Human ventilatory response to CO₂ after 8 h of isocapnic or poikilocapnic hypoxia

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Fatemian, Marzieh, and Peter A. Robbins. Human ventilatory response to CO₂ after 8 h of isocapnic or poikilocapnic hypoxia. J. Appl. Physiol. 85(5): 1922–1928, 1998.—During ventilatory acclimatization to hypoxia (VAH), the relationship between ventilation (Ve) and end-tidal PCO₂ (PETCO₂) changes. This study was designed to determine 1) whether these changes can be seen early in VAH and 2) if these changes are present, whether the responses differ between isocapnic and poikilocapnic exposures. Ten healthy volunteers were studied by using three 8-h exposures: 1) isocapnic hypoxia (IH), end-tidal PO₂ (PETO₂) = 55 Torr and PETCO₂ held at the subject’s normal prehypoxic value; 2) poikilocapnic hypoxia (PH), PETO₂ = 55 Torr; and 3) control (C), air breathing. The Ve-PETCO₂ relationship was determined in hypoxia (PETO₂ = 200 Torr) before and after the exposures. We found a significant increase in the slopes of Ve-PETCO₂ relationship after both hypoxic exposures compared with control (IH vs. C, P < 0.01; PH vs. C, P < 0.001; analysis of covariance with pairwise comparisons). This increase was not significantly different between protocols IH and PH. No significant changes in the intercept were detected. We conclude that 8 h of hypoxia, whether isocapnic or poikilocapnic, increases the sensitivity of the hypoxic chemoreflex response to CO₂.

ventilatory acclimatization; altitude

VENTILATORY ACCLIMATIZATION to hypoxia (VAH) in humans is characterized by a progressive increase in ventilation (Ve) and reduction in end-tidal PCO₂ (PETCO₂), which begins within hours of exposure to hypoxia and continues to develop over a number of days. The underlying changes in respiratory control include a change in slope and intercept for the relationship between Ve and PETCO₂ (3, 8, 14, 15, 17) and an increase in the ventilatory sensitivity to hypoxia (8, 9, 17, 20).

One question that arises is whether these changes in the respiratory control system are driven directly by the hypoxic exposure or whether they are brought about indirectly by the respiratory alkalosis that normally accompanies VAH. In the case of the increase in ventilatory sensitivity to hypoxia, it has now been shown in humans that the early changes in VAH (first 8 h of hypoxia) arise directly from the exposure to hypoxia (11, 12). In the case of the changes in intercept and slope for the relationship between Ve and PETCO₂, the evidence is much less clear. On one hand, evidence obtained in goats suggests that, for hyperventilation to persist on return to euoxia after VAH, a prolonged systemic (central nervous system) hypocapnic alkalosis is required (7). On the other hand, in humans, Tansley et al. (18) reported that an 8-h exposure to hypoxia resulted in a persistent subsequent hyperventilation under hypoxic conditions (at a fixed PETCO₂) and that this effect did not differ between hypoxic exposures carried out under isocapnic and poikilocapnic conditions. A result apparently somewhere between these two was obtained by Eger et al. (6), who found that, after an 8-h period of hypocapnia in humans, there was a leftward shift in the PETCO₂ associated with a Ve of 15 l·min⁻¹·m⁻² under mild hyperoxic conditions but that this leftward shift was greater if the hypocapnic exposure was also associated with exposure to hypoxia. A similar effect on spontaneous PETCO₂ under mildly hypoxic conditions has been reported after a comparison of 26 h of hypoxic ventilation with and without hypoxia (5). None of these studies in humans produces unequivocal evidence as to whether it is the slope or the intercept (or both) of the relationship between Ve and PETCO₂ in hypoxia that is affected.

The present study was designed to look at the effects of 8-h exposures of hypoxia on the slope and intercept of the relationship between Ve and PETCO₂. As in the case of the study by Tansley et al. (18), isocapnic and poikilocapnic exposures were compared to determine whether the respiratory alkalosis normally associated with hypoxia at altitude has an effect on the responses observed.

METHODS

Subjects. Ten healthy volunteers (7 men, 3 women), aged between 18 and 24 yr, took part in the study. The experiment was fully explained in written and verbal forms to all participants. Informed consent was obtained from each subject before each experiment. The study was approved by the Central Oxford Research Ethics Committee.

Protocols. Each subject visited the laboratory at least twice before any of the main experiments was undertaken. In these short visits, the subject was introduced to and familiarized with the apparatus and some initial measurements of control PETCO₂ were obtained. Before each of the main experiments, the subjects were told to make sure that they had a good night’s rest and that they did not exert themselves excessively when coming to the laboratory. No measurements were taken for 10–15 min after the subject’s arrival in the laboratory.

The main experiments were carried out in random order on three separate days at least 1 wk apart. Female subjects were always studied at the same phase of their menstrual cycle. The three 8-h exposures used for each subject were 1) isocapnic hypoxia (IH), 2) poikilocapnic hypoxia (PH), and 3) control (C). For protocol IH, end-tidal PO₂ (PETO₂) was held at 55 Torr and PETCO₂ was held at the subject’s normal (prehypoxic) value. For protocol PH, PETO₂ was held at 55 Torr and...
PETCO₂ was not controlled. For protocol C, the subject was exposed to air as inspired gas. In each of these protocols, the subjects were given a light lunch at ~1:00 PM. If the subjects needed to urinate, they were free to leave the chamber briefly for that purpose. Otherwise, they remained in the chamber for the full 8 h.

Values for air-breathing PETCO₂ were determined before and 0.5 h after each 8-h exposure. After these measurements were made, the ventilatory response to hypercapnia was determined in a protocol lasting 20 min. In this protocol, PETO₂ was held at 200 Torr throughout and PETCO₂ was increased by 3 Torr every 5 min, starting at 1–2 Torr above the control value determined before the start of the chamber exposure. Therefore, the four levels of PETCO₂ desired were ~1.5, ~4.5, ~7.5, and ~10.5 Torr above the control PETCO₂.

Technique. The 8-h exposures were conducted with individual subjects inside a purpose-built chamber with ample room to sit or move around comfortably. Within the chamber the composition of gas can be altered, which obviates the need for subjects to breathe via a face mask or mouthpiece. Fine nasal catheters were held at the opening of each of the subject’s nostrils by a nasal oxygen-therapy mask. The inspired gas was sampled (80 ml/min) via these catheters and analyzed for PO₂ and PCO₂ by a mass spectrometer. The subject also wore a pulse oximeter on a finger to monitor arterial O₂ saturation. The values for PO₂, PCO₂, and O₂ saturation were sampled by a computer every 20 ms. The computer program identified the ends of inspiration and expiration from the PCO₂ profile and recorded the inspired and end-tidal values for PO₂ and PCO₂ together with O₂ saturation at the end of each breath. Before the subject entered the chamber, the composition of the inspired gas necessary to produce the desired end-tidal partial pressures was estimated and set manually. During the experiment, the composition of the inspired gas was altered by the computer every 5 min to maintain the end-tidal partial pressures at the desired level. Manual alteration at other intervals was also possible. This system has been described in greater detail elsewhere (10).

Ventilatory responses to hypercapnia were measured outside the chamber. The subject was seated in an upright position and breathed through a mouthpiece, with his or her nose occluded by a clip. A turbine volume-measuring device fixed in series with the mouthpiece measured the respiratory volumes; a pneumotachograph was used to record flows and timing information. The total dead space associated with the apparatus was 100 ml. Gas was sampled (20 ml/min) from this dead space, close to the mouth, and analyzed by mass spectrometer for PO₂ and PCO₂. A pulse oximeter was attached to a forefinger to monitor the O₂ saturation of the blood. All the data were sampled by a data-acquisition computer every 20 ms, and PETCO₂, PETO₂, inspiratory and expiratory volumes, and durations for each breath were recorded.

To obtain accurate control over PETCO₂ and PETO₂ during the measurements of hypercapnic sensitivity, an end-tidal forcing system was employed. Before the experiment, a “forcing function” containing the predicted inspired gas values required to achieve the desired PETO₂ and PETCO₂ was entered into a second (controlling) computer. During the experiment, actual values of PETO₂ and PETCO₂ were passed breath by breath to the controlling computer from the data-acquisition computer. The actual end-tidal values were compared with the desired values, and a new inspired gas mixture was calculated by using an integral-proportional feedback scheme. The controlling computer generated the new inspired gas mixture by using a fast gas-mixing system that was controlled from the program. This system has been described in more detail elsewhere (13, 16).

Data analysis. Average values for VE and PETCO₂ were calculated for the last minute at each level of hypercapnia. The VE-PETCO₂ response line was obtained by fitting a straight line to each set of four data points by using simple linear regression. Values for the slope and intercept (PETCO₂ at VE = 0) were obtained from this procedure. Mean changes within each protocol (PM – AM) were assessed statistically by using paired t-tests. Comparisons between protocols were undertaken by using analysis of covariance. In the case of the VE-PETCO₂ response curves, this analysis was undertaken on the log of values to help stabilize variance and provide more normal distributions (1). A value of P < 0.05 was used for statistical significance, except for subsequent pairwise comparisons among the three protocols in which P < 0.017 was used to allow for multiple (3) comparisons. The SPSS statistical package was used for these analyses.

RESULTS

Subjects. All subjects completed the series of experiments and provided data that were suitable for analysis. During the 8-h exposures, subjects were generally comfortable and spent their time reading, watching television, or playing computer games. Some subjects reported mild headaches toward the end of some of the exposures; one subject found the level of ventilation rather uncomfortable during the last hour of isocapnic hypoxia, and another felt slightly dizzy toward the end of the pokilocapnic exposure. Control values for PETCO₂ for each subject for each protocol are given in Table 1.

End-tidal gas values in the chamber. Figure 1 shows the end-tidal gases recorded while the subjects were in the chamber, averaged every 5 min, for each of the 10 subjects and for all 3 protocols. The initial and final values for each subject and protocol are given in Table 1. The plots illustrate the quality of the control that was achieved over PETCO₂ and PETO₂ in protocol IH and over PETO₂ in protocol PH. Average values for saturation obtained from the pulse oximeter at the beginning and end of the chamber exposure were 92.8 ± 2.6 and 89.4 ± 1.4% for protocol IH, 92.5 ± 1.5 and 89.8 ± 0.9% for protocol PH, and 97.8 ± 0.7 and 97.8 ± 0.8% for protocol C, respectively.

In protocol PH there appears to be a general trend downward in PETCO₂ over the 8-h period of hypoxia. A comparison of the values for PETCO₂ from the first and last 5 min in the chamber revealed a significant fall of 3.2 Torr [95% confidence interval (CI) 0.4 to 4.9 Torr, P < 0.05, paired t-test]. In protocol C there appears to be a general rise in PETCO₂, but this was not significant (NS) [95% CI –0.4 to 3.5 Torr, P = NS]. To provide comparison among the protocols, an analysis of covariance was performed on the values for PETCO₂ for the last 5 min in the chamber (Table 1) with the values for the first 5 min in the chamber as a covariate, together with subjects as a random factor and protocols as a fixed factor. This revealed that the protocols differed (P < 0.01) with respect to the change in PETCO₂, and pairwise comparisons showed significant differ-
ences between protocol PH and the other two protocols but not between protocol IH and protocol C.

PETCO₂ under air-breathing conditions. Values for air-breathing PETCO₂ 0.5 h after the end of the chamber exposure along with the control values are given in Table 1. PETCO₂ fell after protocol IH by 2.1 Torr (95% CI 1.0 to 3.2 Torr, \( P = 0.005 \)); after protocol PH PETCO₂ fell by 1.8 Torr (95% CI 0.5 to 3.0 Torr, \( P < 0.05 \)) but was unaltered after protocol C (rise of 0.1 Torr, 95% CI –0.9 to 1.2 Torr, \( P = NS \)). A comparison among the protocols was provided statistically by using the control values as a covariate, together with subjects as a random factor and protocols as a fixed factor. There was a significant effect of protocol (\( P < 0.001 \)) on PETCO₂, and pairwise comparisons revealed significant differences between protocol C and the other two protocols, but not between protocols PH and IH.

Ventilatory response to hypercapnia. Figure 2 illustrates the \( \dot{V}E - PETCO₂ \) responses obtained for each of the 10 subjects before and after the 8-h exposure associated with each protocol. The \( \dot{V}E - PETCO₂ \) responses were normalized to pre-chamber control values to account for individual differences in baseline ventilatory drive. The PETCO₂ responses were then averaged for each protocol and for each subject to generate a mean response profile. The mean responses for the isocapnic hypoxia, poikilocapnic hypoxia, and control protocols are shown in Figure 2.

![Figure 1: Control of end-tidal gases in chamber. Deviation of end-tidal PCO₂ (PETCO₂) from prechamber control value (ΔPETCO₂; top row), PETCO₂ (middle row), and end-tidal P0₂ (PETO₂; bottom row) averaged every 5 min from data collected breath by breath over 8 h for all 10 subjects during isocapnic hypoxia protocol (left column), poikilocapnic hypoxia protocol (middle column), and control protocol (right column).](http://jap.physiology.org/)
with each protocol. The data points represent averages over the last minute of each level of PETCO₂. It can be seen that the values for PETCO₂ are generally well matched before and after the hypoxic exposure and among protocols.

The slopes and intercepts for each of the response lines shown in Fig. 2 are given in Table 2. For both protocols IH and PH, 9 of 10 of the response slopes were greater after the hypoxic exposures, whereas for protocol C this was only true for 5 of 10 subjects. The mean increase in slope after protocol IH was 0.8 l·min⁻¹·Torr⁻¹ (95% CI 0.3 to 1.3 l·min⁻¹·Torr⁻¹, P < 0.005) and after protocol PH it was 1.2 l·min⁻¹·Torr⁻¹ (95% CI 0.3 to 2.0 l·min⁻¹·Torr⁻¹, P < 0.05). The mean slope was unaltered in protocol C (increase of 0.0 l·min⁻¹·Torr⁻¹, 95% CI −0.2 to 0.3 l·min⁻¹·Torr⁻¹, P = NS). To compare protocols, an analysis of covariance was performed on the logs of the slopes after the chamber exposure, with the logs of the slopes preceding the chamber exposure as a covariate, the subjects as a random factor, and protocol as a fixed factor. This revealed significant differences among the protocols (P < 0.001). Subsequent pairwise comparisons showed that both hypoxic exposures were significantly different from control (protocol IH vs. protocol C, P < 0.01; protocol PH vs. protocol C, P < 0.001), but there was no significant difference between the two hypoxic protocols.

Inspection of Fig. 2 and Table 2 suggests that there were no significant effects on the value of the intercept (extrapolated PETCO₂ for VE = 0) in any protocol. This was confirmed statistically (decreases in intercept were the following: protocol IH, 0.5 Torr, 95% CI −1.7 to 1.6 Torr, P = NS; protocol PH, 0.0 Torr, 95% CI −1.1 to 1.4, P = NS). Analysis of covariance on the value of the intercept after the chamber exposure, with the value of the intercept before the chamber exposure as a covariate, the subjects as a random factor, and protocol as a fixed factor, confirmed there were no differences among protocols (P = 0.46, NS).
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Slopes and intercepts of V˙E-PETCO₂ response relationship
Table 2.

- **DISCUSSION**

The main findings from this study are 1) 8 h of
hypoxic exposure results in an increase in the slope of
the relationship between V˙E and PETCO₂ in hypoxia
but does not affect the intercept of this relationship
with the PETCO₂ axis; and 2) this increase in slope over
the 8 h of hypoxic exposure does not differ significantly
between isocapnic and poikilocapnic hypoxia, although
the degree of hypocapnia induced was quite limited in
this study. The second finding suggests that the increase
in CO₂ sensitivity in poikilocapnic hypoxia is due
to hypoxia per se rather than to the concomitant
hypocapnia. The results also suggest that, whereas the
increase in slope of the V˙E-PETCO₂ relationship may
begin early in the process of acclimatization to altitude,
this is not the case for the shift in intercept. Thus the
increase in slope of the V˙E-PETCO₂ relationship and the
shift in intercept that occur during acclimatization are
likely to arise, at least in part, from different mecha-
nisms.

Air breathing and chamber values for PETCO₂. The
results for air-breathing PETCO₂ before and after the
chamber exposure demonstrate that a fall in PETCO₂
occurs after sustained hypoxia that is not dependent on
an associated hypocapnic alkalosis during the hypoxic
period. This result is consistent with the previous
observation (18) that hyperoxic V˙E at constant PETCO₂
is increased after 8 h of both isocapnic and poikilocapnic
hypoxia.

A comparison of the values for air-breathing PETCO₂
inside the chamber (protocol C) with the values ob-
tained outside of the chamber revealed that the values
inside the chamber were ~2 Torr higher (Table 1). We
do not have a complete explanation for this. Both the
air-breathing values for PETCO₂ outside the chamber
and the values for PETCO₂ inside the chamber were
obtained by sampling from a nasal catheter. The calibra-
tion gases and software used were identical in both
cases. However, the hardware used for the samples
inside and outside the chamber differed. In particular,
outside the chamber, a different mass spectrometer was
used together with a shorter sampling catheter. Subse-
quent inspection of the calibration data revealed that
the signal from the mass spectrometer associated with
the data from outside the chamber was a little noisier than
that from the mass spectrometer associated with the
data from inside the chamber. Because the software to
determine PETCO₂ operated by detecting peak values
in the CO₂ signal, there is a small amount of bias
introduced such that larger values for PETCO₂ would
be expected with noisier signals for CO₂. However, we do
not think that this mechanism by itself could account
for more than about one-half the difference between the
control values inside the chamber and the values
obtained from outside the chamber. The remaining
difference could possibly be due to the shorter sampling
catheter, although it is difficult really to see why, or to
some other more biological differences, such as how
comfortable and settled the subjects felt inside the
chamber compared with outside. Whatever the reason
for these differences in the control data, they help to
explain why a fall in PETCO₂ was not observed between
the conditions of air breathing outside the chamber and
the first 5 min of poikilocapnic hypoxia inside the
chamber.

Comparison with other studies. Eger et al. (6) studied
four subjects over 8-h periods at different levels of
PETCO₂ (hypocapnia was achieved by hyperventilation)
with and without concomitant hypoxia. They found
that the ratio of the hyperoxic V˙E-PETCO₂ response slope
CO₂ sensitivity after 8 h of hypoxia

CO₂ sensitivity after 8 h of hypoxia

PARA/AM was increased after the hypoxic exposures but not after the euoxic exposures, although the significance of their result was uncertain because the control values for the VePETCO₂ relationship slope differed significantly between the hypoxic and euoxic protocols. Our results remove this uncertainty because the control slopes did not differ among the three protocols of our study (ANOVA, NS, P = 0.73).

Eger et al. (6) report that hypocapnia resulted in a leftward shift of the VePETCO₂ response and that "Hypoxia was associated with a greater shift of the CO₂-response curve than normoxia for a given change in acclimatization PACO₂." At first sight, these results may appear somewhat contradictory to our finding that there was no shift in the VePETCO₂ response, especially in the case of protocol PH. However, there are two important differences between the results of Eger et al. and those of the present study. First, Eger et al. employed much greater degrees of hypocapnia, generated by forced hyperventilation, whereas our levels of hypocapnia were much more modest and resulted solely from the hypoxic stimulus. Second, Eger et al. define shift in relation to an arbitrarily chosen level of ventilation at $15 \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, whereas our study defines shift in relation to the intercept of the VePETCO₂ relationship with the PETCO₂ axis.

To compare the results of Eger et al. (6) with those of the present study in a more direct fashion, we recalculated the intercepts of Eger et al. to correspond with the results of our study. First, Eger et al. employed much greater degrees of hypocapnia, generated by forced hyperventilation, whereas our levels of hypocapnia were much more modest and resulted solely from the hypoxic stimulus. Second, Eger et al. define shift in relation to an arbitrarily chosen level of ventilation at $15 \cdot \text{min}^{-1} \cdot \text{m}^{-2}$, whereas our study defines shift in relation to the intercept of the VePETCO₂ relationship with the PETCO₂ axis.

To compare the results of Eger et al. (6) with those of the present study in a more direct fashion, we recalculated the intercepts of Eger et al. to correspond with the results of our study (ANOVA, NS, P = 0.73).

Engwall and Bisgard (7) reported a rise in CO₂ sensitivity with no change in intercept after isocapnic hypoxia. However, in contrast with the present study, Engwall and Bisgard (7) reported no alteration in the slope of the V̇E-PETCO₂ relationship until 75 h into the experiment, a result in keeping with the results of our study. The interactive term between hypoxia and hypocapnia, which indicates the influence of hypoxia on the shift associated with a given level of hypocapnia, approached significance (P = 0.064) but did not quite reach it.

Cruz et al. (4) compared the effects of 100 h of poikilocapnic hypoxia in one group of four subjects with 100 h of hypoxia with supplemental CO₂ in another group of four subjects. They were unable to detect a significant increase in the slope of the VePETCO₂ relationship over time, although the figure in their paper illustrates a trend in this direction. Similarly, they were unable to detect a change in the intercept of this relationship until 75 h into the experiment, a result in keeping with our study. At 75 h, a reduction in intercept was detected for the poikilocapnic hypoxic exposure but not for the hypoxic exposure with added CO₂.

Mechanisms and animal models. Engwall and Bisgard (7) have reported that, in goats, both isocapnic and poikilocapnic exposures to hypoxia altered the subsequent hypoxic V̇E-PETCO₂ relationship. By way of contrast, Weizhen et al. (19) have reported that, if the conditioning period of hypoxia were restricted so that it affected the central nervous system but not the carotid bodies, then no changes in the hypoxic V̇E-PETCO₂ relationship occurred. These results suggest that it may be the presence of hypoxia at the carotid body that is important for altering the V̇E-PETCO₂ response relationship. Neither of these two studies nor the present study has identified unambiguously whether this alteration in overall CO₂ response arises from a change in the peripheral or the central chemoreflex response to CO₂. On one hand, if hypoxia is exerting its modulatory effects on the CO₂ response at the carotid body, then it might seem more likely that the alteration would be in the peripheral chemoreflex response to CO₂. On the other hand, the responses to CO₂ were measured under conditions of either euoxia (19) or hypoxia (present study and Ref. 7), where the peripheral chemoreflex contribution is generally considered to have been minimized, and this might suggest that the change is an alteration in the central chemoreflex response to CO₂.

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