Long-term Within-Subject Variability of Inspiratory Neural Drive Response to Hypoxia*

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Study objective: We analyze the within-subject variation of mouth occlusion pressure (P_{0.1}) response to progressive isocapnic hypoxic stimulation over long time periods in normal subjects.

Patients and interventions: We studied 21 healthy subjects (14 male and 7 female; aged 40±12 yrs) (mean±SD). Lung volumes, basal P_{0.1}, and P_{0.1} response to hypoxia were measured on two separate occasions 2 months apart, under similar ambient and clinical conditions.

Results: There was no significant change in clinical condition, FVC, FEV1, arterial oxygenation saturation, end-tidal and mixed venous PCO2 levels, or P_{0.1} between the two visits. The mean P_{0.1} responses to hypoxia in the two explorations were 0.032±0.022 and 0.034±0.022 kPa/%, respectively. There was a moderate intrasubject variability of P_{0.1} response to hypoxia, with a coefficient of reproducibility of 0.01 kPa/%. Power calculations to establish the optimal sample size required for hypoxic stimulation are presented.

Conclusion: Long term within-subject variability of P_{0.1} response to hypoxia is moderate. This intrinsic variability needs to be emphasized when interpreting the effects of experimental interventions on hypoxic sensitivity.

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Key words: carotid body; hypoxia; respiratory center; variability

Abbreviations: CR=coefficient of reproducibility; P_{0.1}=mouth occlusion pressure at 1 s after beginning of inspiration; SaO₂=arterial oxygen saturation

Although respiratory response to progressive isocapnic hypoxic stimulation has been important in detailing the effects of respiratory stimulant or depressant drugs, there are fewer data to describe their physiologic variability.

Previous studies have revealed a wide intersubject variation in healthy subjects and in patients with cardiopulmonary diseases. Hypoxic response seems to be influenced by many factors such as age, sex, physical characteristics, exercise, menstrual cycle, smoking habits, and genetic factors.6–8

Within-subject variability of respiratory hypoxic response over short time periods and over long periods (months) is not well known. Some studies examining the variability of ventilatory hypoxic response in healthy subjects revealed that the variation within subjects is lower than between subjects.9–12 However, none of these studies included an examination of long-term intrasubject variability of mouth occlusion pressure response to hypoxia.

This study was designed to determine the within-subject variation of mouth occlusion pressure response to progressive isocapnic hypoxic stimulation over long time periods in healthy subjects.

Materials and Methods

Subjects

Twenty-one normal subjects, 14 male and 7 female (40±12 years), gave their informed consent to be studied. They were judged healthy by history, physical examination, ECG, basal spirometry, and chest fluoroscopy. Their mean height was 170±9 cm and their mean weight was 73±13 kg. Eight volunteers were current smokers and none were taking medication (including oral contraceptives). The women were studied during the estrogenic portion of their menstrual cycles.

Study Design

Two pulmonary function studies were performed, 2 months apart. There was no change in laboratory or subject conditions between the two procedures.
In the trial of hypoxic response was terminated when the subjects reached 80% SaO₂.

Subsequent power calculations were made using the method outlined by Armitage and Berry. This employed the following equation:

$$SD(Z_{1-β}+Z_{1-α}^2)\sqrt{n}$$

where $Z_{1-β}=0.8416$ for 80% power; and $Z_{1-α}=1.96$ for a significance level of 0.05. SD = standard deviation of the differences between hypoxic stimulations.

**RESULTS**

There were no significant differences in the anthropometric and spirometric characteristics between the two explorations (Table 1). Basal SaO₂, mixed venous PCO₂ levels at which the hypoxic response was tested, and baseline end-tidal PCO₂ levels before testing was begun on two visits are also shown in Table 1. None of these parameters changed significantly between the two explorations. No changes in basal P₀.₁ (0.130±0.029 vs 0.139±0.03 kPa) or in P₀.₁ response to hypoxia (0.032±0.022 vs 0.034±0.022 kPa%) were noted (Fig 1). The correlation coefficients between P₀.₁ and SaO₂ for each individual response exceeded 0.95 in all cases.

### Long-term Variability of P₀.₁ Response to Hypoxia

The limits of agreement between hypoxic stimulation 1 vs hypoxic stimulation 2 are graphically reported in Figure 2. There is a moderate inrasubject variation with time that is reflected in the wide 95% range. The CR for the P₀.₁/SaO₂ was 0.010 kPa/ %. The mean coefficient of variability for P₀.₁ response to hypoxia was 8.82±6.15%.

Relative variability did not increase with increasing hypoxic responsiveness. Variability coefficient was not correlated with P₀.₁ response to hypoxia.

### Table 1—Comparison Between the Two Visits*

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>73±13</td>
<td>73±12</td>
</tr>
<tr>
<td>FVC, L</td>
<td>4.52±1.35</td>
<td>4.56±1.80</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>106±12</td>
<td>105±16</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>3.67±0.98</td>
<td>3.78±0.88</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>104±10</td>
<td>107±9</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>82.4±8.1</td>
<td>82.9±8.4</td>
</tr>
<tr>
<td>SaO₂, %</td>
<td>94±3</td>
<td>93±3</td>
</tr>
<tr>
<td>PetCO₂, kPa</td>
<td>4.7±0.9</td>
<td>4.6±0.9</td>
</tr>
<tr>
<td>PpCO₂, kPa</td>
<td>6.0±0.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>P₀.₁, kPa</td>
<td>0.130±0.029</td>
<td>0.139±0.03</td>
</tr>
<tr>
<td>P₀.₁/SaO₂, kPa%</td>
<td>0.032±0.022</td>
<td>0.034±0.022</td>
</tr>
</tbody>
</table>

*Mean ± SD values are presented. PetCO₂=basal end-tidal carbon dioxide; PpCO₂=mixed venous carbon dioxide; P₀.₁/SaO₂=P₀.₁ response to hypoxia.
Figure 1. Individual $P_{0.1}$ responses to progressive isocapnic hypoxia during visit 1 (squares) and visit 2 (circles). Horizontal bars represent mean values.

($r = -0.161$, $p = 0.94$). Moreover, no significant difference was noted between those patients with a $P_{0.1}$ response to hypoxia <0.025 kPa/\% and those with a $P_{0.1}/\text{SaO}_2 \geq 0.025$ kPa/\% (6.43 ± 6.41 vs 9.85 ± 6.35%; $p = 0.247$). Neither were significant differences found for other $P_{0.1}/\text{SaO}_2$ cutoff points.

Figure 2. Plot of within-subject differences vs mean of $P_{0.1}$ response to hypoxia. The continuous line represents the mean difference; the dashed lines represent 2 SDs around the mean.

Power Calculation for the Sample Size

The plot of calculated detectable difference vs sample size for $P_{0.1}/\text{SaO}_2$ is shown in Figure 3 using 80% power. This would suggest that in hypoxic sensitivity studies, there would be little advantage in terms of increased power by increasing the number of subjects above approximately 20.

Discussion

Our study indicates that $P_{0.1}$ response of healthy subjects to progressive isocapnic hypoxic stimulation presents a moderate intrasubject variation over long time periods.

Data describing variability of respiratory response to hypoxia are scanty.9-12,20 Moreover, several methodologic differences limit the comparison of results. Previous studies have only measured the ventilatory response to hypoxia. Different methods of stimulation (steady-state,20 rebreathing,9-11 or rebreathing after hyperoxia12) and the analysis of ventilatory response (hyperbolic model9,11 or linear regression10,12) were employed. Because in many patients, ventilatory response may fail to reflect the true output at the respiratory centers,16 we measured $P_{0.1}$ response, which is known to reflect respiratory neuromuscular function more directly than ventilation.16,21

Sample sizes of previous studies9-12,20 were very small, ranging from three12 to nine subjects.9 Only Sahn et al11 analyzed within-subject variability over a long period of time (5 months), while the other authors studied the day-to-day variability. Finally, statistical analysis employed in these reports included comparisons of means.9, analysis of vari-
ance,10,11 and coefficient of variability determination.11,12 None of them determined the CR.

Despite important differences between our study and previous ones, the coefficient of variability for $P_{0.1}$ response to hypoxia found in our study (8.82 ± 6.15%) is similar to the coefficient of variability for ventilatory response to hypoxia reported by White et al12 (8.9 ± 2.3%) and lower than the one described by Sahn et al11 (19.4, ranging from 7.6 to 63.8%).

Factors that may influence hypoxic sensitivity in humans include acid-base status, metabolic rate, and catecholamines.4 Respiratory response to hypoxia is highly sensitive to variations in arterial pH induced by P$\text{CO}_2$ changes.22 In this way, Re buck and Woodley23 showed that to obtain the maximal linearity of hypoxic response, the mixed venous P$\text{CO}_2$ level does not vary more than 1 to 3 mm Hg from day to day. In our subjects, there was no change in mixed venous P$\text{CO}_2$ between the days of testing (Table 1). This suggests that factors other than acid-base status contributed to variability of hypoxic sensitivity.

The effects of variations in progesterone8 or cortisone11 levels are minimized because the subjects were tested at the same time of day and during the estrogogenic portion of the menstrual cycle. Although Sahn et al11 did not find a significant relationship between the daily changes in metabolic rate and ventilatory response to hypoxia, the influence of basal metabolic changes on variability of hypoxic sensitivity cannot be excluded. In consequence, the within-subject variability of $P_{0.1}$ response to hypoxia could be due to small variations of the metabolic rate or to changes in the sympathetic tone.

This study has used conventional power calculations to determine the likely sample size needed for adequate power in hypoxic sensitivity studies. Our data would indicate (Fig 3) that in order to obtain a power of 80% to detect a significant difference for $P_{0.1}$ response to hypoxia (0.003 kPa/%), 20 subjects appear to be an optimal minimal sample size, although with a moderate potential for type 2 statistical errors (relatively subtle changes may occur that cannot be detected with this method). Likely, differences in $P_{0.1}$ response to hypoxia <0.003 kPa/% are scarcely relevant.2,13 Besides the change it is desired to detect, power calculation is dependent on the design of the study. Our observations can be used to estimate the number of subjects required for different study designs to obtain an 80% power: 20 would be needed in each treatment arm of a parallel group study, though only 10 would be needed in each treatment group in a crossover study design. Indeed, the usefulness of our results is limited to those who would use this technique for studies of responses to hypoxia either over time or with different treatments. Moreover, it cannot be applied to individuals who are demographically different.

In conclusion, this study has drawn attention to the presence of moderate long-term intrasubject variability in hypoxic sensitivity, as assessed using $P_{0.1}$ response. This intrinsic variability needs to be emphasized when interpreting the effects of experimental interventions on hypoxic sensitivity.

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