Human pulmonary vascular response to 4 h of hypercapnia and hypocapnia measured using Doppler echocardiography

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Balanos, George M., Nicholas P. Talbot, Keith L. Dorrington, and Peter A. Robbins. Human pulmonary vascular response to 4 h of hypercapnia and hypocapnia measured using Doppler echocardiography. J. Appl. Physiol. 94: 1543–1551, 2003. First published December 13, 2002; 10.1152/japplphysiol.00890.2002.—Hypercapnia has been shown in animal experiments to induce pulmonary hypertension. This study measured the sensitivity and time course of the human pulmonary vascular response to sustained (4 h) hypercapnia and hypocapnia. Twelve volunteers underwent three protocols: 1) 4-h euoxic (end-tidal $P_{O_2} = 100$ Torr) hypercapnia (end-tidal $P_{CO_2}$ was 10 Torr above normal), followed by 2 h of recovery with euoxic eucapnia; 2) 4-h euoxic hypocapnia (end-tidal $P_{CO_2}$ was 10 Torr below normal) followed by 2 h of recovery; and 3) 6-h air breathing (control). Pulmonary vascular resistance was assessed at 0.5- to 1-h intervals by using Doppler echocardiography via the maximum tricuspid pressure gradient during systole. Results show progressive changes in pressure gradient over 1–2 h after the onset or offset of the stimuli, and sensitivities of 0.6 to 1 Torr change in pressure gradient per Torr change in end-tidal $P_{CO_2}$. The human pulmonary circulatory response to changes in $P_{CO_2}$ has a slower time course and greater sensitivity than is commonly assumed. Vascular tone in the normal pulmonary circulation is substantial.

Pulmonary vascular effects of hypocapnia in the right apical lobe of conscious sheep were explored by Sheehan and Farhi (22). They found that hypocapnia increased blood flow to the lobe substantially, and they suggested that variations in both $CO_2$ and $O_2$ about normal values play an important role in the physiological matching of ventilation to perfusion when these values are perturbed in the lung by gravity.

Because hyperoxia has been found to have little vasodilatory effect on the pulmonary circulation, it appears to be widely thought that, in the words of Fishman (6), “because of the low initial tone, attempts to vasodilate the normal pulmonary circulation are destined to be fruitless.” In this study, we set out to compare the effects of sustained (4 h) hypocapnia and hypercapnia on the human pulmonary circulation, with a view to establishing whether normal tone can be reduced and whether changes in end-tidal $P_{CO_2}$ ($P_{ETCO_2}$) produce substantial or small proportionate changes in an index of pulmonary vascular resistance (PVR). A second aim was to define the time course of the pulmonary vascular responses to these stimuli and of the recovery from these stimuli during a subsequent 2 h of eucapnia.

METHODS

Subjects. Twelve healthy volunteers (5 women, 7 men, age 24.8 ± 3.3 yr, mean ± SD) participated in the study. The suitability of subjects for the experiments was confirmed by echocardiographic visualization of tricuspid regurgitation during ventricular systole, which is commonly detected in most healthy individuals. Informed, written consent was obtained on each experimental day. Ethical permission was granted by the Central Oxford Research Ethics Committee.

Protocols. Subjects underwent three protocols on three separate days. In the hypercapnia protocol, subjects were exposed to euoxic hypercapnia for 4 h [end-tidal $P_{O_2}$ ($P_{ETO_2}$) = 100 Torr and $P_{ETCO_2}$ = 10 Torr above normal], followed by 2 h of euoxic eucapnia ($P_{ETO_2}$ = 100 Torr, normal $P_{ETCO_2}$). Hypercapnia was achieved by elevating the inspired $P_{CO_2}$ ($P_{ICO_2}$) above normal. In the hypocapnia protocol, subjects were exposed to euoxic hypocapnia for 4 h ($P_{ETO_2}$ = 100 Torr, $P_{ETCO_2}$ = 10 Torr below normal), followed by 2 h of euoxic eucapnia ($P_{ETO_2}$ = 100 Torr, normal $P_{ETCO_2}$). Hypocapnia was achieved by mechanical hyperventilation via a facemask using a Siemans-Elma Servo Ventilator 900B. During the control protocol, subjects breathed air for 6 h. All three protocols started at the same time of day. The order of the protocols was varied between subjects.

Subjects reported to the laboratory 60 min before the beginning of each experiment. During this period, the subject’s normal $P_{ETCO_2}$ was measured, baseline echocardiographic measurements were made, and 5 ml of blood were
taken from an arm vein for measurement of hemoglobin concentration (Hb). Throughout all three protocols, subjects were asked to wear a comfortable facemask. This was the means of achieving passive hyperventilation during the hypocapnia protocol and for collecting expired gases in all three protocols.

Control of end-tidal gases. During all three protocols, subjects were either seated or lying down in a chamber in which the inspired PO₂ (PᵢO₂) and PᵢCO₂ could be changed so as to maintain desired end-tidal values. Respired gas was sampled continuously from a nasal cannula within the facemask and was analyzed with a mass spectrometer. Inspired and end-tidal values were recorded on a computer for each breath. Computer-automated control of PᵢT₀₂ and PᵢTᵢCO₂ was achieved by adjustment of the composition of the gas in the chamber every 5 min, as previously described (7). In the hypocapnia protocol, an elevation in PᵢTᵢCO₂ was achieved by increasing PᵢCO₂. Ventilation remained spontaneous and consequently increased to above normal values. In the presence of a sound machine with a S4 two-dimensional transducer (2)

tidal values were recorded on a computer for each breath. 

Repeated-measures ANOVA were then performed on the data from just the control protocols to check that time did not have a significant effect in these protocols. Separate repeated-measures ANOVA was undertaken to determine whether there was an interaction between time and protocol. Separate repeated-measures ANOVA were then performed on the data from just the control protocols to check that time did not have a significant effect in these protocols. 

Cardiac output. Cardiac output (Q) was measured by using an apical five-chamber view with Doppler mode at a display screen sweep speed of 100 mm/s. Doppler sampling of the flow was made just below the orifice of the aortic valve. The flow was quantified automatically by using the velocity-time integral, which is the mean distance through which blood travels in the outflow tract during ventricular contraction.

The diameter just below the aortic valve orifice was measured from a parasternal long axis view, and the area at this site (A) was calculated. Q was then calculated by using Eq. 2

\[
Q = VTI \times A \times HR
\]

where VTI is the velocity-time integral.

Collection of expired gas. Mixed expired gas was collected to enable us to estimate the mixed venous PO₂ (PᵥO₂) and PᵥCO₂ (PᵥCO₂) (see Estimation of gas composition of mixed venous blood below). The aim of the collection was to measure the rate of O₂ consumption (Vₒ₂) and CO₂ elimination (VₑCO₂) that are required for this calculation.

A Douglas bag was connected either to a two-way valve of the mask (hypocapnia and control protocols) or to the expiratory port of the ventilator (hypocapnia protocol) for collection of mixed expired gas. The duration of each collection was measured and timed to coincide with echocardiography measurements. After each collection, two 50-ml samples from the Douglas bag were analyzed for concentrations of O₂ and CO₂ by using a mass spectrometer. The volume and temperature of the bag contents were measured with a dry spirometer and thermometer, respectively.

Estimation of gas composition of mixed venous blood. It is known that variations in PᵥO₂ can induce changes in pulmonary vascular tone independently of alveolar gas PO₂ (14). Whether variations in PᵥCO₂, independent of alveolar PCO₂ (PᵥCO₂; i.e., PᵢTᵢCO₂) have an effect on pulmonary vascular tone appears not to have been addressed in the literature. Because of the possibility that changes in either PᵥO₂ or PᵥCO₂ might be at least partly responsible for changes in ΔPᵥmax measured during alveolar hypocapnia or hypocapnia, we estimated the mixed venous blood gases by using measurements of VO₂, VₑCO₂, Q, and [Hb] (g/l), as explained in the APPENDIX.

Statistical analysis. Repeated-measures ANOVA was undertaken to determine whether there was an interaction between time and protocol. Separate repeated-measures ANOVA were then performed on the data from just the control protocols to check that time did not have a significant effect in these protocols.

To determine the time at which steady state had been reached after the induction of hypocapnia or hypcapnia, a family of linear models was used, where the next model differed from the previous model by the inclusion of the next additional factor for time (t), starting from t = 0. With the use of the notation defined by Armitage (1), the models are given by

\[
V_v = μ + S_1 + ε_v, (j = 0)
\]

\[
V_v = μ + S_1 + h_1 + ε_v, (j = 1)
\]

\[
V_v = μ + S_1 + h_1 + h_2 + \ldots + h_{n-1} + ε_v, (j = n - 1)
\]

The diameter just below the aortic valve orifice was measured from a parasternal long axis view, and the area at this site (A) was calculated. Q was then calculated by using Eq. 2

\[
Q = VTI \times A \times HR
\]

where VTI is the velocity-time integral.
where \( j \) is the index of the model, \( V \) is the dependent variable, \( \mu \) is the mean, \( S \) indicates the contribution of a particular subject (index \( i \)), \( h_1 \) to \( h_{n-1} \) are the factors that indicate the contribution of each time point, \( v \) is the number of time points at which data were collected, and \( \epsilon \) is the residual error. As each model was introduced sequentially, the reduction in squared error over the previous model was assessed for statistical significance (F ratio test). The time at which steady state had been reached was taken as the first time point for which a significant reduction in squared error did not arise.

Values given are means \( \pm \) SE unless otherwise stated. Statistical significance was assumed at \( P < 0.05 \).

**RESULTS**

*Gas control.* Figure 1 illustrates values for \( P_{\text{IO}} \), \( P_{\text{ICO}} \), \( P_{\text{ET}} \), and \( P_{\text{ETCO}_2} \). For the hypercapnia and hypocapnia protocols, it can be seen that the steps into and out of the conditions of altered \( P_{\text{ETCO}_2} \) were achieved relatively rapidly and that euoxia was maintained throughout, apart from brief deviations in \( P_{\text{ETCO}_2} \) of \(-10\) Torr during the first 15 min of the hypercapnia and hypocapnia protocols. On average, \( P_{\text{ETCO}_2} \) was maintained at 9.1 Torr above subjects’ normal values during hypercapnia and at 10.1 Torr below subjects’
normal values during hypocapnia. During the control protocol, the end-tidal gases changed little over the 6-h period.

Pulmonary vascular response to hypercapnia, and during recovery. ΔP_{max} increased gradually during hypercapnia (Fig. 2). The response was significantly different compared with control (P < 0.001, protocol by time, repeated-measures ANOVA). Steady state had not been achieved by 2 h (P < 0.01, ANOVA). For the period 3–4 h after the beginning of hypercapnia, the sensitivity of the change in ΔP_{max} relative to the value at t = 0 was 0.95 Torr per Torr change in P_{ETCO2}. On return to eucapnic conditions, ΔP_{max} was restored to control levels after 1.5 h.

Pulmonary vascular response to hypocapnia and during recovery. ΔP_{max} decreased gradually during hypocapnia (Fig. 2). The response was significantly different compared with control (P < 0.001, protocol by time, repeated-measures ANOVA). Steady state had not been achieved by 1.5 h (P < 0.05, ANOVA). For the period 3–4 h after the beginning of hypocapnia, the sensitivity of the change in ΔP_{max} relative to the value at t = 0 was 0.63 Torr per Torr change in P_{ETCO2}. On return to eucapnic conditions, ΔP_{max} was restored to control levels after 1.0 h.

HR, stroke volume, and Q responses. Figure 3 shows the response of HR, stroke volume, and Q during the hypercapnia, hypocapnia, and control protocols. Q during hypercapnia was significantly higher when compared with control (P < 0.005, protocol by time, repeated-measures ANOVA). A similar statistical comparison revealed a significant difference for HR (P < 0.005, protocol by time, repeated-measures ANOVA) but not for stroke volume.

Q during hypocapnia was also significantly different when compared with control (P < 0.005, protocol by time, repeated-measures ANOVA). In Fig. 4, absolute differences are plotted between hypercapnia and control, and hypocapnia and control. This is done so that a clearer picture of the net response of each condition can be presented, and also for effects caused by initial anxiety and digestion to be cancelled out.

Calculated P_{VCO2}, P_{VO2} and mixed venous pH. Figure 5 shows the results of the calculations performed according to the Appendix to estimate P_{VCO2} and P_{VO2}. The results from some of these calculations were compared with the predictions of the blood-gas nomograms of Olszowska et al. (19). In all cases, the calculations and predictions agreed. Both P_{VCO2} and P_{VO2} during the hypocapnia protocol and P_{VCO2} during the hypercapnia protocol were significantly different when compared with the control protocol (P < 0.002, protocol by time, repeated-measures ANOVA). P_{VO2} during the hypercapnia protocol did not change significantly. The changes in P_{VCO2} and P_{VO2} when switching into and out of hypercapnia and hypocapnia were quick. Steady state was achieved by 30 min in all cases except P_{VO2} during the return to eucapnia after hypocapnia.

The calculated mixed venous pH values showed that pH during the hypercapnia protocol was lower (7.31 ± 0.01), and during the hypocapnia protocol was higher (7.44 ± 0.01), when compared with the control protocol (7.35 ± 0.01).

DISCUSSION

The main findings of our study are that the human pulmonary vascular responses to hypercapnia and hypocapnia consist, respectively, of constriction and dilatation that take 1.5–2 h to reach a steady level when resolved with measurements every 0.5–1 h over a 4-h period. The time courses for recovery in eucapnia are similar. The cardiovascular responses to the two stimuli differed qualitatively. Hypercapnia generated a rise in Q by changing HR; hypocapnia produced a fall in Q by changing stroke volume. The finding of marked vasodilatation in response to hypocapnia demonstrates that there is normally substantial vascular tone in the human pulmonary circulation.

Relationship between ΔP_{max} and pulmonary vascular tone. At a cellular level, the physiological response that is of greatest relevance to our observations on the intact pulmonary circulation is the change that occurs in pulmonary artery smooth muscle activity in response to hypercapnia and hypocapnia. Our echocardiographic measurement of ΔP_{max} is an indirect index of this smooth muscle activity, which we here call pulmonary vascular tone. In a previous study, we argued that changes in ΔP_{max} reflect changes in pulmonary vascular tone but not alterations in PVR brought about by changes in Q (2). This study adds support to this observation; during the first 3 h of the control protocol, Q fell and then rose by ~900 ml/min (Fig. 3). This was associated
with a concurrent fall and then rise in ΔP<sub>max</sub> of only ~0.9 Torr (Fig. 2).

Effects of mixed venous blood gases on pulmonary vascular tone. There is evidence from animal experiments that both alveolar P<sub>O2</sub> (P<sub>A</sub>O<sub>2</sub>) and P<sub>V</sub>O<sub>2</sub> affect pulmonary vascular tone (13, 15). In the present experiment, P<sub>V</sub>O<sub>2</sub> in the hypocapnia protocol was ~4 Torr lower than in the control and hypercapnia protocols (Fig. 5). One explanation for the lower sensitivity of the change in ΔP<sub>max</sub> in response to a fall in P<sub>ET</sub>CO<sub>2</sub> than to a rise in P<sub>ET</sub>CO<sub>2</sub> is that the relative mixed venous blood hypoxia in the hypocapnia protocol induced a degree of hypoxic pulmonary vasoconstriction that partially offset the effect of hypocapnia.

The question of the extent to which changes in P<sub>V</sub>CO<sub>2</sub> can lead to changes in pulmonary vascular tone that are independent of P<sub>A</sub>CO<sub>2</sub> appears not to have been addressed in the literature. It is notable that the changes in P<sub>V</sub>CO<sub>2</sub> occurring in this experiment are of a similar magnitude to the corresponding changes in P<sub>ET</sub>CO<sub>2</sub>. It is not possible from this experiment to distinguish between the separate effects of these stimuli.

The absence of any significant change over time in calculated values of P<sub>V</sub>CO<sub>2</sub> and P<sub>V</sub>O<sub>2</sub> suggests that the delay in reaching steady state during hypercapnia (3 h) and hypocapnia (2 h) cannot be attributed to gradual changes in the gas stimuli in mixed venous blood. The relatively slow time course of the responses seen with
half-hourly measurements is consequently likely to arise from a slow component within the physiological response to CO₂ itself.

Relative contributions of O₂ and CO₂ to ventilation-perfusion matching in the lung. In the healthy lung, variations in regional ratios of ventilation and perfusion are thought to generate a range of values of P_AO₂ of 80–140 Torr and a range of P_ACO₂ values of 42–20 Torr while breathing air at sea level (24). Pulmonary vasoconstriction in response to regional hypoxia and hypercapnia is recognized as a mechanism that is potentially capable of reducing mismatch of ventilation and perfusion, although much of the literature that has examined this phenomenon has concentrated on the oxygen signal.

Thus, for example, Mélot et al. (16), who studied humans by using the multiple inert gas elimination technique, deduced that “in normoxic conditions, active hypoxic regulation of gas exchange results in hardly or nondetectable improvements in arterial blood gases.” The weakness of the hypoxic pulmonary vasoconstriction in normoxia was attributed by these authors to the fact that its potential contribution to improving matching of perfusion to ventilation was greatest for values of P_AO₂ of 60 Torr, i.e., below the usual range experienced in healthy lungs ventilated with air at sea level. In experiments on sheep, Sheehan and Farhi (22) gave attention to both the O₂ and CO₂ signals in the matching of perfusion to ventilation. When a model deduced from experiments on sheep was applied to

Fig. 4. Mean differences (Δ) in heart rate (A), stroke volume (B), and cardiac output (C) between the hypercapnia and control protocols (circles), and between the hypocapnia and control protocols (squares). Closed symbols, measurements made under hypercapnic (hypercapnia protocol) or hypocapnic (hypocapnia protocol) conditions; open symbols, measurements made under eucapnic conditions. Data are means ± SE for n = 12 subjects. These data are derived from those in Fig. 3.
humans, Sheehan and Farhi concluded that “as we stand, local blood flow control by alveolar gases halves the alveolar-arterial PO2 and PCO2 differences imposed by gravity,” suggesting that the regional responses to hypercapnia and hypocapnia make a substantial contribution to the regulation of blood flow.

The relative magnitudes of the pulmonary vascular effects in humans of sustained changes in PO2 and PCO2 cannot be assessed from the existing literature. Studies have been limited to brief exposures lasting up to 30 min. Thus, for example, Kilburn et al. (11) studied patients with chronic pulmonary diseases by exposing them for 10–20 min to an inspired CO2 level of 10%. They measured pulmonary artery pressure by using direct cannulation and concluded that mean pulmonary artery pressure rose by 0.85 Torr per Torr rise in arterial PCO2 in patients who were chronically hypercapnic, and by 0.59 Torr per Torr rise in arterial PCO2 in patients who were normally eucapnic. More recently, Kiely et al. (10) used echocardiography to assess hemodynamic changes after 30 min in healthy humans rendered hypercapnic (PETCO2 = 7 kPa) by inspiring CO2 mixed with air. Mean pulmonary artery pressure rose by ~0.4 Torr per Torr rise in PETCO2. In neither study was PETCO2 independently controlled.

The sensitivity of the whole pulmonary circulation to hypercapnia and hypocapnia measured in the present study was a change in ΔPmax of 0.6–1 Torr per Torr rise in PACO2. In a similar echocardiographic study in humans, we have previously measured the sensitivity of the pulmonary circulation to a fall in PAO2 from 100 to 50 Torr to be a rise in ΔPmax of 15 Torr (2). The vasoconstrictor response to hypoxia is unlikely to be linear with respect to P02. For data from animal experiments, hypoxic pulmonary vasoconstriction has been modeled as a sigmoidal function of PAO2, in which the sensitivity of the vascular response increases as PAO2 decreases from ~100 Torr toward ~30 Torr (13). If the human response is similar in this respect, it is to be anticipated that the sensitivity of the pulmonary vascular response to hypoxia in the PAO2 range of 80–140 Torr given for the normal erect lung (24) would be considerably less that the value of the 0.3 Torr change in ΔPmax per Torr change in PAO2, which is the average over the range of 50–100 Torr in our previous study (2).

In contrast to this probably weak vascular response to hypoxia within the physiological range of PAO2 in the healthy lung, the results presented here show a vigorous response to changes in PACO2 within the physiological range. This suggests that, in the healthy lung, CO2 is a more important regulator of perfusion than O2.

APPENDIX

Mixed PvO2 and PvCO2 were estimated from measurements of PETO2, PETCO2, Q, [Hb], VO2, and VCO2 by using the following relationships.

Concentration of oxygen in arterial and mixed venous blood. The concentration of O2 (CO2) in blood was related to the PO2 in blood according to the equation

\[ CO_2 = \frac{\alpha_O2 \cdot PO2 + [Hb] \cdot c \cdot (PO2)^n}{(PO2)^n + (P50)^n} \]  (4)

where \( \alpha_O2 \) is the solubility of O2 in blood (0.03 ml STP \cdot 1^{-1} \cdot Torr^{-1}), \( c \) is the O2 binding capacity of hemoglobin (1.31 ml STP/g), \( P50 \) is the PO2 required to obtain 50% O2 saturation of hemoglobin, and \( n \) is the Hill coefficient for adult hemoglobin (2.8). These physiological variables have been attributed to a variety of values by different experimenters; the above values are in accord with the recommendations of Nunn (18) and Stryer (23). \( P50 \) was taken to equal 26 Torr at pH 7.4 and PCO2 = 40 Torr, and is independently a function of pH and PCO2 according to the relationship presented by Kelman (8).

\[ \Delta \log_{10} P50 = -0.40 \cdot \Delta pH + 0.06 \cdot \Delta \log_{10} PCO2 \]  (5)

During changes in pH entirely generated in vitro by changes in PCO2, this relationship has been shown (21) to take the form

\[ \Delta \log_{10} P50 = -0.48 \cdot \Delta pH \]  (6)

Consequently, we calculated values of \( P50 \) separately from Eq. 6 for both arterial blood and mixed venous blood according to the pH calculated for both of these, as indicated below.
The concentrations of O\textsubscript{2} in arterial (Ca\textsubscript{O2}) and mixed venous (Cv\textsubscript{O2}) blood were related to Vo\textsubscript{2} and Q by the relationship
\[ Ca\textsubscript{O2} - Cv\textsubscript{O2} = Vo\textsubscript{2}/Q \] (7)

It was assumed that Ca\textsubscript{O2} could be obtained from Eq. 4 by substituting Pet\textsubscript{O2} as the appropriate PO\textsubscript{2}. This assumes that Pet\textsubscript{O2} and Pa\textsubscript{O2} are close, as one would expect, in healthy participants.

Concentration of CO\textsubscript{2} in arterial and mixed venous blood. The concentration of CO\textsubscript{2} in the blood was calculated by using the method of Kelman (9). According to this method, the concentration of CO\textsubscript{2} in the plasma, both as molecular CO\textsubscript{2} and as bicarbonate, is calculated from the Henderson-Hasselbalch equation in the form
\[ CCO\textsubscript{2,plasma} = a\textsubscript{CO2}\cdot Pa\textsubscript{CO2} [1 + 10^{\text{pH}-pK}] \] (8)

and the total CO\textsubscript{2} carried in blood, including that carried as carbamino-CO\textsubscript{2} bound to hemoglobin, is obtained by multiplying the plasma concentration by an empirically derived ratio of the cell concentration of CO\textsubscript{2} (C\textsubscript{CO2,cell}) to the plasma concentration (C\textsubscript{CO2,plasma}). This latter ratio is itself a slight function of pH and the degree of saturation of hemoglobin with oxygen. For the purposes of the calculation undertaken here, it is sufficient to take the following values of this ratio
\[ C\textsubscript{CO2,cell}/C\textsubscript{CO2,plasma} = 0.59 \] (9)

for euoxic arterial blood with pH close to 7.4, and
\[ C\textsubscript{CO2,cell}/C\textsubscript{CO2,plasma} = 0.62 \] (10)

for mixed venous blood with a hemoglobin saturation of \(\sim75\)% and a pH in the range of 7.3–7.4. Assuming a value of hematocrit of 0.4, we obtained from Eqs. 8, 9, and 10 the following expressions for the whole blood concentrations of CO\textsubscript{2}, both reacted and unreacted
\[ Ca\textsubscript{CO2} = 0.84 \cdot a\textsubscript{CO2}\cdot Pa\textsubscript{CO2} [1 + 10^{\text{pH}-pK}] \] (11)

for arterial blood, and
\[ Cv\textsubscript{CO2} = 0.85 \cdot a\textsubscript{CO2}\cdot Pv\textsubscript{CO2} [1 + 10^{\text{pH}-pK}] \] (12)

for mixed venous blood. The value of pK\textsubscript{2} was taken to be 6.1, and a\textsubscript{CO2} the solubility of molecular CO\textsubscript{2} in plasma, was taken to equal 0.69 ml STP \cdot l^{-1} \cdot Torr\(^{-1}\) (18).

The concentrations of CO\textsubscript{2} in arterial and mixed venous blood were related to the CO\textsubscript{2} elimination of the body and Q by the relationship
\[ Ca\textsubscript{CO2} - Cv\textsubscript{CO2} = V\textsubscript{CO2}/Q \] (13)

It was assumed that Ca\textsubscript{CO2} could be obtained from Eq. 11 by substituting Pet\textsubscript{CO2} as the appropriate PCO\textsubscript{2}. This assumes that Pet\textsubscript{CO2} and arterial PCO\textsubscript{2} are close.

Calculation of arterial and mixed venous values of pH. It remained to estimate the change in pH that occurs as blood passes through the lungs. We assumed that the participant’s arterial blood has zero base excess, i.e., at a PCO\textsubscript{2} of 40 Torr the pH would equal 7.4. We then derived the pH of arterial and venous blood by using the results of Lloyd and Michel (12). These give mathematical expressions for the changes in plasma pH that occur with changes in PCO\textsubscript{2} and oxyhemoglobin saturation (SO\textsubscript{2}) in human blood in vitro. Given that blood passing through the pulmonary capillaries has only minimal opportunity to exchange bicarbonate with interstitial fluid, it is appropriate to use the relationships derived for in vitro blood rather than in vivo blood.

The calculation was based on the observation that there is a linear relationship between pH and plasma bicarbonate concentration (the Davenport diagram) in the blood in response to changes in PCO\textsubscript{2}
\[ pH = A + B[H\textsubscript{CO2}] = A + B\alpha\textsubscript{CO2}PCO2[10^{\text{pH}-pK}] \] (14)
in which \(\alpha\textsubscript{CO2}\) is the solubility of molecular CO\textsubscript{2} in plasma, here in units of mmol \cdot l\(^{-1}\) \cdot Torr\(^{-1}\) (and equals 0.0308), and B (l/mmole) is primarily a function of [H\textsubscript{b}], according to the relationship (12)
\[ B = -0.005 - 0.26/([H\textsubscript{b}]) \] (15)

where [H\textsubscript{b}] is in units of meq/l of O\textsubscript{2} binding sites. We converted this to a form that can include [H\textsubscript{b}] in units of g/l, noting that the molecular weight of hemoglobin is 64,458 (18) and that each molecule is associated with four binding sites for hemoglobin, so that [H\textsubscript{b}] = 16.1[H\textsubscript{b}]
\[ B = -0.005 - 4.19/([H\textsubscript{b}]) \] (16)

The intercept (A) in Eq. 14 is itself primarily a function of SO\textsubscript{2} according to the relationship (12)
\[ \Delta A/\Delta SO2 = -0.07 \] (17)

assuming a [H\textsubscript{b}] of \(-145\) g/l. In Eqs. 16 and 17, a dependence of both B and A on SO\textsubscript{2} is a second-order effect and was neglected for the purposes of the present calculation. For the purposes of calculating the change in SO\textsubscript{2} (\(\Delta SO2\)) between arterial and mixed venous blood, within Eq. 17 we made the assumption that the dissolved component of O\textsubscript{2} could be ignored and set
\[ \Delta SO2 = (Vo\textsubscript{2}/Q) / ([H\textsubscript{b}]+c) \] (18)

It should be noted that this assumption was limited to the calculation of A only and was not otherwise used in the calculation of the properties of mixed venous blood.

The above equations were solved iteratively to obtain values of arterial pH, venous pH, and the values of P\textsubscript{50} for hemoglobin in both arterial and mixed venous blood. From these, P\textsubscript{Vo2} and PV\textsubscript{CO2} were obtained by iteration.

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