Human ventilatory response to acute hyperoxia during and after 8 h of both isocapnic and poikilocapnic hypoxia

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Human ventilatory response to acute hyperoxia during and after 8 h of both isocapnic and poikilocapnic hypoxia. J. Appl. Physiol. 82(2): 513–519, 1997.—During 8 h of either isocapnic or poikilocapnic hypoxia, there may be a rise in ventilation (VE) that cannot be rapidly reversed with a return to higher PO2 (L. S. G. E. Howard and P. A. Robbins, J. Appl. Physiol. 78: 1098–1107, 1995). To investigate this further, three protocols were compared: 1) 8-h isocapnic hypoxia (end-tidal Pco2 (PETCO2) held at pre-study value, end-tidal PO2 (PETO2) = 55 Torr), followed by 8-h isocapnic euoxia (PETO2 = 100 Torr); 2) 8-h poikilocapnic hypoxia followed by 8-h poikilocapnic euoxia; and 3) 16-h air-breathing control. Before and at intervals throughout each protocol, the VE response to eu-capnic hyperoxia (PETCO2 held 1–2 Torr above pre-study value, PETO2 = 300 Torr) was determined. There was a significant rise in hyperoxic VE over 8 h during both forms of hypoxia (P < 0.05, analysis of variance) that persisted during the subsequent 8-h euoxic period (P < 0.05, analysis of variance). These results support the notion that an 8-h period of hypoxia increases subsequent hyperoxic VE, even if acid-base changes have been minimized through maintenance of isocapnia during the hypoxic period.

In humans, ventilatory acclimatization to altitude is characterized by a progressive rise in ventilation (VE) accompanied by a fall in end-tidal Pco2 (PETCO2), which begins within hours of exposure to hypoxia and is mostly complete within a few days. There appear to be at least two distinct processes underlying acclimatization. First, there appears to be a general increase in ventilatory sensitivity to hypoxia (14, 18), and, second, there is a leftward shift of the VE-PETCO2 response curve (12) that persists under hyperoxic conditions and hence should be considered as distinct from the increase in hypoxic sensitivity.

To assess the importance of the hypocapnia and associated alkalosis in driving the acclimatization processes, a recent study from our laboratory (9, 10) investigated a period of 8 h of hypoxia (end-tidal PO2 (PETO2) of 55 Torr) during which the PETCO2 was not allowed to fall but held constant at prehypoxic values. The rise in VE over the 8 h was shown to be dramatic, which demonstrated, first, that substantial changes could be detected in VE over a much shorter exposure to hypoxia than is required to produce full acclimatization, and second, that hypocapnia was not required for these progressive responses to hypoxia (i.e., responses that develop over hours) to occur. Intermittent brief tests of hypoxic sensitivity involving rapid (90-s period) alteration of PETO2 at fixed PETCO2 (held constant at 1–2 Torr above initial prehypoxic values) demonstrated that there was an associated increase in the sensitivity of VE to rapid variations in PO2 throughout the 8-h period. Similar results were observed in experiments involving 8 h of hypoxia (PETO2 = 55 Torr), during which the PETCO2 was allowed to fall naturally (the brief measurements of hypoxic sensitivity again being made at a PETCO2 of 1–2 Torr above prehypoxic levels). This increase might be thought of as corresponding to the early stages of the rise in ventilatory sensitivity to hypoxia associated with acclimatization to altitude.

In addition to the above findings, the results from these experiments also raised the possibility that, in both the isocapnic and poikilocapnic exposures, there might be a component of the rise in VE with 8 h of hypoxia that is not rapidly reversed by a rise in PO2, possibly not even by a period of hyperoxia. Such a change could be thought of as corresponding to the leftward shift of the VE-PETCO2 curve. Thus the first question this study set out to investigate was whether there is indeed a progressive rise in VE that develops over 8 h of hypoxia and that is not rapidly reversed by a rise in PO2, even with the use of hyperoxia.

In the previous studies (9, 10), the underlying changes in respiratory control with poikilocapnic and isocapnic hypoxic exposures appeared similar. This suggests that any changes in hyperoxic VE that may occur might be similar for the two types of hypoxic exposure. If this were the case for a change in hyperoxic VE, it would imply that not only is the hypocapnia that normally occurs with exposure to hypoxia not necessary to generate changes in hypoxic sensitivity but also that the hypocapnia is not necessary to generate an increase in VE that persists under conditions of hyperoxia (i.e., one which may be thought of as corresponding to a leftward shift of the VE-PETCO2 curve). However, related work carried out on goats does not support this notion (5). In goats, an effect of hypoxia on VE that was not rapidly reversible by an elevation of PO2 was only found after poikilocapnic hypoxia and not after exposure to isocapnic hypoxia. This leads to the second question to be addressed: If there is a rise in hyperoxic VE, does this occur in both poikilocapnic and isocapnic exposures or is it confined to poikilocapnic exposure where there is an associated alkalosis, as suggested by evidence from the experiments on goats?

Full recovery of the leftward shift of the hyperoxic VE-PETCO2 curve generated by acclimatization requires several days (12). This observation raises our third question, which is, if there is a rise in hyperoxic VE induced by 8-h hypoxia, does recovery occur over a similar 8-h time scale or does the rise in hyperoxic VE persist for longer, as is the case with the leftward shift occurring with acclimatization to altitude? In particu-
lar, we ask whether it persists during an 8-h period of euoxia after the hypoxic exposure.

Therefore, this study aims to answer the following questions. Over an 8-h period of hypoxia in humans 1) are there progressive changes in the V̇E observed during brief periods of hyperoxia? 2) If there are changes, do they differ between isocapnic and poikilocapnic hypoxic exposures? 3) If there are changes, do they persist during a subsequent period of 8 h after a return to euoxia?

METHODS

Subjects. Twelve healthy subjects (7 men, 5 women) aged between 18 and 27 yr volunteered to take part in the study. The study requirements were fully explained in written and verbal forms to all participants in such a way that they were naive as to the exact purpose of the experiment. Each subject gave informed consent before participation in the study. The research had approval from the Central Oxford Research Ethics Committee.

Experimental technique. Most of the experiment was conducted with individual subjects inside a specially built chamber that allowed them to be seated comfortably or move around if they wished. The design of this chamber avoided any need for a mouthpiece. The subjects wore a pulse oximeter attached to a finger to monitor arterial O2 saturation. Respired gas was sampled (80 ml/min) via fine catheters held at the opening of each nostril by a nasal O2-therapy mask. The samples were continuously analyzed for PO2 and PCO2 by mass spectrometry. The data (PCO2, PO2, and O2 saturation) were sampled by computer at a rate of 50 Hz. Inspiratory and end-tidal values for PCO2 and PO2 were identified by computer from the PCO2 data and recorded for each breath. At the start of the experiment, the inspired gas composition necessary to produce the desired PETO2 and PETCO2 was estimated and set manually before the subject entered the chamber. Once the subject had entered the chamber, the inspired composition was altered automatically every 5 min or at manually overridden intervals to minimize the error between the actual and desired end-tidal gases. This system has been described in greater detail elsewhere (8).

Measurements of the ventilatory response to hyperoxia were made outside the chamber by using a dynamic end-tidal forcing system. Subjects, each with the nose occluded with a clip, were seated in an upright position and breathed through a mouthpiece. Respiratory volumes were measured by using a turbine volume-measuring device fixed in series with the mouthpiece; flows and timing information were obtained by using a pneumotachograph. The total dead space associated with the apparatus was 100 ml. Gas was sampled continuously from this dead space close to the mouth at a rate of 20 ml/min and analyzed by mass spectrometry for PO2 and PCO2. A pulse oximeter was attached to the forehead to monitor the O2 saturation of the blood. The data were recorded by computer at a sampling rate of 50 Hz, the computer also being used to detect the ends of the expiratory and inspiratory phases from the flow data to determine the expiratory and inspiratory duration, volumes, and PETO2 and PETCO2.

A “forcing function” containing the predicted inspired gas values required to achieve the desired PETO2 and PETCO2 was entered into a second computer before the hyperoxic test. During the test, actual values of PETO2 and PETCO2 were passed breath by breath to this controlling computer from the data-acquisition computer. These measured values were compared with the desired values, and a new inspired gas mixture was calculated breath by breath using an integral-proportional feedback scheme. The controlling computer adjusted the inspired PO2, PCO2, and partial pressure of N2 via a series of valves connected to gas supplies. This system for controlling PETCO2 and PETO2 has been described in greater detail elsewhere (11, 13).

Protocols. After one or two preliminary visits, during which a control value for PETCO2 was obtained and the subject was familiarized with the apparatus, each participant visited the laboratory on three further occasions, each session lasting ~19 h. On each visit, one of three protocols was performed in a randomly determined order, each lasting 16 h. In the isocapnic protocol (protocol I), subjects were exposed to 8 h of hypoxia during which PETO2 was held at 55 Torr and PETCO2 was allowed to vary during the first 8 h, followed by 8 h of isocapnic euoxia (PETO2 = 100 Torr). In the protocol (protocol I), PETO2 was held at 55 Torr and PETCO2 was allowed to vary during the first 8 h, followed by 8 h of poikilocapnic euoxia (again, PETO2 = 100 Torr). In the control protocol (protocol C), subjects were exposed to 16 h with air as the inspired gas.

Assessment of acute hyperoxic ventilatory response. The first hyperoxic test was performed before the start of the first 8-h period. The second hyperoxic test was performed 20 min after the subjects were placed in the chamber. The third and fourth measurements were made after 4 and 8 h, respectively. After the 8-h measurement, the subjects were returned to the chamber for the recovery period, with hyperoxic tests performed after 20 min, 4, and 8 h, respectively.

The protocol for measuring the hyperoxic ventilation consisted of a 7-min “lead-in” in which PETO2 was held at the present value desired in the chamber (i.e., 100 Torr for protocol C and tests before and before hypoxic exposure and 55 Torr during hypoxia). This lead-in period was followed by 10 min of hypoxia, in which PETO2 was held at 300 Torr, followed by a “lead-out” of the same form as the lead-in. PETO2 was held at 1–2 Torr above the subject’s hypoxic value throughout every test in all three protocols.

Statistical analysis. Average V̇E values were taken over the second 5 min of each hyperoxic period. An analysis of variance employing a general linear model was used to test for differences between the hyperoxic V̇E over time and among protocols. The analysis was undertaken in two parts relating to the first 8 h and the second 8 h. With the use of the notation defined by Armitage (2), the model took the form

\[
(V̇E)_{ijkl} = \mu + S_i + (C-1)p_j + (I-P)k + T_l + S(C-1)T_{il}\]

where \(\mu\) is the mean value, \(S_i\) is subject, (C-1)p, is the term for the comparison of protocol C with the hypoxic protocols, (1-I-P), is the term for the comparison of protocols I and P, T, is the time (a covariate), and e is the error term. Subjects were treated as a random factor, and sums of squares were calculated sequentially from left to right. Statistical analysis was undertaken by using Statistical Package for Social Studies software.

RESULTS

Subjects. Of the 12 subjects studied, 8 provided data that were suitable for analysis. One subject withdrew from the study before completing the three protocols, two were rejected from the analysis process because of inadequate gas control brought about by technical difficulties, and one subject hyperventilated when asked
to breathe through a mouthpiece. While in the chamber, subjects were generally comfortable, spending their time reading, watching television, or playing computer games, although some did report mild headaches or discomfort from the high levels of \( V \dot{E} \) during the last hours of hypoxia.

End-tidal gas control in the chamber. Figure 1 shows the end-tidal gases recorded from each of the eight subjects while they were in the chamber, averaged every 5 min, for each of the three protocols (I, P, C). These plots illustrate the quality of control achieved. The mean values for \( P_{\text{ETO}_2} \) and \( P_{\text{ETCO}_2} \) and SD on the basis of averages taken every 5 min for each protocol are given in Table 1.

Assessment of acute hyperoxic response. Figure 2 is an example of the data from a hyperoxic test (subject 983,

<table>
<thead>
<tr>
<th>Protocol</th>
<th>( P_{\text{ETO}_2} ) (Torr)</th>
<th>( P_{\text{ETCO}_2} ) (Torr)</th>
<th>( V \dot{E} ) (l min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocapnic hypoxia</td>
<td>39.2 ± 3.2</td>
<td>56.3 ± 2.2</td>
<td>45.0 ± 7.1</td>
</tr>
<tr>
<td>Poikilocapnic hypoxia</td>
<td>39.1 ± 2.8</td>
<td>56.5 ± 2.6</td>
<td>46.5 ± 8.2</td>
</tr>
<tr>
<td>Control</td>
<td>39.6 ± 3.7</td>
<td>104.8 ± 4.5</td>
<td>45.0 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 8 \) subjects. \( P_{\text{ETCO}_2} \), end-tidal \( P_{\text{CO}_2} \); \( P_{\text{ETO}_2} \), end-tidal \( P_{\text{O}_2} \); \( t \), time.
This illustrates the good gas control for \( \text{PETO}_2 \) and \( \text{PETCO}_2 \), obtained with the mouthpiece system and a slightly rising \( \dot{V}E \) response over the second 5–10 min of the acute hyperoxic exposure.

Figure 3 shows the ventilatory responses to acute hyperoxia during the first 8 h of each protocol (tests 1–4) averaged over all eight subjects. It can be seen that gas control was well matched both within each protocol and across protocols. In isocapnic and poikilocapnic hypoxia, there is the appearance of a rise in hyperoxic \( \dot{V}E \) at 4 and 8 h when compared with 0 and 20 min. This appearance was not present in protocol C. The significance of this observation was determined by applying the general linear model described in the methods to the 5-min averages for \( \dot{V}E \) from these protocols (mean values for these \( \dot{V}E \) are given in Table 2). With the use of this linear model, a significant effect of time was detected, which was different between hypoxic protocols and control (\( P < 0.05 \)). No significant difference between the two types of hypoxic protocol was detected.

Figure 4 shows the ventilatory responses to acute hyperoxia during the 8-h recovery period of each protocol (tests 4–7). Again, gas control was well matched both within and among protocols. Little recovery of \( \dot{V}E \) toward prehypoxic levels was apparent over the 8-h period for the hypoxic protocols. Statistically, the mean values for \( \dot{V}E \) from the last 5 min of hyperoxia differ significantly between hypoxic protocols and control (\( P < 0.05 \)), with no significant difference between the two types of hypoxic exposure. However, there is no significant change over time for \( \dot{V}E \) for either hypoxic or control protocols. Mean values for these \( \dot{V}E \) are given in Table 3.

### DISCUSSION

The main findings of this study are that over the course of an 8-h exposure to hypoxia, there is a progres-

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Isocapnic Hypoxia</th>
<th>Poikilocapnic Hypoxia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.5 ± 2.6</td>
<td>13.7 ± 1.0</td>
<td>15.4 ± 1.9</td>
</tr>
<tr>
<td>20</td>
<td>15.8 ± 2.0</td>
<td>14.0 ± 1.0</td>
<td>18.0 ± 2.3</td>
</tr>
<tr>
<td>240</td>
<td>22.0 ± 3.9</td>
<td>18.9 ± 2.0</td>
<td>18.0 ± 2.1</td>
</tr>
<tr>
<td>480</td>
<td>20.8 ± 3.8</td>
<td>21.0 ± 2.2</td>
<td>16.3 ± 2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE given in l/min; \( n = 8 \) subjects. \( \dot{V}E \), ventilation.
sive increase in $\dot{V}E$ observed during brief periods of acute hyperoxia and that this increase persists over an ensuing 8 h period of euoxia. These findings were not shown to be significantly different between isocapnic and poikilocapnic hypoxic exposures, suggesting that these changes do not depend on acid-base alterations in the blood and are, therefore, the result of some other effect of hypoxia.

Assessment of acute hyperoxic response. A small progressive rise in $\dot{V}E$ is observed throughout these 10-min tests. Although we are unsure of its origin, it is observed during the control experiments and is, therefore, unlikely to be attributable to the hypoxic exposures. One possibility is that it is related to the slight elevation of $\text{PETCO}_2$ in these tests.

Comparison with other studies. In humans, it is well recognized that after the prolonged poikilocapnic hypoxia associated with altitude, there is an increase in $\dot{V}E$ that is not totally relieved by either return to sea level or by hyperoxia (see Ref. 3 for review). Furthermore, it is generally accepted that this effect is due, either wholly or in part, to the acid-base adjustments that occur in response to the initial respiratory alkalosis. The findings from our study suggest that such changes in $\dot{V}E$ can occur with much shorter exposures to hypoxia, during which little by way of compensatory acid-base changes to the initial respiratory alkalosis would be expected, and with isocapnic hypoxia, where the initial respiratory alkalosis has been prevented.

Our results are broadly consistent with a previous study in humans by Eger et al. (4), in which subjects were made hypoxic by breathing gas from a facemask for 8 h. They found a leftward shift in the hyperoxic $\dot{V}E$-$\text{PETCO}_2$ response curve after 8 h of hypoxia at various

### Table 3. Mean values of last 5 min of hyperoxic $\dot{V}E$ (2nd 8 h)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Isocapnic Hypoxia</th>
<th>Poikilocapnic Hypoxia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>480</td>
<td>20.8 ± 3.8</td>
<td>21.0 ± 2.2</td>
<td>16.3 ± 2.0</td>
</tr>
<tr>
<td>500</td>
<td>19.0 ± 2.3</td>
<td>21.4 ± 1.8</td>
<td>17.7 ± 2.2</td>
</tr>
<tr>
<td>720</td>
<td>19.5 ± 2.6</td>
<td>18.7 ± 1.8</td>
<td>15.9 ± 2.4</td>
</tr>
<tr>
<td>960</td>
<td>20.0 ± 2.0</td>
<td>18.4 ± 1.5</td>
<td>16.8 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$ subjects. $\dot{V}E$ is measured in l/min.
levels of $P_{\text{CO}_2}$. However, one difference in the results from their study as compared with ours was that they found the leftward shift was greater with lower levels of $P_{\text{ET}_\text{CO}_2}$. Our results are also consistent with an earlier study (9) of the change in ventilatory sensitivity to hypoxia over 8 h of either isocapnic or hypocapnic hypoxia. In addition to the increase in hypoxic sensitivity observed in this study, there was also an increase in the parameter of the model that reflected baseline (or hyperoxic) $V_{\text{E}}$. The changes in baseline $V_{\text{E}}$ were not significantly different between the isocapnic and hypocapnic exposures.

Comparisons with animal studies need to be drawn with care because of the very different time courses that may be associated with ventilatory acclimatization to altitude. In the goat, which acclimatizes very rapidly, there is an increase in $V_{\text{E}}$ in euoxia and hypoxia after 4 h of poikilocapnic hypoxia (5). However, in contrast to our study, such a change was not observed if the exposure to hypoxia was isocapnic. Thus the investigators attributed this change in $V_{\text{E}}$ with poikilocapnic exposure to the associated respiratory alkalosis.

Underlying mechanisms. The results from this study indicate that, in humans, an 8-h period of hypoxia may alter $V_{\text{E}}$ in subsequent hypoxia. Our finding that, in humans, the shift in hyperoxic $V_{\text{E}}$ is similar in both the isocapnic and poikilocapnic exposures to hypoxia suggests that additional factors other than acid-base changes are associated with this response to hypoxia. There are a number of possibilities. First, although the isocapnic exposure will have attenuated any acid-base changes in the systemic circulation, it is nevertheless possible that pH changes still occur in the vicinity of the central chemoreceptors. In awake chronically instrumented goats, a fall in pH at the medullary surface was reported with both isocapnic and poikilocapnic hypoxia (19). Interestingly, there appeared to have been little effect on $V_{\text{E}}$ during the development of the acidosis. On the other hand, another study of hypoxia in awake goats (1) suggests that inhibiting the production of lactate by using intravenous infusion of dichloroacetate results in enhanced hyperventilation, which would suggest that brain lactic acidosis induced by hypoxia may in fact depress breathing. This study found that the progressive rise in carotid body activity in such a way as to explain the persistence of the effect in the subsequent 8-h recovery by this mechanism. Also set against this is a further study in awake goats (17), which investigated the effects of systemic [central nervous system (CNS)] hypoxia in the absence of carotid body hypoxia and found only mild hyperventila
tion of rapid onset and no progressive or persistent changes, i.e., no acclimatization.

A second possible explanation of our results is related to the phenomenon of “hyperventilation-induced hypopnea.” Smith et al. (15) have found that spontaneous hyperventilation occurs in humans after a period of increased breathing (produced by a ventilator) during which $CO_2$ is kept at normal levels. They attributed this effect to direct facilitation of CNS activity by hyperventilation. However, although this phenomenon may have some relevance to the isocapnic hypoxic conditioning in which an increase in $V_{\text{E}}$ is observed, its role in poikilocapnic hypoxia, in which the rise in $V_{\text{E}}$ is much more modest, is unclear.

A third possible mechanism underlying our results is that hypoxia exerts effects directly on the CNS. Gallman and Millhorn (7) have suggested that there are two opposing effects after a period of central hypoxia in peripherally chemodenervated anesthetized cats. Their findings are 1) facilitation of $V_{\text{E}}$ by mild hypoxia; 2) inhibition of $V_{\text{E}}$ by more severe hypoxia; the mesencephalon seems to be important and, furthermore, it appears that in the absence of the mesencephalon neither prolonged inhibition nor prolonged facilitation can be produced after a period of hypoxia. The two effects appear to be simultaneous, the level of hypoxia determining which predominates. It is possible that we are seeing predominantly the effects of mild hypoxia on the brain in our study, resulting in the facilitation of $V_{\text{E}}$ posthypoxia. Again, however, the findings in conscious goats (17) suggest that central effects are of rapid onset and offset, and it is therefore difficult to establish their role in acclimatization. Furthermore, studies involving carotid body resection in awake cats (6) and awake goats (16) indicate that carotid bodies are required for acclimatization to occur; thus it seems unlikely that the phenomenon we observe can be explained through an entirely central effect of hypoxia.

A fourth possible mechanism is that hypoxia causes a progressive rise in carotid body activity in such a way that a component of this response is not rapidly reversible by hypoxia. However we know of no evidence to support this possibility.

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