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Full Length Reseach Paper

Assessment of airway inflammation with exhaled nitric oxide in obese Tunisian women: Obesity and airway inflammation in Tunisian women

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Obesity and asthma are associated disorders but the mechanisms responsible for this relationship are unclear. Exhaled nitric oxide has been proposed as a non invasive marker of airway inflammation in asthma. The aim of this study was to investigate the relationship between exhaled nitric oxide, body mass index (BMI) and airway hyper-responsiveness to methacholine. Fifty two (21 lean and 31 obese) healthy women were recruited and examined in the Clinical Laboratory of Physiology located in the Medical School of Sousse. The average ages (± s.e.) of the two groups of lean and obese were 35.6±2.1 and 37.4±1.9 years, respectively. Their corresponding mean BMI (± s.e.) were 22.1±0.3 and 37.7±1.2 kg.m^{-2}, respectively. Pulmonary function tests, exhaled NO and airway hyper-responsiveness were investigated in the two groups. Our data indicate that: (1) exhaled NO levels were significantly correlated BMI (r=0.85; p<0.001) as well as with the efficacy of the MCH agonist promoting the maximal response (r=0.45; p=0.001); and 2) the mean of exhaled NO was significantly higher in obese than in lean group (p<0.001). We concluded that obesity induced airway hyper-reactivity in obese women through airway inflammation as evidenced by the significant association between exhaled NO and BMI.

Key words: Airway hyper-reactivity, asthma, inflammation, obesity, women.

INTRODUCTION

It is well known that obesity has become a major disease and its prevalence is increasing worldwide among children and adolescents (Gordon-Larsen et al., 2004; Dehghan et al., 2005). Tunisia is experiencing a rapid increase in adult and child overweight (Ghannem et al., 2001; Beltaifa et al., 2009). Obesity is also becoming a serious risk factor that may play an important role in the induction and exacerbation or persistence of asthma (Weiss and Shore, 2004). This was investigated by recent studies which showed that obesity affects pulmonary function performance and induces airway hyper-responsiveness (Shin et al., 2008; Chouchane et al., 2010; Celtin et al., 2012). Several potential mechanisms may play a role in the relationship between obesity and asthma severity. Some investigators have proposed immunological and inflammatory interventions (Fantuzzi, 2005) by which obesity could interact with asthma. For instance, the adipose tissue produces and releases two hormones, leptin and adiponectin, that exert pro-inflammatory (Huang and Li, 2000) and anti-inflammatory (Ouchi et al., 2003) roles, respectively. Interestingly, serum leptin is known to increase markedly in obesity, and is found at higher levels in asthmatics than in non-asthmatics (Guler et al., 2004), regardless of the extent of obesity. In contrast, serum adiponectin levels have the tendency to be reduced in obese subjects (Chandran et al., 2003). Further evidence of a direct association between obesity and airway hyper-reactivity has been shown in an experimental study in which ozone-induced airway hyper-reactivity and bronchial inflammation in obese mice were more pronounced than that observed in lean wild-type mice (Shore et al., 2003). Several invasive and non-invasive methods have been proposed to assess airway inflammation, including bron-

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choalveolar lavage, biopsy and induced sputum (Fahy et al., 1995). Although the examination of induced sputum for cell and fluid-phase constituents is a relatively non-invasive and safe technique (Karakurt et al., 2001), it requires a consistent cooperation of the subjects. On the other hand, the measurement of exhaled nitric oxide (eNO), a recognized marker of airway inflammation in airway diseases in general and asthma in particular, is a simple and easy non-invasive procedure, and has emerged as a potential clinical tool that uses a range of commercially available analyzers (Berlyne et al., 2000). In a previous study, we have shown that obesity induced broncho-pulmonary hyper-responsiveness in healthy women (Parker et al., 1965). In the present study, we hypothesize that obesity plays a significant role in the induction of asthma by altering pulmonary function performance and enhancing airway inflammation. To this end, our research was focused on healthy non-smoking groups of women (21 lean, 31 obese) to 1) determine quantitatively the efficacy and the potency of the broncho-constricting agonist in relation to the Body Mass Index (BMI) of our female subjects and to analyze whether the fraction of eNO is associated with the BMI as well as with the pulmonary function decrements.

MATERIALS AND METHODS

Study subjects

A total of 52 volunteers and healthy women (31 obese and 21 lean) were recruited from Sousse city and its suburban small towns. Only females were included in this study because the obesity of males in Tunisia is low, and unlike women, the large majority of men are heavy cigarette smokers. Exclusion criteria admitted in the study were high blood pressure, a medical history of asthma, chronic respiratory, heart or any other diseases. With the exception of birth control pills and vitamin supplements, women who regularly took medication of any kind were excluded from the study. All our participating subjects never smoked cigarettes, and were neither pregnant nor breastfeeding. They were characterized as lean if their BMI was < 25 kgm^{-2} and obese if their BMI was ≥ 30 kgm^{-2}.

Each of the 52 subjects who completed the study were first asked to fill in medical and smoking history forms, and then participated in a health screening session which included an overall medical examination assessed by a physician, and measurements of their heights, weights and resting blood pressures. All research sessions, including health screening and physiological measurements were carried out at the Farhat Hached hospital of the Medical School of Sousse. The experimental protocol and informed consent procedures were approved by the Research Ethics Committee of this institution.

Study design

The main objective of this study is to determine whether a relationship exists between obesity and airway inflammation.

Drugs

MCH chloride, a cholinergic neurotransmitter analog of acetylcholine, showed greater duration and selectivity of action than did the parent compound and was well tolerated without producing systemic effects (Parker et al., 1965, O'Connor et al., 1987). Owing to stability constraints, MCH was distributed (Sigma-Aldrich, Steinheim, Germany) as a crystalline powder in sterile and sealed vials and was kept refrigerated in desiccators. The nebulized solutions were prepared with sterile saline and stored at $4^{o}C$ to avoid contamination and decomposition.

Pulmonary function tests (PFT) and methacholine challenge (MCH)

To screen subjects for their respiratory performance conditions, their pulmonary function testing parameters, including forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and FEV1/FVC were measured using a commercial whole body plethysmograph (ZAN Messgerate GmbH, Oberthulba, Germany).To undergo a MHC challenge test, the results of the subject's pulmonary function parameters had to be within normal limits, defined as an FEV1/FVC ratio > 75% of the predicted Knudsen value (Quanjer, 1988). The MCH challenge test is a standard clinical method for assessing bronchopulmonary responsiveness (BPR). As a first step, the subject started breathing saline as a control solution, and then inhaled MCH in ascending aerosolized doses (Ryan et al., 1981). The aerosolization of MCH was produced by an air-compressed nebulizer, and the inhalation of the broncho-constrictor was achieved using an FDC 88 dosimeter (MEDIPROM, Paris, France). As the nebulizer was equipped with a solenoid valve, the opening of which occurred only during inspiration, the subject was instructed simply to breathe the aerosolized bolus of MCH through the nebulizer at her vital capacity. The inhalation of MCH was performed successively every 5 min in cumulative doses ranging from 100 to 3000 µg. Thirty and ninety seconds after each MCH bolus inhalation, BPR was assessed twice by measuring the decrease in FEV1 in response to the cumulative doses of the broncho-constricting agonist , using a commercial spirometer (Pal MINATO, OSAKA, JAPAN). The best value of the two measurements of FEV1 was retained at the end. A cumulative concentration response curve (CCRC) was then obtained for each subject and plotted

Table 1. Anthropometric and pulmonary function parameters of the subject population. Data are given as mean ± standard error (s.e.).

	Lean (n=21)	Obese (n=31)	P-value
Age (years)	35.67±2.10	37.42±1.97	0.507
Height (m)	1.62±0.01	1.57±0.008	0.003
Weight (Kg)	58.9±1.7	95.4±3.6	<0.001
BMI (Kg.m-²)	22.18±0.39	37.70±1.25	<0.001
FVC (l)	3.19±0.13	3.22±0.11	0.444
FEV1 (l)	2.80±0.09	2.69±0.08	0.366
FEV1/FVC (%)	88.71±1.55	84.30±0.96	0.026
SBP (mm Hg)	120.48±1.46	113.23±1.56	0.003
DBP (mm Hg)	64.76±2.24	63.87±2.20	0.714

Significance was defined as $p < 0.05$. Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1s; SBP, systolic blood pressure; DBP, diastolic blood pressure.

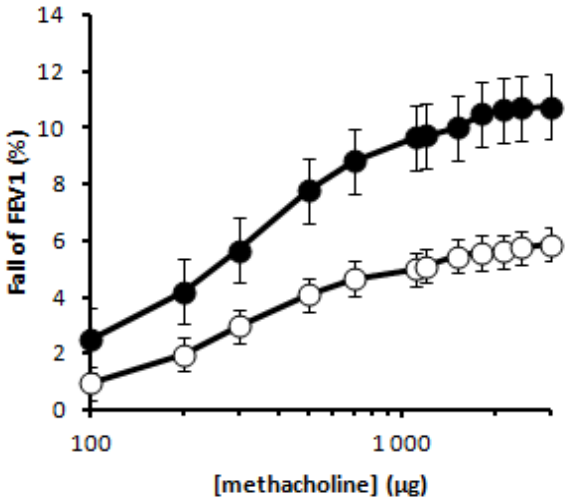


Figure 1. Mean cumulative concentration response curves of the % fall in FEV1 in response to methacholine challenge (MCH) in lean and obese women. These results indicate that MCH increased the decrement of FEV1 in a dose-dependent manner in the two groups, with the obese patients responding significantly higher than the lean subjects. Symbols: :obese group, :lean group.

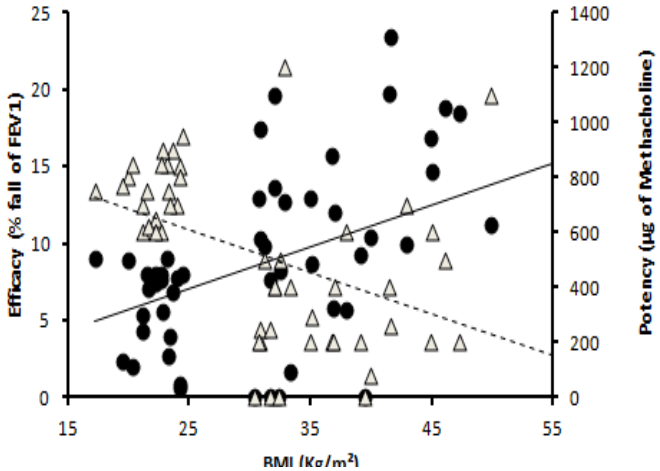


Figure 2: Correlation of both individual efficacy (Emax) and potency (ED50), with individual body mass index (BMI). These correlations were positively (—) and negatively(---) significant (r=0.45, P=0.001) and (r=-0.72, P<0.0001), respectively. Symbols: Emax, Potency.

in a semi-log_{10} scale curve to determine two quantitative response parameters characterizing the BPR of each subject: (1) the efficacy of the MCH agonist promoting the maximal response (E_{max}); and (2) its potency or effective dose producing 50% of the maximal response (ED_{50}). E_{max} was determined from the plateau of the dose–response curve, and ED_{50} was estimated from the position of the approximate sigmoid curve corresponding to 50% of the plateau.

Measurement of exhaled NO (eNO)

A fast-responding chemiluminescence NO analyzer (Medisoft, Dinant, Belgium) was used to determine the fraction of nitric oxide in the exhaled breath. eNO was measured using a restricted breathing technique which employed expiratory resistance and positive mouth pressure to close the volume and exclude nasal NO, and a constant expiratory flow of 50 $mL.s^{-1}$ (ATS/ERS, 2005). Subjects inhaled to total lung capacity and exhaled whilst targeting a pressure from 5 to 20 mmHg. These eNO measurements were taken prior to the PFT and to the methacholine challenge.

Analysis

Data analysis was carried out using the Statistical Package for the Social Sciences software (SPSS 11.0, Chicago, IL, USA). A U Mann-Whitney test was used to analyze differences in response parameters between lean and obese subjects, and correlations between variables were performed using the Spearman rank corr-

Figure 3. Mean fraction of exhaled nitric oxide (FeNO) in lean and obese subjects. These results indicate that FeNO increased significantly in obese than in the lean group (P<0.0001).

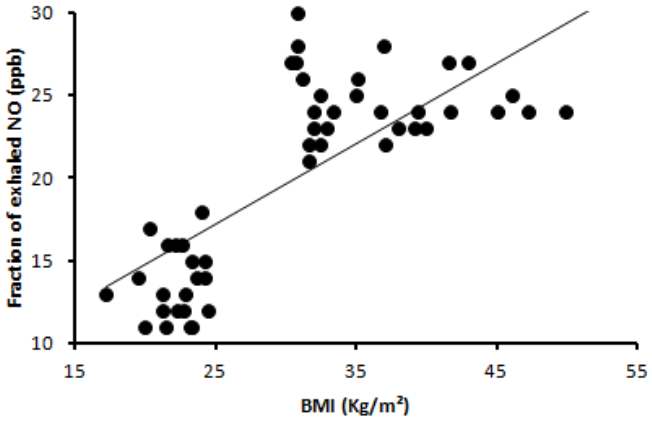
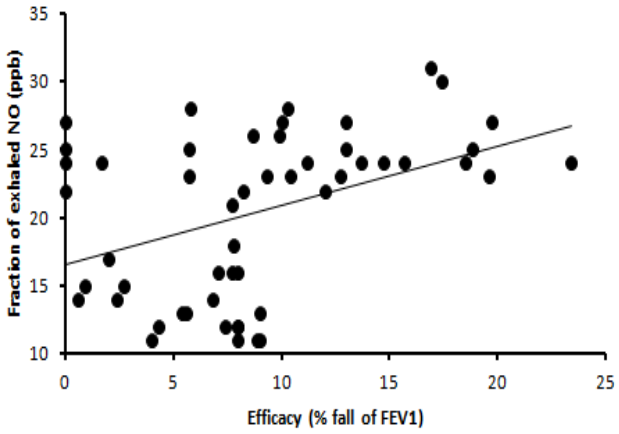


Figure 4. Correlation between body mass index (BMI) and fraction of exhaled nitric oxide (FeNO). This correlation was significant (r=0.85, P<0.0001).



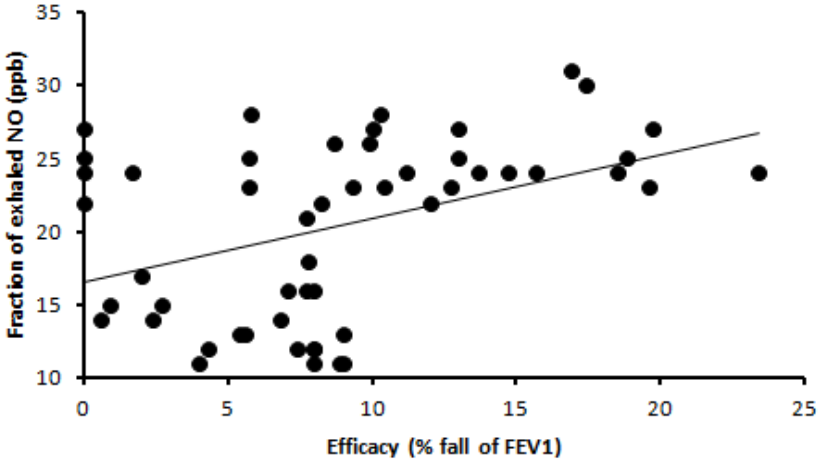


Figure 5. Significant correlation between % fall of FEV1 (efficacy) and fraction of exhaled nitric oxide (FeNO) (r=0.4, P=0.004).

elation test. In all statistical tests, the level of significance was set at p=0.05.

RESULTS

Subject characteristics are summarized in Table 1. It contains an analysis of the values of anthropometrical, ventilator variables and blood pressure, each pooled for the 21 lean and 31 obese women. Means of the forced vital capacity (FVC), forced expiratory volume in 1s (FEV1) and diastolic blood pressure (DBP) values were not significantly different between the two groups. However, although the adjusted mean of FEV1/FVC ratio was significantly lower in obese than in lean subjects, it was within normal range of healthy individuals according to the predicted values of Knudsen (Quanjer, 1988) which take into account the age and height of the subjects. The results for the AHR of the two groups of women to cumulatively increasing doses of MCH are graphed in Figures 1 and 2. Judging from these plots, three convergent and prominent features associating obesity with AHR can be singled out: (1) the CCRC points in Figure 1 illustrate that the decrease in FEV1, averaged for all subjects over the explored range of MCH doses, was consistently and significantly more pronounced in the obese than in the lean subjects; (2) the overall mean of maximum fall in FEV1 (efficacy or E_{max}) induced by MCH challenge was significantly higher in the obese group (10.76%) than in the lean group (5.88%), and the individual efficacies were positively correlated with BMI (Figure 2); and (3) the potency of MCH (ED_{50}) was significantly lower in the obese than in the lean subjects, and the individual potencies were negatively correlated with BMI (Figure 2). Similar to FEV1 decrements, Figure 3 shows that the fraction of exhaled NO (FeNO), averag-

ed for all subjects, was steadily and significantly higher in the obese (overall mean is 24.9 ppb) than in the lean subjects (overall mean is 13.6 ppb). Additionally, our results of both exhaled NO and AHR show that there are similar correlations of eNO (Figure 4) and FEV1 decrement (Figure 2) with BMI that resulted in a clear cross-correlation between the exhaled NO and the fall of FEV1 in response to MCH challenge (Figure 5).

DISCUSSION

Obesity and asthma are chronic diseases that affect millions of people all over the world. Moreover, recent data emphasize that obesity is an important risk factor for asthma (Vieira et al. 2005; GINA, 2006; Chouchane et al., 2010, Tam et al., 2011). Accordingly, this study was designed to determine whether airway inflammation is the pathway via which obesity acted to induce bronchopulmonary hyper-responsiveness that we had observed in our previous investigation (Chouchane et al., 2010). Our new data clearly indicate that eNO concentration is higher in obese than in lean group (24.87 versus 13.62 ppb, respectively) (Figure 3). Also, the efficacy of methacholine and the fraction of eNO are significantly correlated with the BMI of our healthy female subjects, suggesting the potential implication of airway inflammation in the induction of airway hyper-responsiveness by obesity. The rationale for using eNO is because it is a simple technique to use and it is a noninvasive biomarker for several respiratory and inflammatory diseases as well as in obstructive sleep apnea (OSA) (Olopade et al., 1997; Demange et al., 2010). A number of interesting hypotheses and pathways have recently emerged that individually or collectively can explain the important link between obesity and airway inflammation. First, NO is derived from L-arginine by the enzyme NO-synthase (NOS), of which at least three distinct isoforms exist in the human body (Kharitonov and Barnes, 2000). But the expression of the inducible NO synthase (iNOS) predominates in bronchial epithelial cells, its activity is induced by inflammatory mediators and its expression is significantly increased in asthma, but the exact mechanism leading to this overexpression remains poorly understood (Cuzzocrea et al., 2000). Thus, the excess production of NO resulting from the expression of inducible NOS by inflammatory cells of the airways has a double meaning. First, the excess of NO in the airways may be considered a reflection of airway inflammation. Second, NO and free radicals of oxygen (ROS) formed from NO, are a source of aggression in cellular metabolism by oxidative stress they induce (Dinh-Xuan et al., 1998). This explanation is consistent with the crucial role of inflammation in obesity. One additional link between obesity and asthma may be the adipokines and their effects on airway inflammation as shown in recent studies (Sood, 2010, Khan, 2013).Our findings indicate that methacholine challenge and eNO measurement are good markers for assessing airway inflammation induced by obesity. But, further investigations are needed to discern the influence of the oxidant status on the airway inflammation in obese patients and to clarify the relationship between leptin/adiponectin and other inflammatory cytokines involved commonly in asthma and obesity.

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