Deep inspiration avoidance and airway response to methacholine: Influence of body mass index

Louis-Philippe Boulet MD FRCPC, Hélène Turcotte MSc, Geneviève Boulet DEC, Barbara Simard BSc, Patricia Robichaud BSc

OBJECTIVE: To evaluate the effects of deep inspiration avoidance response to methacholine inhalation in 23 nonobese (body mass index between 18 kg/m² and 30 kg/m²) and 27 obese (body mass index ≥ 30 kg/m²) subjects.

METHODS: Each subject had four methacholine challenges. In tests A and B, the first postmethacholine forced expiratory volume in 1 s (FEV₁) was measured at 30 s and 3 min postinhalation, respectively; tests C and D were single-dose tests (using the final dose of test B), with the first postmethacholine FEV₁ being obtained at 3 min, without (test C) or with (test D) 20 min of deep inspiration avoidance before inhalation.

RESULTS: The mean provocative concentrations inducing a 20% fall in FEV₁ on tests A and B were 80.6 mg/mL and 28.5 mg/mL (P < 0.0001) in nonobese subjects, respectively, and 56.3 mg/mL and 21.5 mg/mL (P < 0.0001) in obese subjects, respectively. No significant differences were observed in test A or B between control and obese subjects. Mean falls in FEV₁ for tests C and D were 20.3% and 40.0% (P = 0.0003) in nonobese subjects, respectively, and 18.5% and 23.6% (P > 0.05) in obese subjects, respectively.

CONCLUSIONS: As previously observed in patients with asthma, the present study found that nonasthmatic obese subjects had no increase in the fall in FEV₁ after deep inspiration avoidance before methacholine, whereas nonobese subjects did, suggesting that obesity alters airway function. No significant changes were found between groups for symptom perception.

Key Words: Airway responsiveness; Asthma; Deep inspiration; Methacholine

Several studies (1,2) have reported an increased prevalence of asthma or asthma-like symptoms in obese subjects. As a corollary, weight loss has been associated with an improvement in lung function and a reduction in asthma severity (3,4). However, it is still unclear how obesity affects airway function and asthma severity. Among the possibilities proposed in a recent paper published in the National Heart, Lung, and Blood Institute workshop report (5) on obesity and asthma, several factors have been considered: inhaled corticosteroids, allergic mechanisms, neurogenic mechanisms, and possible mechanical factors. With regard to these last factors, reduced airway distension during inspiratory manoeuvres and loss of airway smooth muscle (ASM) stretch-relaxation have been considered, as has also been suggested in patients with asthma (6). It has indeed been proposed that the failure to regularly stretch the ASM can result in a potentially hyperresponsive state for the muscle (7,8). Healthy subjects do not develop airway narrowing in response to bronchoconstrictor agents (agents that induce bronchoconstriction in asthmatic patients) but do develop an exaggerated airway responsiveness when they avoid deep inspirations (8,9). In patients with asthma, however, deep inspirations have little or no influence on the airway response to methacholine.

Limited information is available on the influence of obesity on airway responses to bronchoconstrictor agents. In attempting to elucidate the possible influences of obesity on airway function, we hypothesized that obesity is associated with an altered airway response in the form of a reduced influence of deep inspiration.

Effets de l’absence d’inspirations profondes sur la réponse bronchique à la méta-choline : Influence de l’indice de masse corporelle

OBJECTIF : Évaluer les effets de l’absence d’inspirations profondes sur la réponse à la méta-choline chez des sujets normaux sans allergie et sans asthme : 23 non-obèses (18 kg/m² < indice de masse corporelle < 30 kg/m²) et 27 obèses (indice de masse corporelle ≥ 30 kg/m²).

MÉTHODES : Les sujets ont eu quatre tests de provocation bronchique à la méta-choline. Lors des tests A et B, pendant lesquels les doses croissantes de méta-choline étaient inhalées, la première mesure du volume expiratoire maximal en une seconde (VEMS) était prise, respectivement, 30 s et 3 min après la fin de chaque inhalation. Lors des tests C et D (tests de provocation à dose unique avec la dose finale du test B), la première mesure de VEMS était obtenue 3 min après la fin de l’inhalation de méta-choline.

RÉSULTATS : La concentration moyenne provoquant une chute de 20 % du VEMS était de 80,6 mg/mL après le test A et de 28,5 mg/mL après le test B (p < 0,0001) chez les sujets non-obèses, et de 56,3 mg/mL et 21,5 mg/mL (p < 0,0001) chez les sujets obèses. Il n’y avait pas de différence significative entre les groupes pour la CPm, observée au test A ou au test B.

CONCLUSION : Le principal point d’intérêt de cette étude est que, tel qu’observé précédemment chez les sujets asthmatiques, les sujets obèses n’ont pas d’augmentation de chute du VEMS en l’absence de prise d’inspirations profondes précédant la provocation à la métacholine alors que les sujets non-obèses, dans les mêmes conditions, voient leur VEMS diminuer de façon significative, suggérant que l’obésité affecte la fonction respiratoire. Aucune différence significative n’a été observée entre les groupes en ce qui a trait à la perception des symptômes induits.
Table 1: Subjects’ characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonobese subjects BMI &lt;30 kg/m² (n=23)</th>
<th>Obese subjects BMI ≥30 kg/m² (n=27)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, men/women</td>
<td>4/19</td>
<td>5/22</td>
<td></td>
</tr>
<tr>
<td>Mean age, years</td>
<td>32.2 (19 to 59)</td>
<td>34.6 (21 to 50)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>103.9±12.3</td>
<td>97.6±13.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>(range)</td>
<td>(87 to 122)</td>
<td>(70 to 147)</td>
<td></td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>104.4±1.5</td>
<td>98.6±13.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>(range)</td>
<td>(90 to 119)</td>
<td>(72 to 147)</td>
<td></td>
</tr>
<tr>
<td>Mean PC₂₀, mg/mL</td>
<td>80.6 (±SEM 80 to 87.5)</td>
<td>56.3 (±SEM 47.5 to 67.0)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>(range)</td>
<td>(16 to &gt;128)</td>
<td>(9 to &gt;128)</td>
<td></td>
</tr>
<tr>
<td>Mean BMI, kg/m²</td>
<td>22.3 (range 19 to 28)</td>
<td>37.3 (range 30 to 49)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BMI: Body mass index; FEV₁: Forced expiratory volume in 1 s; FVC: Forced vital capacity; PC₂₀: Provocative concentration inducing a 20% fall in FEV₁.

inspiration on the methacholine response. Among other factors that potentially modulate the airway response to methacholine is the time of the first forced expiratory volume in 1 s (FEV₁) after the first inhalation of methacholine. We previously described that when the first FEV₁ was obtained at 3 min compared with 0.5 min postmethacholine, there was an increased airway response to methacholine in both normal and asthmatic subjects, although it was more marked in the latter group (10).

The goal of the present study was to determine whether obese subjects, in comparison with nonobese subjects, have changes in airway responses similar to those found in asthma. We therefore looked at the influence of avoidance of deep inspirations before or after methacholine inhalation on airway response. We also investigated whether the perception of symptoms was different between obese and nonobese subjects.

METHODS

Subjects
Subjects were enrolled from advertisements in local newspapers and at Laval University, Ste-Foy, Quebec. Fifty nonatopic, non-smoking subjects who had normal airway responsiveness (ie, the provocative concentration of methacholine inducing a 20% fall in FEV₁ [PC₂₀] was greater than 8 mg/mL) (Table 1) were enrolled (11). The group was divided into 23 nonobese (body mass index [BMI] less than 30 kg/m²) and 27 obese subjects (BMI 30 kg/m² or greater) (12). All subjects were nonsmokers and none had ever had a diagnosis of asthma or reported respiratory symptoms consistent with asthma. They showed no evidence of other pulmonary disease and had no contraindication to the tests. None of the subjects reported respiratory airway infection in the month preceding the study. The study was approved by an institutional ethics committee. All subjects signed an informed consent form.

Study design
A previously developed study design was used that was originally developed to look at the differences in airway responses between normal and asthmatic subjects following avoidance of deep inspiration before and/or after methacholine (10). Subjects completed four visits at the same time of day on four separate days within a 10-day period and, for those considered obese (ie, having a BMI 30 kg/m² or greater), another visit was required for body plethysmography, within one month of the first visit (12,13).

During the first visit, subjects completed a standardized respiratory questionnaire to evaluate their pulmonary function, symptoms, family and personal medical history, atopy, smoking and environment. They had skin-prick tests with a battery of common airborne allergens and blood sampling for eosinophil cationic protein measurements.

To determine the frequency of deep inspirations in both groups when inspiratory manoeuvres were not restricted, the subjects were asked, while they were quietly seated on a chair, to breathe normally through a facial mask for 20 min. Their breathing pattern (volume and frequency of breaths) was recorded with a pneumotachograph.

A standard methacholine challenge with complete expiratory manoeuvres was performed with FEV₁ measured 30 s, 90 s, 3 min and 4 min from the end of methacholine inhalation (test A) (11). Baseline FEV₁ had to be greater than 70% predicted. Briefly, after the baseline FEV₁ measurements were made in triplicate, normal saline (0.9%) was inhaled with a Wright nebulizer (Roxon, Canada), with a mouthpiece and filter on the expiratory line, followed by increasing doubling concentrations of methacholine. These were given for 2 min at 7 min intervals (ie, a 5 min interval between the end of one inhalation and the start of the next). The lowest FEV₁ value postsaline was retained as the baseline value to estimate the percentage fall in FEV₁ after each next step. The lowest FEV₁ was measured after each concentration and the test was stopped either when there was a fall of 20% in FEV₁ compared with the postsaline value or when the highest concentration of methacholine (128 mg/mL) had been administered. If the FEV₁ fell between 17.5% and 20% from baseline, the methacholine challenge was stopped and the PC₂₀ was extrapolated.

A second visit included a standard methacholine challenge, including the inhalation of normal saline (0.9%), with FEV₁ measured only at 3 min and 4 min and not at 30 s and 90 s (test B) from the end of saline and methacholine inhalations. At the end of the inhalation, the patient continued to avoid deep inspirations for 3 min, after which the first FEV₁ was obtained.

For visits 3 and 4, tests C and D were performed in a random order.

During test C, a single-dose methacholine test was performed. Baseline FEV₁ was measured in triplicate and the lowest baseline value was retained to estimate the percentage fall in FEV₁. There was no saline inhalation. The dose of methacholine chosen for the test was the final dose that had induced a 20% fall in FEV₁ on test B, and FEV₁ was measured only at 3 min and 4 min from the end of inhalation. At the end of the inhalation, the patient continued to avoid deep inspirations for 3 min, after which the first FEV₁ was obtained.

Test D was the same as test C except that after baseline measures of FEV₁, the inhalation of the single dose of methacholine was preceded by 20 min of deep inspiration avoidance. During that period, the patient avoided any deep inspirations, including sneezing, coughing, laughing or any abnormal respiratory movement for 20 min before the onset of the inhalation of the single dose of methacholine. To ensure that no deep inspiration was taken, the respiratory volume was checked with a pneumotachograph.

The perception of symptoms during tests was evaluated on a perception scale from 0 to 10 (14).
Body plethysmography was performed in subjects with a BMI of 30 kg/m² or greater, with measures of total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV), inspiratory capacity, expiratory reserve volume (ERV), maximum inspiratory and expiratory pressures and airway resistance.

Data analysis
Results are expressed as mean ± SEM values and as medians for respiratory symptoms. Baseline FEV₁ for the four tests were compared using ANOVA. To analyze the effect of the time interval between the end of inhalation and the first FEV₁ measurement, paired t-tests were used to compare log PC₂₀ and fall in FEV₁ between tests A and B, and to compare the fall in FEV₁ between tests C and D to determine the influence of 20 min deep inspiration avoidance before methacholine inhalation (15).

Unpaired t-tests were used to compare the breathing pattern (total number of breaths in the 20 min period, total volume inspired, mean time duration/breath and mean volume/breath) between nonobese and obese subjects.

RESULTS
Breathing pattern during a 20 min period: A pilot study
In a preliminary analysis of a group of five obese and five nonobese subjects, the spontaneous pattern of breathing during a 20 min period was examined to determine whether there were differences in the magnitude and frequency of deep inspirations between the two groups.

There were no significant differences between the means of the total number and total volume of breaths in 20 min in nonobese (278±25 and 45.2±12.3 L, respectively) and obese subjects (277±41 and 36.5±5.4 L, respectively; both P>0.05), between the mean duration of one breath (4.5±0.4 s and 4.6±0.6 s, respectively; P>0.05), and the mean volume inspired/breath (0.161±0.040 L and 0.145±0.031 L, respectively; P>0.05). The number of inspirations greater than mean tidal volume was not significantly different between obese and nonobese subjects (Figure 1, P>0.05). In this group of 10 subjects, volumes measured by body plethysmography were available for four obese and four nonobese subjects: the obese subjects had a lower FRC (a mean of 84% of predicted values compared with a mean of 101% in nonobese subjects) and mean TLC values were 94% and 108%, respectively. For this reason, for the same amplitude of breath, they may not be as close to TLC as the nonobese subjects were.

Subjects' characteristics and pulmonary function
Subjects' characteristics are summarized in Table 1. The 50 subjects participating in the study consisted of nine men (four with a BMI less than 30 kg/m² and five with a BMI of 30 kg/m² or greater) and 41 women (19 with a BMI less than 30 kg/m² and 22 with a BMI of 30 kg/m² or greater) aged 19 to 59 years (mean age 33.5 years), with a BMI ranging from 18 kg/m² to 49 kg/m². The mean BMI for the nonobese subjects was 22.3 kg/m² (range 18 kg/m² to 28 kg/m²) and, for the obese subjects, it was 37.3 kg/m² (range 30 kg/m² to 49 kg/m²). All subjects completed the study. The two groups were similar with regard to age and sex. None of the subjects in either the nonobese or obese group were currently smoking; five subjects in the nonobese group and eight in the obese group were ex-smokers for more than one year (P>0.05), with respective medians of 4.0 pack-years and 2.4 pack-years (P>0.05), and medians of five and seven years of smoking cessation, respectively. The number of years of smoking was not significantly different between the groups.

Standard methacholine inhalation test (first FEV₁ at 30 s)
There were no significant differences between control and obese subjects with regard to baseline FEV₁ at first visit, with respective values of 103.9±2.3% and 97.9±3.0%, nor in baseline airway responsiveness, with a geometric mean PC₂₀ of 80.6 mg/mL and 56.3 mg/mL, respectively (P>0.05). Baseline FEV₁ and forced vital capacity (FVC) were normal in all subjects; they were not significantly different between the two groups, nor at baseline between the four tests in each subject (P>0.05). There was a significant difference between sexes for baseline airway responsiveness, with a mean PC₂₀ of 120 mg/mL in men and 50.4 mg/mL in women (P=0.03).

On body plethysmography, respiratory volumes of obese subjects were (as a percentage of predicted values): TLC 106±2%, FRC 84±5%, RV 110±5%, inspiratory capacity 124±4% and
Mean airway resistance was 116±7% of predicted value. Baseline FEV1 (percentage of predicted values) did not vary significantly between the two groups (P=0.05). For a similar dose of methacholine, avoidance of deep inspirations before methacholine in test D increased the fall in FEV1 compared with in test C in nonobese subjects (open circles indicate subjects with a body mass index less than 30 kg/m2; r_s= –0.231, P>0.05; Figure 2). The change in the number of doubling concentrations of methacholine was not correlated with age or BMI (P>0.05), but was correlated with PC20 on test A, the greater the drop in the doubling concentrations of methacholine the greater the fall in FEV1 (Spearman rank correlation [r_s]=0.47, P=0.02) but not in nonobese subjects (r_s=0.27, P=0.20).

**DISCUSSION**

The main finding of the present study is that in nonasthmatic obese subjects, contrary to what is observed in nonobese subjects, but similar to what has been previously described in asthmatic patients, there were no differences in the fall in FEV1 with or without previous avoidance of deep inspirations.
finding suggests the presence of altered airway protective mechanisms against bronchoconstricting agents, which could contribute to respiratory symptoms experienced by obese subjects. Furthermore, in both groups, the airway response to methacholine was similarly increased if the first postinhalation FEV₁ was measured at 3 min instead of 0.5 min.

This exploration of airway function was motivated by the previously observed increased prevalence of asthma and airway hyperresponsiveness (AHR) in obese subjects, and a marked improvement in asthma with weight reduction (1-4). We deliberately selected obese patients without asthma or AHR to explore any possible early change in airway function that could reflect an increased tendency to develop AHR over time or to develop asthma-like symptoms. Our observations suggest that the airways of obese subjects are not behaving normally, but instead like those of asthmatic patients, showing a loss of the bronchoprotection of deep inspiration manoeuvres.

The impairment of bronchial ASM relaxation following deep inspiration has been considered to result in increased airway responsiveness. However, the subjects studied had no asthma symptoms or frank AHR, possibly because their baseline level of airway responsiveness was too low for such an abnormality to translate into symptoms or AHR. This abnormality may nevertheless predispose those subjects, following progressive increases in airway responsiveness from antigenic exposure or other factors, to develop AHR at an earlier stage than nonobese subjects.

The mechanisms by which the bronchoprotection of deep inspiration manoeuvres is lost are uncertain, but this airway behaviour can be influenced by thickening or inflammation of the peribronchial adventitia, loss of lung elastic recoil or reduced tidal lung expansion (7). The last of these can occur with aging, obstructive or restrictive diseases, cervical spine injury or obesity.

Tidal stretching of the airway ASM is a potent bronchodilator and can detach cross bridges, reduce muscle stiffness and result in a decreased force generation and shortening (5). Obesity has been associated with a reduction in tidal volume (16) and, in obese subjects, tidal volume failed to increase during stresses such as exercise (17). It is possible that in obese subjects, deep inspiration does not result in the same effects on ASM as in normal subjects. In our study, the volume inspired per breath and the number of breaths/min were similar between nonobese and obese subjects. However, obese subjects were probably breathing at lower lung volumes than nonobese subjects, which seems to be confirmed by the low percentage predicted FRC and ERV values obtained by body plethysmography, an observation in keeping with previous findings showing that obesity leads to a reduction in FRC and ERV (18,19). In our initial analysis, as in previous studies, for which we had measurements of TLC and FRC in some of the obese and nonobese subjects, FRC was lower in obese subjects and normal in the others, whereas TLC was normal in both groups. For this reason, for the same amplitude of breath, the obese subjects may not have been as close to TLC as the nonobese subjects. The reduction in FRC and the low tidal volume may therefore alter ASM behaviour and the consequence of deep inspiration (20). As recently noted (5), the extent to which airway mechanical changes occur in subjects over a wide range of body weights and ages is an important research question. The above effects may be enhanced by breathing near the closing volume. In keeping with this hypothesis, a reduction in FRC, as found in obese subjects, has been associated with an increase in airway resistance and responsiveness to methacholine (19,21). A low ERV has been associated with an abnormal distribution of ventilation and an increased closing volume (5).

A number of studies (22,23) have shown an association between the symptoms of asthma and obesity in both adults and children. Asthma-like symptoms have also been reported (24) in obese subjects without AHR. The mechanisms by which these are induced are uncertain, but in a study (25) of obese healthy men with a normal FEV₁ and a FEV₁/FVC greater than 80%, the presence of resting dyspnea was associated with reduced maximum static expiratory mouth pressures, maximum voluntary ventilation and forced expiratory flow between 25% and 75% of vital capacity, suggesting peripheral airway dysfunction (25).

In our study, although the perception of symptoms at baseline was similar between obese and nonobese subjects, nonobese subjects had higher scores for perception of chest tightness at PC₂₀ on the first methacholine inhalation test (test A). The fact that similar scores were not observed after test B could be the result of an adaptation due to the longer delay before the first FEV₁ measurement or because that symptom had already been experienced during test A. In obese subjects, the sensation of tightness may already be present and considered normal.

Furthermore, we observed a small, cumulative effect of methacholine that did not differ between obese and nonobese subjects. Such a cumulative effect has been previously reported, and we recently found it to be more marked in asthmatic patients than in normal subjects (10,26).

Other mechanisms have been suggested to explain the relationship between asthma and obesity, such as neurogenic mechanisms, hormonal influences, an increased prevalence of gastroesophageal reflux or a predisposition to develop allergy and airway inflammation (5,6,27-29). A possible alteration to immune responses has been suggested for obese patients, who have increased concentrations of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1-beta, IL-5, IL-6, leptin and C-reactive protein (5,30-33). IL-6 and TNF-α have been found to be expressed during adiposis and correlate with total fat mass (34,35). Leptin, a hormone produced by adipocytes, is present in greater quantities in overweight subjects and has been associated with an increased production of TNF-α, IL-6 and IL-12 from lipopolysaccharide-stimulated macrophages (33). The possibility that obesity is associated with an increased airway inflammatory response for a given stimulus is also supported by recent observations (36) that leptin increases ozone-induced neutrophil influx and eotaxin release in bronchoalveolar lavage of mice.

Finally, it has been proposed that obesity can increase the incidence of asthma, especially in women (37). We noted in the present study that airway responsiveness was higher in men than in women, and that RV, measured by body plethysmography, was significantly higher in women than in men, although we had mostly women in our study.

CONCLUSIONS
Nonasthmatic obese subjects with normal baseline airway responsiveness showed an abnormal airway behaviour in the form of a lack of change in airway response following avoidance of deep inspirations, as has been previously shown in asthmatic patients. This altered airway behaviour may contribute to the increased prevalence of asthma-like symptoms and AHR observed in obese subjects.
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