inappropriate treatment and refrain children
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5 from performing sports leading to a vicious circle impeding improvement of both obesity and
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7 respiratory symptoms.
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10 Interestingly in the ObA and Ob groups we documented lower basal values of MEF₇₅ when
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12 compared to healthy controls. Furthermore PEF values were significantly different across the four
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14 groups with a tendency of lower values in the two obese groups. In fact, by performing a multiple
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16 analysis of covariance obesity was significantly and independently related to MEF₇₅ and to PEF,
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18 while this association was not found for asthma. Similar data were documented by two previous
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20 studies^{34,35} where obesity was associated with a significant reduction in FEF₂₅₋₇₅. These data
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22 suggests that obesity might play a primary role on upper airway flows, and despite being possibly
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24 explained by fatty infiltration of muscles narrowing directly the upper airway, recent literature
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26 suggest that this functional parameter might be used as an early predictor of sub-clinical asthma.³⁶
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30 In order to obtain a complete evaluation of the bronchial status we performed also FeNO analysis.
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32 In effect, recent studies revealed that FeNO is a potentially useful measure to evaluate the role of
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34 airways inflammation in asthma, as it represents the forerunner of the most important event namely
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36 remodelling of bronchial airways,³⁷ offering therefore a logical complementary item to the only use
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38 of clinical and functional data. In our study, as expected, both groups of asthmatic children
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40 presented highly elevated FeNO values when compared to Ob children and normal controls
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42 reflecting the underlying bronchial inflammation process. However, data from animal and human
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44 studies clearly demonstrate that the enhancement of normal adipose tissue in obesity leads to a
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46 systemic pro-inflammatory state.³⁸ Adipose tissue from obese individuals expresses a number of
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48 pro-inflammatory molecules, such as leptin, tumor necrosis factor- α , IL-6, transforming growth
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50 factor- β 1, and C-reactive protein, and there appears to be significant overlap between the immune
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52 function of adipocytes and the function of T-lymphocytes and macrophages, particularly with
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54 respect to the elaboration of inflammatory cytokines.^{39,40} Although a conclusive relationship
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56 between obesity and airway inflammation has not yet to been described, it could be argued that the
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3 role of the pro-inflammatory state underlying the main metabolic and cardiovascular complications
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5 in obesity might play a pivotal role in determining airways inflammation independently of any
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7 allergic mechanisms. This might explain the findings of our study where FeNO values were
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9 significantly higher in Ob children when compared to healthy controls, and the findings of
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11 Santamaria et al.⁴¹ who found a positive correlation between FeNO and BMI in healthy children.
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13 However it needs to be acknowledged that in the multiple analysis of covariance obesity was not a
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15 predictor for increased FeNO values as only asthmatic status remained as such. This apparent
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17 contrasting findings might be partially explained by the age group of our study population as
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19 obesity related inflammation might not have reached its full implication and a possible involvement
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21 of the respiratory system might require a long standing history of obesity.
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27 In this respect an important limitation of this study is the cross sectional design which does not
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29 allow to speculate on the full clinical implications of our findings. In fact, as puberty is a well
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31 known risk factor for developing obesity related complications it might be of crucial interest to
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33 obtain longitudinal data starting in pre-puberty and lasting through all pubertal stages. This would
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35 allow to fully explore this intriguing relationship and to not underestimate the possible role of
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37 obesity on the development of bronchial inflammation.
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41 In conclusion, while AHR and airways inflammation are clearly associated to an asthmatic status,
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43 obesity seems to induce reduction of upper airway flows combined with a certain degree of early
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45 pro-inflammatory airways changes. Longitudinal data are required in order to clarify whether these
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47 changes might represent risk factor for asthma later on in life.
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3 **The reciprocal influences of asthma and obesity on lung function in**
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10 *Consilvio Nicola Pietro, MD; Di Pillo Sabrina, MD; Verini Marcello, MD; Tommaso de Giorgis,*
11 *MD; Anna Cingolani, MD; **Fabio Masuccio, MD**; Francesco Chiarelli, PhD; Angelika Mohn, MD.*
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17 Department of Pediatrics, University of Chieti, Chieti, Italy.
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50 **Correspondence:**

51 Angelika Mohn
52 Department of Pediatrics
53 University of Chieti
54 Via Dei Vestini 5
55 66100 Chieti
56 Tel +39 0871 358827
57 Fax +39 0871 584731
58 Email amohn@unich.it
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ABSTRACT

As the incidence of obesity and asthma increase consistently, the reciprocal influences of both on lung function, airways hyperresponsiveness (AHR) and bronchial inflammation were analysed in a group of 118 caucasian prepubertal children.

Anthropometric measurements and respiratory parameters (Flow/Volume curves, “free run” Exercise Challenge Test, FeNO) were evaluated and the study population was divided into four groups according to Body Mass Index and the presence or absence of asthma: *Obese Asthmatic, Normal-weight Asthmatic, Obese non-Asthmatic, Normal-weight non-Asthmatic* children.

MEF75% was significantly lower in obese asthmatic and non-asthmatic children compared to healthy controls and the influence of obesity on MEF75% was confirmed by multiple linear regression. After the “free-run” Exercise Challenge Test all analysed respiratory parameters decreased significantly in asthmatic obese and non-obese children, in contrast in non-asthmatic obese children no differences were found. FeNO analysis demonstrate significantly higher values in asthmatic children compared to obese non-asthmatic and healthy controls. Furthermore FeNO was significantly higher in obese non-asthmatic children when compared to healthy controls.

In conclusion, while AHR and airways inflammation are clearly associated to an asthmatic status, obesity seems to induce early proinflammatory airways changes combined with reduction of higher intrathoracic airway flows.

INTRODUCTION

Over the past 20 years the incidence and prevalence of obesity have steadily increased. The most recent data from the Centers for Disease Control's (CDC) Third National Health and Nutrition Examination Survey (NHANES III) revealed a prevalence of obesity – defined as a body mass index (BMI) in excess of 95% of the age specific distribution – of 14% in children aged 6 – 11 surveyed between 1988 and 1994.¹ In comparison NHANES II, conducted between 1976 and 1980, found a prevalence of obesity of 7.6% for the same population. This has been accompanied by a similar rise in the association rates of asthma. The CDC self-reported prevalence of asthma in children aged 5 – 14 rose from 42.8 per 1000 in 1980 to 74.4 per 1000 in 1994.² Epidemiologists have reported that both obesity and asthma are “endemic” in developed countries^{3,4} and “epidemic” in developing countries undergoing their epidemiological transition phase.⁵ Accordingly, the World Health Organization (WHO) includes both obesity and asthma among the major chronic disorders representing world-wide public health priorities.⁶

Given the dramatic rise in the prevalence of obesity and asthma, it is not surprising that there has been an increasing body of literature on the association between BMI and asthma.⁷

Obesity may have effects on both the upper and lower airway;⁸ and this hypothesis is supported to a certain extent by the observation that BMI, the best single measure of body adiposity,⁹ seems to be a reliable predictor of allergic respiratory symptoms and airways hyperresponsiveness (AHR).^{10,11,12}

Infiltration by eosinophils, mast cells and T lymphocytes is a key feature of airway inflammation in asthma¹³ and an increased eosinophil number in induced sputum of asthmatic children is tightly linked to current asthma symptoms and AHR.¹⁴ Recently, exhaled Nitric Oxide (eNO) has been shown to have a good correlation with airway eosinophilic inflammation¹⁵ and its fractional concentration in exhaled air (FeNO) has been accepted to be a useful indicator of airway inflammation. On the other hand recent literature emphasises the role of obesity in inducing a proinflammatory state as obesity is associated with increased levels of leptin and proinflammatory

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3 cytokines such as interleukin (IL)-6 and IL-1 which might be implicated in a possible up-regulate
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5 inflammation of the airway system.¹⁶
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8 The reported association between increased BMI and onset of AHR has been elegantly
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10 demonstrated by Litonjua et al.¹⁷ in a longitudinal study of aging; and has been further confirmed
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12 by two large cross-sectional studies.^{18,19} This is in contrast with epidemiologic studies where this
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14 association has not been universally observed, indicating that different factors such as duration of
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16 obesity, physiological changes of airway structures might play an important role on the
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18 development of this relationship.
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22 Prepubertal children might represent a special study population in order to investigate the reciprocal
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24 and independent influences of obesity and asthma on lung function testing, and bronchial indices of
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26 flogosis as both diseases should not necessarily having caused physiological changes commonly
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28 observed in older subjects.
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31 We therefore evaluated AHR and bronchial inflammation expressed by FeNO in obese and non
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33 obese asthmatic children in comparison with non asthmatic obese and normalweight prepubertal
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35 children.
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METHODS

Study subjects

Clinical, anthropometric measurements and respiratory parameters of a group of 118 Caucasian pre-pubertal children referred to the Department of Pediatrics, Children Obesity Clinic and to the Allergological and Pneumological Unity of the Pediatric Department, University of Chieti, Italy, between May 2008 and August 2008, were evaluated.

According to Body Mass Index [BMI], and based on the presence or absence of asthma, the study population was divided into four groups:

1. *Obese Asthmatic Prepubertal Children [ObA] (16 M and 14 F);*
2. *Normal-weight Asthmatic Prepubertal Children [NwA] (16 M and 14 F);*
3. *Non-Asthmatic Obese Prepubertal Children [Ob] (14 M and 16 F);*
4. *Non-Asthmatic Normal-weight Prepubertal Children [Nw] (14 M and 14 F)*

- Pubertal stage was defined on the basis of breast development in girls and genital development in boys according to the criteria of Tanner (all subjects had pre-adolescent characteristics corresponding to stage 1).

- A complete physical examination including [height, height Standard Deviation Score (SDS), weight, weight-SDS, BMI, BMI-SDS]. Children with values between -2 and 2 SD were defined normal-weight, while children with BMI above 2 SD were defined obese.^{20,21}

- Asthma was classified according to GINA guidelines²² as intermittent asthma, mild persistent, moderate persistent and severe persistent asthma. All subjects had been admitted for intermittent/mild persistent asthma and the diagnosis was made by a pediatric respiratory physician on the basis of clinical history of repeated episodes of coughing, dyspnea and wheezing, according to ATS-ERS criteria.²³ To evaluate the bronchial reactivity was made an Exercise Challenge Test. Moreover, to evaluate the bronchial inflammation all children underwent FeNO analysis. To evaluate the allergic sensitization we made Skin Prick Test (SPT) and serum specific IgE measurements for the most common respiratory allergens.

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3 Asthmatic children had received an inhaled corticosteroids (ICS) therapy, but were free from
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5 therapy for at least 2 weeks before the study evaluation; and all obese children were therapy-free.
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8 All the subjects were symptom-free and had no clinical evidence of cardiopulmonary disease in a
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10 preceding evaluation. Exclusion criteria from the study for all groups were: presence of upper or
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12 lower respiratory infection or exacerbation of asthma during the previous 4 weeks, not well
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14 controlled asthma according to current Global Initiative for Asthma (GINA) classification of
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16 asthma,²⁴ congenital abnormalities of cardiorespiratory system, chest or skeletal deformities, or
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18 neuromuscular system disease, chronic and autoimmune disease, gastroesophageal reflux, presence
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20 of medical condition in which exercise was forbidden.
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24 Written informed consent was obtained from all parents and oral consent from all children.
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27 The study was approved by Ethical Committee of University of Chieti.
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31 **Methods**

32 *Anthropometric measurements*

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34 Body weight was determined to the nearest 0.1 Kg, and height was measured in triplicate with
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36 Harpenden stadiometer to the nearest 0.1 cm.
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39 We used BMI for fatness indexes (the weight in kilograms divided by the square of the height in
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41 meters); and also BMI expressed as a continuous variable in standard deviation score (BMI-SDS)
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43 units (z-score), determined as difference between the individual observed value and the reference
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45 mean for age and sex, divided by the corresponding standard deviation.²⁵
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52 *Functional respiratory data*

53 Evaluations were made by Flow/Volume curves (KitMicro Spirometer 7.3a – COSMED), according
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55 ATS/ERS Guidelines,²⁶ and the main spirometric tests were considered: Forced Expiratory Volume
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57 in 1 sec (FEV1), Forced Vital Capacity (FVC), Peak Expiratory Flow (PEF) and Maximal
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3 Expiratory Flows at 75%, 50% and 25% of the Forced Vital Capacity (MEF75%, MEF50%,
4 MEF25%). All the tests were expressed as a percentage of theoretical values for eight.
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8 To evaluate the bronchial hyperresponsiveness measurements are performed in the standing
9 position, with nose-clip until two consecutive technically acceptable curves are achieved. After the
10 free-run, spirometry was measured in duplicate sets at 1, 3, 5, 10 and 20 minutes post-exercise. The
11 better of the two curves was selected as the representative value at each interval, but differences
12 between the two values of FEV1 had to be <5%. The target was to achieve 6 minutes of "free run"
13 to increase heart-rate to 80% in the maximum for at least 4 minutes according to ATS/ERS
14 recommendations for exercise challenge test in older children.^{27,28} Exercise response was defined as
15 the greatest decrease in FEV1 expressed as a percentage of the baseline values. A
16 bronchoconstriction response to exercise was considered as positive when the FEV1 decrease from
17 baseline was greater than 13%.²⁹
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34 *Allergic sensitization*

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36 Was evaluated by Skin Prick Test (SPT) and serum specific IgE measurements for the most
37 common respiratory allergens: Dust Mite (*Dermatophagoides Pteronyssinus* and *Farinae*), Grass,
38 Parietaria, *Artemisia Vulgaris*, Olive, Cypress, Lime, Stone, Elm, Plane, Cat and Dog dander,
39 Alternaria Alternata and *Aspergillus Fumigatus* (moulds). Determination of Allergen-Specific IgE
40 was made by an Immunoenzymatic Allergo-sorbent Test (Cap test Pharmacia).^{30,31}
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50 *Respiratory inflammation marker*

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52 FeNO was determined with an on-line method using a single breath exhalation and a sensitive
53 chemiluminescence assay (Ecomedics CLD 88), according to ATS-ERS.³² Patients made an
54 inspiration of eNO-free air via a mouthpiece immediately followed by full exhalation at a constant
55 rate (50 ml/sec) for at least 5 seconds. The mean of three readings at the end of the expiration
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3 (plateau phase) was taken as the representative value for each measurements. 12 ppb or more were
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5 considered positive, according to ATS-ERS criteria.³³
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10 *Statistical analysis*

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12 All values were expressed as **Means and SD**. Kruskal Wallis Test was performed for evaluating
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14 differences between the four groups (ObA, NwA, Ob, Nw). Statistical significance level was
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16 $p < 0.05$. Subsequently, differences within the single groups were tested by Mann-Whitney Test,
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18 adjusting the significance level by $p < 0.0125$. Differences in functional respiratory parameters **pre-**
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20 **and post-“free-run” exercise Challenge Test** were analysed using Wilcoxon Signed Ranks Test
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22
23 within each group.
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27 **A multiple linear regression analysis was performed to asses the independent contribution of**
28
29 **obesity (BMI-SDS) and asthma on respiratory inflammation and respiratory functional parameters.**
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31 **FeNO, PEF% and MEF75% were the dependent variables and BMI-SDS and asthma were the**
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33 **independent variables.** Statistical significance level was $p < 0.05$.
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36 All calculations were made with the computer program SPSS (Statistical Package for the Social
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38 Science), version 16.0 software for Windows.
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RESULTS

Anthropometric measurements and respiratory functional data are reported in **table 1**.

The four groups were similar for age, gender, and **height**.

All the asthmatic children were **allergic** to Dermatophagoides Pteronyssinus (**Df. pt**).

As expected BMI and BMI-SDS was similar between obese asthmatic and obese non-asthmatic children, but was significantly different between obese and normal-weight children (**ObA vs Ob: $p>0.05$** ; ObA vs NwA: $p= 0.0004$; ObA vs Nw: $p=0.0008$; Ob vs NwA: $p=0.0001$; Ob vs Nw: $p=0.0002$).

Baseline Respiratory Functional Parameters

No significant differences were found for FVC%, FEV1%, MEF50% and MEF25% between the four groups.

Despite detecting a trend of difference in PEF% between the four groups, no differences were found when performing sub-analysis between ObA vs NwA, ObA vs Ob, ObA vs Nw, NwA vs Ob, NwA vs Nw, and Ob vs Nw.

We found that MEF75% was significantly lower in OA group and OnA group compared to NnA group ($p=0.004$ and $p=0.0002$, respectively) (**Table 1**).

Respiratory Functional Parameters after “free run” Exercise Challenge Test

No significant differences were found in FEV1%, FVC%, MEF50% and MEF25% after the exercise test among the four groups.

After the “free-run” exercise Challenge Test we found statistically significant differences in PEF% and MEF75%. In fact NnA had significantly higher values of these two parameters compared to OA ($p=0.001$ for PEF% and $p=0.002$ for MEF75%); furthermore obese non-asthmatic children had significantly lower value of MEF75% compared to the NnA group ($p=0.007$) (**Table 1**).

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3 As shown in Table 2, analysing functional respiratory data within each group after the “free-run”
4 exercise Challenge Test, we found that all analysed parameters, with the exception of FVC% for the
5 OA group and of MEF25% for the NA group, decreased significantly in asthmatic obese and non-
6 obese children (without clinical and functional evidence of obstruction – all indices were in the
7 normal range for age and height). In contrast in non-asthmatic obese children we did not detect any
8 statistical significant differences in all functional respiratory parameters after “free run” exercise
9 Challenge Test.
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20 21 22 *Respiratory inflammation marker*

23 FeNO values were significantly different across all four groups ($p=0.0002$).

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25 In fact FeNO values were significantly higher in the ObA group compared to Ob group and to the
26 Nw group ($p=0.011$ and $p=0.0001$, respectively); and were significantly higher in the NwA group
27 compared to Ob group and to the Nw group ($p=0.0002$ and $p=0.0003$, respectively). We did not
28 detect any statistical significant difference in FeNO values between the asthmatic children
29 ($p=0.201$). Furthermore FeNO was significantly higher in the Ob group when compared to the Nw
30 group ($p=0.0004$) (Figure 1).
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43 *Independent contribution of BMI-SDS and asthma on respiratory inflammation and respiratory* 44 *functional parameters*

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47 In a multiple linear regression analysis with FeNO as the dependent variable and BMI-SDS and
48 asthma (asthmatic vs non-asthmatic children) as the independent variables, asthma was significantly
49 and independently related to FeNO values, whereas no association was found for obesity (BMI-
50 SDS). In a second multiple linear regression model with PEF% as the dependent variable and BMI-
51 SDS and asthma as the independent variables, BMI-SDS was significantly and independently
52 related to PEF% values. A further multiple linear regression analysis with MEF75% as the
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dependent variable and BMI-SDS and asthma as the independent variables, showed again that BMI-SDS was significantly and independently related to MEF75% values (Table 3).

For Peer Review

DISCUSSION

This is the first study comparing obese asthmatic with obese non-asthmatic children and healthy controls with the aim to analyse the reciprocal influences of obesity and asthma on lung function in prepubertal children. Although the effect of obesity on pulmonary function tests has been extensively investigated in adults, the number of similar studies in children is limited and confirm the controversial results between obesity and AHR observed in adults.

In order to evaluate the relationship between obesity and AHR and to detect whether breathlessness experienced by obese children during physical activities is effectively due to the airway hyper-responsiveness we performed a “free-run” Exercise Challenge test. While basal values of spirometric indexes were normal in all examined groups, in asthmatic obese and non-obese children free run induced AHR documented by reduction of all functional respiratory parameters. In contrast in obese children no changes in these functional respiratory parameters were detected. In addition, in a multiple linear regression we found that asthma remained the only predictor for AHR. In agreement to our data, epidemiological studies do not uniformly support the conclusions that obesity leads to airflow limitation, AHR or asthma. In this respect Schachter and coworkers showed in a group of 1971 adults, that although BMI was associated with a clinical diagnosis of asthma and subjective symptoms of dyspnea and wheeze, airflow obstruction or AHR subsequent to histamine challenge could not be detected in the sole obese subjects.³⁴ Similar findings were obtained by Bibi et al.³⁵ who demonstrated in a large population of prepubertal children that broncodilation could not be induced by salbutamol inhalation in non-asthmatic obese subjects.³⁶ These data demonstrate that obesity should not be considered as being a risk factor for AHR in prepubertal children. Furthermore asthma like symptoms especially during physical activity should not be overestimated by parents and medical staff. This is of paramount relevance as it might induce inappropriate treatment and refrain children from performing sports leading to a vicious circle impeding improvement of both obesity and respiratory symptoms.

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3 Interestingly in obese asthmatic children and obese non-asthmatic children we documented lower
4 basal values of MEF75% when compared to healthy controls. Furthermore by performing a
5 multiple linear logistic regression analysis obesity, defined as BMI-SDS, was significantly and
6 independently related to MEF75%; while no association was found for asthma. Similar data were
7 documented by two previous studies^{37,38} where obesity was associated with a significant reduction
8 in FEF₂₅₋₇₅. These data suggests that obesity might play a primary role on higher intrathoracic
9 airway flows and despite being possibly explained by fatty infiltration of muscles narrowing
10 directly the upper airway, recent literature suggest that this functional parameter might be used as
11 an early predictor of subclinical asthma. In order to obtain a complete evaluation of bronchial status
12 in this study we performed also FeNO analysis. In fact recent studies revealed that FeNO is a
13 potentially useful measure to evaluate the role of airways inflammation in asthma, as it represents
14 the forerunner of the most important event namely “remodelling” of bronchial airways.³⁹ In fact
15 FeNO, as recommended in the Consensus Report Practall for the Diagnosis, Management and
16 Treatment of Childhood Asthma⁴⁰ offers a logical complementary item to the only use of clinical
17 and functional data, evaluating the bronchial eosinophilic inflammation. In our study, as expected,
18 both groups of asthmatic children presented highly elevated FeNO values when compared to normal
19 controls reflecting the underlying bronchial inflammation process. Data from animal and human
20 studies clearly demonstrate that the enhancement of normal adipose tissue functions in obesity leads
21 to a systemic proinflammatory state.⁴¹ Adipose tissue from obese individuals expresses a number of
22 proinflammatory molecules, such as leptin, tumor necrosis factor- α (TNF- α), IL-6, transforming
23 growth factor- β 1 (TGF- β 1), and C-reactive protein (CRP), and there appears to be significant
24 overlap between the immune function of adipocytes and the function of T-lymphocytes and
25 macrophages, particularly with respect to the elaboration of inflammatory cytokines.^{42,43} Although
26 a conclusive relationship between obesity and airway inflammation has not yet to been described, it
27 could be argued that the role of the proinflammatory state underlying the main metabolic and
28 cardiovascular complications in obesity might play a pivotal role in determining airways

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3 inflammation independently of any allergic mechanisms. This might explain the findings of our
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5 study where FeNO values were significantly higher in obese non-asthmatic children compared to
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7 healthy controls and the findings of Santamaria et al.⁴⁴ who found a positive correlation between
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9 FeNO and BMI in healthy children. However it needs to be acknowledged that in the multiple
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11 regression analysis obesity was not a predictor for increased FeNO values. This apparent
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13 contrasting findings might be partially explained by the age group of our study population as
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15 obesity related inflammation might not have reached its full implication and a possible involvement
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17 of the respiratory system might require a long standing history of obesity. As puberty is a well
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19 known risk factor for developing obesity related complications it might be of crucial interest to
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21 obtain longitudinal data starting in prepuberty and lasting through all pubertal stages in order to not
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23 underestimate the possible role of obesity on the development of bronchial inflammation and
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25 therefore to fully explore this intriguing relationship.
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31 In conclusion, while AHR and airways inflammation are clearly associated to an asthmatic status,
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33 obesity seems to induce early proinflammatory airways changes combined with reduction of higher
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35 intrathoracic airway flows. Longitudinal data are required in order to clarify whether these changes
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37 might represent risk factor for asthma later on in life.
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43 Acknowledgements

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47 Di Luca D, Diomedi A, for their daily support in clinical research and care of children.
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Table 1. Anthropometric measurements and respiratory parameters of the study population.

	ObA	NwA	Ob	Nw	p^{\dagger}
Clinical and Anthropometric characteristics					
Gender (M/F)	16/14	16/14	14/16	14/14	NS*
Age (years)	7.03 [6.05 – 8.02]	7.06 [6.77 – 8.05]	8.04 [7.05 – 9.02]	6.1 [6.03 – 8.09]	NS
Weight (Kg)	35 [31 – 45.25]	25.75 [22 – 30.2]	42 [36.5 – 48]	24 [21 – 31.5]	0.0001
SDS-Height	0.5 [0.06 – 1.2]	0.5 [0.05 – 1.1]	0.56 [0.05 – 1.55]	0.48 [0.051 – 1.2]	NS
SDS-BMI	4.6 [3.85 – 5.45]	0.4 [-0.25 – 1.3]	5 [4.55 – 7.1]	0 [-0.4 – 0.1]	0.0001
Respiratory inflammation marker					
FeNO (ppb)	5.1 [2.8 – 10.3]	7.4 [3.5 – 17]	3.5 [2.3 – 4.3]	2.3 [2.1 – 2.4]	0.0002
Baseline Respiratory Functional Parameters					
FVC (Z-score)	-0.26 [-0.78 – 0.53]	-0.11 [-0.48 – 0.33]	-0.28 [-1.12 – 0.02]	0.25 [-0.76 – 1.99]	NS
FEV₁ (Z-score)	-0.14 [-0.84 – 0.31]	0.025 [-0.35 – 0.44]	0.12 [-0.74 – 0.73]	-0.13 [-0.66 – 2.01]	NS
FEV₁/FVC (Z-score)	0.11 [-0.57 – 0.69]	0.27 [-0.58 – 1]	0.69 [-0.37 – 1.45]	0.85 [0.62 – 1.10]	0.025
FEF₂₅₋₇₅ (Z-score)	-0.13 [-0.49 – 0.25]	0.05 [-0.32 – 0.75]	0.35 [-0.30 – 0.93]	0.4 [0.08 – 0.56]	NS
Respiratory Functional Parameters after “free run” Exercise Challenge Test					
post-FEV₁ (Z-score)	-0.97 [-2.12 – 0.33]	-1.11 [-1.60 – -0.37]	0.09 [-0.69 – 0.85]	-0.03 [-1.57 – -0.03]	0.009
post-FVC (Z-score)	-1.01 [-1.71 – 0.31]	-1.15 [-1.53 – -0.30]	-0.43 [-1.15 – 0.36]	0.20 [-1.11 – 0.96]	NS
post-FEV₁/FVC (Z-score)	-0.63 [-1.12 – 0.48]	0.18 [-0.80 – 0.81]	0.68 [-0.14 – 1.95]	1.35 [-0.61 – 1.53]	0.01
post-FEF₂₅₋₇₅ (Z-score)	-0.22 [-0.65 – 0.13]	-0.06 [-0.55 – 0.51]	0.3 [-0.21 – 0.49]	0.42 [-0.91 – 0.62]	NS
Fall in FEV₁ >12%	5/30 16.7%	7/30 23.3%	2/30 6.7%	0/28 0%	0.008*

Values are Median [Interquartile Range]. NS, no significant difference ($P > 0.05$). M, male; F, female.

\dagger Kruskal Wallis Test

*Pearson's Chi-square (X^2) Test

BMI= Body Mass Index;

FVC= Forced Vital Capacity; FEV₁= Forced Expiratory Volume in 1 sec; FEV₁/FVC= Forced Expiratory Volume in 1 sec/ Forced Vital Capacity; FEF₂₅₋₇₅= Forced Expiratory Flows between 25% and 75% of the Forced Vital Capacity

All the tests are expressed as Z-score.

Table 2. Comparison of the respiratory parameters after the 6MWT within each group.

	ObA	NwA	Ob	Nw
<i>FVC (Z-score)</i>	-0.26 [-0.78 – 0.53]	-0.11 [-0.48 – 0.33]	-0.28 [-1.12 – 0.02]	0.25 [-0.76 – 1.99]
<i>post-FVC (Z-score)</i>	-1.01 [-1.71 – 0.31]	-1.15 [-1.53 – -0.30]	-0.43 [-1.15 – 0.36]	0.20 [-1.11 – 0.96]
<i>p[§]</i>	NS	0.0001	NS	NS
<i>FEV₁ (Z-score)</i>	-0.14 [-0.84 – 0.31]	0.025 [-0.35 – 0.44]	0.12 [-0.74 – 0.73]	-0.13 [-0.66 – 2.01]
<i>post-FEV₁ (Z-score)</i>	-0.97 [-2.12 – 0.33]	-1.11 [-1.60 – -0.37]	0.09 [-0.69 – 0.85]	-0.03 [-0.75 – 2.46]
<i>p[§]</i>	NS	0.0001	NS	NS
<i>FEV₁/FVC (Z-score)</i>	0.11 [-0.57 – 0.69]	0.27 [-0.58 – 1]	0.69 [-0.37 – 1.45]	0.85 [0.62 – 1.10]
<i>post-FEV₁/FVC (Z-score)</i>	-0.63 [-1.12 – 0.48]	0.18 [-0.80 – 0.81]	0.68 [-0.14 – 1.95]	1.35 [-0.61 – 1.53]
<i>p[§]</i>	0.03	NS	NS	NS
<i>FEF₂₅₋₇₅ (Z-score)</i>	-0.13 [-0.49 – 0.25]	0.05 [-0.32 – 0.75]	0.35 [-0.30 – 0.93]	0.4 [0.08 – 0.56]
<i>post-FEF₂₅₋₇₅ (Z-score)</i>	-0.22 [-0.65 – 0.13]	-0.06 [-0.55 – 0.51]	0.3 [-0.21 – 0.49]	0.42 [-0.91 – 0.62]
<i>p[§]</i>	NS	0.02	NS	NS

Values are Median [Interquartile Range]. NS, no significant difference ($P > 0.05$).

[§] Wilcoxon Signed Ranks Test

FVC= Forced Vital Capacity; FEV₁= Forced Expiratory Volume in 1 sec; FEV₁/FVC= Forced Expiratory Volume in 1 sec/ Forced Vital Capacity; FEF₂₅₋₇₅= Forced Expiratory Flows between 25% and 75% of the Forced Vital Capacity

All the tests are expressed as Z-scores.

Table 3. Independent effect of asthma and obesity on lung function and bronchial inflammation.

Source	Dependent Variable	Type III Sum of Squares	F	p [#]
Asthma	<i>FeNO</i>	655,174	3,957	0.048
	<i>MEF₇₅ (%)</i>	505,481	1,038	NS
	<i>PEF (%)</i>	33,466	0,184	NS
	<i>FEV₁/FVC (Z-score)</i>	18,871	0,623	NS
Obesity	<i>FeNO</i>	119,116	0,719	NS
	<i>MEF₇₅ (%)</i>	4156,102	8,534	0.005
	<i>PEF (%)</i>	1131,609	6,232	0.015
	<i>FEV₁/FVC (Z-score)</i>	5,290	0,175	NS

NS, no significant difference ($P > 0.05$).

[#] Multiple analysis of covariance (MANCOVA)

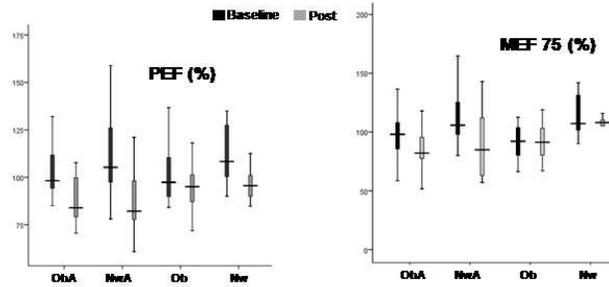
FeNO= Fractional exhaled Nitric Oxide; MEF₇₅= Maximal Expiratory Flow at 75% of the Forced Vital Capacity; PEF= Peak Expiratory Flow; FEV₁/FVC= Forced Expiratory Volume in 1 sec/ Forced Vital Capacity

FEV₁/FVC ratio are expressed as Z-score.

MEF₇₅ and PEF are expressed as percentage of theoretical values for age, gender, and height.

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Figure 1. Differences in PEF and MEF₇₅ obtained at baseline and after 6MWT.



Values are Median [Interquartile Range].

PEF= Peak Expiratory Flow; MEF₇₅= Maximal Expiratory Flow at 75% of the Forced Vital Capacity.

All the tests are expressed as %-predicted.

254x190mm (96 x 96 DPI)

Review



UNIVERSITY OF CHIETI
- ITALY -
www.unich.it/cliped/

Pediatric Department
(Chief: Prof. Francesco Chiarelli)



Chieti, Italy, March 6th 2010

Dear Editor,

Thank you for giving us the possibility of revising and resubmitting our manuscript PPUL-09-0317 entitled **“The reciprocal influences of asthma and obesity on lung function testing, AHR and airway inflammation in prepubertal children.”**

We thank you and the referees for the detailed review and for the precious comments, which we have addressed thoroughly thereby improving our manuscript substantially.

We have tried to answer the major comments of referee 1 and 2 and please find enclosed a point-by-point answer sheet for each of them.

In agreement with your suggestion we have now calculated and expressed the results of the lung function tests as Z-score, according to the equations proposed by Stanojevic et al. (AJRCCM 2008). However, we continue to present the values of MEF₇₅ and PEF as %-expected because for these parameters the equations for Z-score were not available and the main differences detected in our study regarded these indices of lung function.

Furthermore, as requested we added all necessary information concerning our inclusion and exclusion criteria in the method section. We have modified the introduction in order to define in a clear way the main hypothesis of our study. Finally the most important limitation consisting of the cross sectional nature of this study has been discussed.

Please find enclosed the revised version of our manuscript where the changes have been marked-up.

We hope that our findings on this intriguing relationship between obesity and asthma are of interest to your readership and hope that the manuscript is now acceptable for publication in your prestigious journal.

Do not hesitate to contact us should further changes be necessary

Yours Sincerely,

Angelika Mohn

Department of Pediatrics
University of Chieti
Ospedale Policlinico
Via dei Vestini 5
66100 Chieti, Italy
Fax: +39 0871-574831
E-mail: amohn@unich.it