Factors affecting oxygen saturation during
methacholine challenge in a mixed population

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Bronchial challenge with methacholine or histamine is associated with a reduction in arterial oxygen tension, which can be appreciable. In this study, oxygen saturation was monitored during methacholine challenge in subjects with and without respiratory disease, over a wide age range, in order to identify factors predicting a large fall in saturation during the challenge.

Two hundred and twenty subjects aged 24–86 years were included, comprising 15 healthy adult volunteers, and 205 adults from a random sample of the local adult population taking part in a survey of bronchial responsiveness. Subjects with ischaemic heart disease or baseline FEV₁ <60% predicted were excluded. Methacholine challenge was performed by the Newcastle Dosimeter technique; oxygen saturation (SaO₂) was monitored using a pulse oximeter and finger probe.

Of the 220 subjects, 27% were current smokers and 39.5% were ex-smokers; 26% reported asthma or bronchitis. Mean baseline FEV₁ was 100% predicted; mean baseline saturation was 97%. Mean fall in saturation was 3.2% (range 0–17.5%). Multiple regression analysis revealed that fall in saturation during methacholine challenge is related to baseline FEV₁, baseline SaO₂, log of total methacholine dose inhaled, and fall in FEV₁ during challenge. Change in saturation was not related to subject age, smoking history, reported asthma or bronchitis, or the presence of respiratory symptoms.

Methacholine challenge produces a significant fall in oxygen saturation, but this is not greater in subjects who are old or have low baseline saturation.

Introduction

Bronchial challenge with methacholine or histamine is widely used in research and clinical practice (1). Monitoring of arterial oxygen partial pressure either directly or indirectly, using a transcutaneous electrode or pulse oximeter, has shown that subjects undergoing such challenge experience varying degrees of hypoxia or desaturation (2–14). In most cases, this is small but, in a few subjects, it can be appreciable. Identification of those patients at risk of a significant fall in oxygenation or saturation during bronchial challenge would be useful. However, most published studies have included small numbers of highly selected individuals, and have not identified factors predicting significant hypoxia or desaturation.

In an attempt to identify such factors, the authors have monitored oxygen saturation during methacholine challenge in a large number of subjects over a wide age range, including smokers and non-smokers, and subjects with and without airways obstruction.

Methods

SUBJECTS

Two hundred and twenty subjects (123 females) between the ages of 24 and 86 years completed the protocol. Fifteen were volunteers from the hospital staff (age 24–33 years, mean age 27.1 years, six females); the remaining 205 subjects were a random sample of the local adult population, recruited for an epidemiological survey of bronchial responsiveness in the local community (age range 45–86 years, mean age 63.7 years, 117 females). Exclusion criteria for the epidemiological survey were as follows: cognitive impairment, immobility (inability to attend hospital for tests), non-Caucasian race (because of known inter-racial differences in bronchial responsiveness) (15), history of ischaemic heart disease, severe baseline airflow obstruction (FEV₁ <60% predicted) (16), abnormal 12-lead ECG, current medication with oral steroids or anti-cholinergic drugs, and current.
medication with oral or topical \( \beta \)-adrenergic antagonists. The study was approved by the Central Manchester Health Authority Ethical Committee, and written informed consent was obtained from all subjects.

Details of respiratory symptoms, history of obstructive airways disease (asthma or bronchitis), smoking history and current medications were obtained by postal questionnaire. Attendance for methacholine challenge was delayed until >6 weeks after a recent upper airways infection or exacerbation of wheezing.

Methacholine challenge was performed by the Newcastle Dosimeter method (17,18). Subjects were asked to avoid caffeine-containing drinks for 12 h, and to omit bronchodilator medication for 12 h (inhaled preparations), 24 h (oral preparations) or 48 h (long-acting preparations) prior to bronchial challenge. Doubling doses of nebulized methacholine were inhaled at 5-min intervals by the subject, seated and wearing a noseclip. FEV\(_1\) (mean of three recordings reproducible within 10%) was measured before each subsequent dose. End-points were a 20% decrease in FEV\(_1\), or administration of a maximum cumulative dose of 6.4 mg methacholine. Results were expressed as the methacholine dose producing a 20% fall in FEV\(_1\) (PD\(_{20}\)), and as the simplified slope of the FEV\(_1\) dose–response curve (DRS) (19).

Oxygen saturation (\( \text{SaO}_2 \)) was monitored using a finger oximeter (Biox 3700e, Ohmeda, Louisville, U.S.A.). The 95% confidence limits for background variability in \( \text{SaO}_2 \) in the authors’ laboratory (measured in young controls over 2 min) are \( \pm 2.7\% \) (20). Since \( \text{SaO}_2 \) was observed to increase following the three FEV\(_1\) manoeuvres, presumably secondary to a reduction in ventilation/perfusion mismatch resulting from repeated maximal inhalations, \( \text{SaO}_2 \) measurements were recorded immediately before each set of FEV\(_1\) manoeuvres.

**Statistical Analysis**

Values for methacholine dose, PD\(_{20}\), and DRS were transformed to logarithms prior to analysis to achieve a normal distribution. One subject had a slight increase in FEV\(_1\) during methacholine challenge, producing a negative dose–response slope; to allow logarithmic conversion of this result, a constant of 0.43 was added to all dose–response slope values (21).

Smoking history was expressed as number of pack years smoked, where 1 pack year=20 cigarettes day\(^{-1}\) for 1 yr. Comparison of subgroups was performed by grouped \( t \)-test. Multiple regression analysis was used to assess the influence of various parameters on oxygen saturation. Where inter-relationships were observed between independent variables in regression equation, interaction factors were included (21).

In order to avoid the age and height bias associated with the expression of FEV\(_1\) as percent of predicted values, baseline FEV\(_1\) was expressed as standardized residuals (SR) (22). These were calculated using the equation \( \text{SR} = (\text{recorded value} - \text{predicted value})/\text{RSD} \), where RSD is the residual standard deviation about the regression equation used to calculate the predicted values (23). The prediction equations used for the calculation of standardized residuals were derived from urban white U.K. adults over a wide age range comparable to that of the current study (24).

Increased bronchial responsiveness was defined as PD\(_{20}<400\mu \text{g methacholine} \) (25). Chronic airflow obstruction was defined as FEV\(_1/\text{FVC}\% <65\% \) for subjects aged <65 years; for those aged \( \geq 65 \) years, a predicted value and lower limit of normal for FEV\(_1/\text{FVC}\% \) was calculated as described by Enright et al. (26). In all cases, significance was defined at the 5% level.

**Results**

Of the 220 subjects taking part, 60 (27%) were current smokers and 87 (39.5%) were ex-smokers. Fifty-seven subjects (26%) gave a history of obstructive airways disease; 20 subjects reported asthma, 28 subjects reported bronchitis, and nine subjects reported both diagnoses. Respiratory symptoms (cough, sputum, wheeze or breathlessness at rest/walking on the level) were reported by 109 subjects.

Mean baseline FEV\(_1\) for the 220 subjects was 100% predicted (range 63–177%) (16). Thirty-three subjects had airflow obstruction. One hundred and fifty-three subjects (69.5%) achieved a PD\(_{20}\); 91 subjects had increased bronchial responsiveness.

Mean baseline \( \text{SaO}_2 \) was 97·1% (range 91.5–99.5%). Baseline \( \text{SaO}_2 \) was higher in females than males [females 97.3% (SD 1.5), males 96.9% (SD 1.3), \( P=0.005 \)]. Mean fall in FEV\(_1 \) (\( \triangle \text{FEV}_1 \)) during the methacholine challenge was 20.2% (range -5.9–42.7%); mean fall in \( \text{SaO}_2 \) (\( \triangle \text{SaO}_2 \)) was 3.2% (range -4.0–17.5%). \( \triangle \text{SaO}_2 \) was >10% in only four subjects (Fig. 1).

**Factors Affecting Oxygen Saturation**

**Baseline \( \text{SaO}_2 \)**

There was no difference in baseline \( \text{SaO}_2 \) between subjects reporting asthma, bronchitis or no history of
Factors affecting oxygen saturation

Fall in $\text{SaO}_2$ (%)

Fig. 1 Distribution of fall in oxygen saturation ($\text{SaO}_2$) during methacholine challenge.

Obstructive airways disease. Baseline $\text{SaO}_2$ was lower in subjects with spirometric evidence of airflow obstruction [96.1% (SD 2.3)] than in those without airflow obstruction [97.1% (SD 1.2); $P=0.002$]. Subjects reporting one or more respiratory symptoms had lower baseline $\text{SaO}_2$ [96.5% (SD 1.5)] than asymptomatic subjects [97.2% (SD 1.2); $P=0.0005$].

Multiple regression analysis with baseline $\text{SaO}_2$ as the dependent variable showed an independent positive relationship with FEV$_1$ (standardized residuals), and independent negative relationships with age, and with pack years smoked (Table 1). Baseline $\text{SaO}_2$ was also independently associated with sex ($\text{SaO}_2$ higher in women). Interaction factors for the relationships between age and pack years, and sex and pack years, did not alter the results. However, an interaction factor for the relationship between FEV$_1$ and pack years was strongly associated with baseline $\text{SaO}_2$ ($B=0.02$, $SE=0.03$, $P<0.0001$), removing the independent relationships of baseline $\text{SaO}_2$ with FEV$_1$ and pack years. The square of the coefficient of multiple regression ($R^2$) was 0.31.

Table 1 Factors associated with baseline oxygen saturation: multiple regression analysis

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>SE</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$ (standardized residuals)</td>
<td>0.45</td>
<td>0.08</td>
</tr>
<tr>
<td>Age</td>
<td>$-0.03$</td>
<td>0.008</td>
</tr>
<tr>
<td>Pack years</td>
<td>$-0.01$</td>
<td>0.004</td>
</tr>
<tr>
<td>Sex</td>
<td>$-0.02$</td>
<td>0.019</td>
</tr>
</tbody>
</table>

No relationship was found between baseline saturation and bronchial responsiveness (log dose–response curve), haemoglobin, reported asthma or bronchitis, or respiratory symptoms $R^2=0.31$.

Table 2 Factors associated with change in oxygen saturation during methacholine challenge: multiple regression analysis

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>SE</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall in FEV$_1$ (%)</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>Log methacholine dose</td>
<td>0.95</td>
<td>0.21</td>
</tr>
<tr>
<td>Baseline saturation</td>
<td>0.35</td>
<td>0.14</td>
</tr>
<tr>
<td>FEV$_1$ (standardized residuals)</td>
<td>$-0.41$</td>
<td>0.2</td>
</tr>
</tbody>
</table>

No relationship was found between fall in saturation and bronchial responsiveness (log dose–response curve), age, sex, pack years, reported asthma/bronchitis, or respiratory symptoms $R^2=0.18$.

Fall in $\text{SaO}_2$ between subjects with and without airflow obstruction, or between symptomatic and asymptomatic subjects.

Multiple regression analysis showed $\nabla \text{SaO}_2$ to be positively associated with baseline saturation, fall in FEV$_1$ during challenge (as % of baseline), and the log of the methacholine dose given; $\nabla \text{SaO}_2$ was negatively associated with baseline FEV$_1$ (Table 2). When interaction factors were added to the regression for the relationships between fall in FEV$_1$ and log methacholine dose ($B=-0.04$, $SE=0.007$, $P=0.008$), and between baseline saturation and baseline FEV$_1$ ($B=-0.0004$, $SE=0.002$, $P=0.04$), these replaced the individual variables as significant determinants of $\nabla \text{SaO}_2$. $R^2$ was 0.16.

There was no relationship between $\nabla \text{SaO}_2$ and age, sex, pack years, bronchial responsiveness or haemoglobin.

Discussion

Previous smaller studies have shown a relationship between the change in oxygenation during bronchial
challenge and either the change in FEV$_1$ (2-4), the dose of challenge agent inhaled (5), the pre-challenge level of oxygenation (6), or none of these parameters (7). The current study has shown that the change in saturation is related to all of the above parameters, as well as the baseline FEV$_1$. However, the low coefficient of multiple regression indicates that only 18% of the variation in SaO$_2$ is attributable to the measured parameters, and so other unidentified factors must be involved.

The present study population included subjects over a wide age range. Most other studies have included only children or small numbers of young adults. The only other study to have included elderly subjects (age range 19-77 years) had a mean age of 46 years, suggesting that few older subjects were included (7).

No other study of oxygen saturation during bronchial challenge has included adults with chronic airflow obstruction. Although subjects with FEV$_1$ <60% predicted were excluded, 15% of the population had spirometric evidence of chronic airflow obstruction. Asthma and chronic bronchitis might be expected to have different effects on oxygen saturation, but no difference was found during methacholine challenge between subjects reporting these diagnoses. However, subtle differences might be detected using a more sensitive technique for the measurement of oxygenation.

Direct measurement of partial pressure of oxygen (PaO$_2$) in arterial blood samples is inconvenient and uncomfortable for patients. The use of a transcutaneous electrode to measure partial pressure of oxygen (PtCO$_2$) gives similar results during bronchial challenge (9); measurement of oxygen saturation by pulse oximeter gave comparable results in one study (10), but not another (11). Because of the sigmoid oxygen saturation curve, large changes in SaO$_2$ only occur once the PaO$_2$ has fallen below 60 mmHg (27); thus monitoring of saturation is relatively insensitive in well-oxygenated subjects. Despite this, all but one study using SaO$_2$ during bronchial challenge showed a statistically significant mean fall in SaO$_2$ of around 3%, which is consistent with the results of the present study (9,7,11-13).

The fact that $\nabla$ SaO$_2$ during bronchial challenge is not related to $\nabla$ FEV$_1$ alone but also to methacholine dose suggests that methacholine may be altering SaO$_2$ both indirectly, via reduction in airways calibre, and also directly, by some other mechanism. Such mechanisms could include alteration of ventilation-perfusion distributions, as have been recorded during bronchial challenge (6).

The authors had hoped to identify baseline factors which could be used to identify subjects at risk of hypoxia during methacholine challenge. However, although $\nabla$ SaO$_2$ is associated with baseline FEV$_1$ (confirming the expectation that subjects with chronic airflow obstruction may become hypoxic during methacholine challenge), there is also a positive association with baseline SaO$_2$. This unexpected relationship has been noted previously in a study of PaO$_2$ during methacholine challenge in asthmatic patients (6), and may represent a form of regression to the mean.

Elderly subjects in the study population, despite having lower baseline FEV$_1$ and oxygen saturation, were not at increased risk of desaturation during methacholine challenge. This reinforces the assertion that bronchial challenge with methacholine is a safe and useful technique in this age group (17). A fall in SaO$_2$ with increasing age has been reported previously (28), but was not demonstrated in adults aged >65 years with chronic bronchitis (29).

In summary, in the majority of cases, the fall in oxygen saturation occurring during methacholine challenge is small; older patients and those with low baseline SaO$_2$ are not at increased risk of desaturation. However, it is not clear whether these results can be extrapolated to subjects excluded from this study, for example those with baseline FEV$_1$ <60% predicted or with ischaemic heart disease.

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References
4. van Broekhoven P, Hop WC, Rasser E, de Jongste JC, Kerrebijn KF. Comparison of FEV1 and transcutaneous oxygen tension in the measurement of airway
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