Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise

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Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise. J Appl Physiol 90: 1798–1810, 2001.—To calculate cardiac output by the indirect Fick principle, CO2 concentrations ([CO2]) of mixed venous ([CvCO2]) and arterial blood are commonly estimated from PcO2, based on the assumption that the CO2 pressure-concentration relationship (PcO2-CO2) is influenced more by changes in pH, concentration and blood oxyhemoglobin saturation than by changes in pH. The purpose of the study was to measure and assess the relative importance of these variables, both in arterial and mixed venous blood, during rest and increasing levels of exercise to maximum (Max) in five healthy men. Although the mean mixed venous Pco2 rose from 47 Torr at rest to 59 Torr at the lactic acidosis threshold (LAT) and further to 78 Torr at Max, the CvCO2 rose from 22.8 mM at rest to 25.5 mM at LAT but then fell to 23.9 mM at Max. Meanwhile, the mixed venous pH fell from 7.36 at rest to 7.30 at LAT and to 7.13 at Max. Thus, as work rate increases above the LAT, changes in pH, reflecting changes in buffer base, account for the major changes in the Pco2-CO2 relationship, causing CvCO2 to decrease, despite increasing mixed venous Pco2. Furthermore, whereas the increase in the arteriovenous CO2 difference of 2.2 mM below LAT is mainly due to the increase in CvCO2, the further increase in the arteriovenous CO2 difference of 4.6 mM above LAT is due to a striking fall in arterial CO2 from 21.4 to 15.2 mM. We conclude that changes in buffer base and pH dominate the Pco2-CO2 relationship during exercise, with changes in Hb and blood oxyhemoglobin saturation exerting much less influence.

lactic acidosis threshold; maximum oxygen consumption; carbon dioxide transport; arteriovenous carbon dioxide difference; cardiac output

Our laboratory recently demonstrated that direct Fick principle calculated cardiac output using CO2 ([CO2]) agrees well with cardiac output using O2 ([O2]) in normal subjects at rest and during exercise (51), as predicted by Fick (16). Other investigators, to avoid blood sampling, have calculated CO2 indirectly (7, 12, 15, 29, 36). This approach, although superficially attractive, pays insufficient attention to the effect of pH and change in buffer base on the accuracy and precision of the estimation of CO2 concentration ([CO2]) from PcO2, particularly during work rates at which lactate is increased. Blood CO2 is influenced by pH, PcO2, Hb concentration, and oxyhemoglobin saturation (SO2). During exercise, the CO2 pressure-concentration relationship (PcO2-CO2 relationship) may be more complex than the near-linear relationship depicted in textbooks and the original reports (8, 38, 56), because it is assumed that there is no change in buffer base as CO2 increases. A frequently referenced report, which presents formulas for refining the influences of CO2, Hb, and SO2 on CO2 at rest and during exercise, concludes that “the relationship (Pco2-CO2) is only slightly influenced by changes in pH” (34). Although some investigators recommend correction for acid-base changes (18, 27), most investigators have calculated the arteriovenous CO2 difference [mixed venous CO2 (CvCO2)-arterial CO2 (CaCO2)] for estimating cardiac output by the indirect Fick method from mixed venous CO2 (PvCO2) and arterial CO2 (PaCO2), correcting only for changes in Hb and SO2, but ignoring pH changes (6, 9–11, 26, 31–33, 35, 37, 42).

It is well known that acidemia due to lactic acidosis occurs with symptom-limiting exercise, both in normal subjects and patients (3, 4, 21, 50–55), resulting in an almost stoichiometric decrease in bicarbonate concentration ([HCO3−]) as lactate increases (46, 48, 55). This intimate relationship challenges the concept that changes in pH only slightly influence CO2 during exercise. We measured pH, Pco2, Hb, and SO2 in both arterial and mixed venous blood during progressively increasing work rate exercise to maximum (Max) in normal subjects to determine the relative importance of the changes in pH, Hb, and SO2 on the Pco2-CO2 relationship and on each component in CO2 transport. We hypothesized that, by ignoring changes in acid-base balance during exercise, major errors result in estimates of CO2 from Pco2 at work rates above the lactic acidosis threshold (LAT). This could translate into ma-
METHODS

Subjects, Protocol, and Measures

Subjects. The research protocol was approved by the Human Subjects Committee at Harbor-UCLA Medical Center. Informed consent was obtained from five healthy nonsmoking male subjects that participated in the study.

Catheter placement. A flow-directed pulmonary artery catheter (Arrow International, Reading, PA) was introduced via a femoral vein sheath (Cordis, Miami, FL), which had been inserted percutaneously into the right femoral vein and positioned in the main pulmonary artery under direct fluoroscopic guidance. An arterial catheter was placed percutaneously into the left brachial artery. Each catheter was attached to an infusion apparatus (Continu-Flo, Baxter Health Care, Deerfield, IL), which provided a slow, continuous flow (15 ml/h) of heparinized normal saline (1,000 U heparin/l) and allowed periodic bolus flushings.

Exercise protocols. An increasing work rate exercise test was performed on an electromagnetically braked cycle ergometer (type 18070, Gould-Godart, Bilthoven, the Netherlands). The rate of work rate increase (25–40 W/min) depended on a preliminary, noninvasive increasing work rate exercise test designed to achieve exhaustion in ~10 min. Gas-exchange and heart rate measurements were averaged for each 30-s period during 3 min of rest and 3 min of unloaded pedaling and during the progressively increasing work rate test to maximum tolerance. Pedal frequency was maintained at 60 rpm.

Respiratory gas analysis. The subjects respired through a mouthpiece during the test. Expired air was directed to a Fleisch type 3 pneumotachograph via a breathing valve (100-ml dead space). The PO2, PCO2, and partial pressure of N2 at the mouthpiece were continuously measured by mass spectrometry (MGA-1100, Perkin Elmer, Pomona, CA). Minute ventilation (BTPS) and O2 uptake (VO2) and CO2 production (VCO2) (both STPD) were calculated as whole breath averages for each 30-s period during exercise and during the progressively increasing work rate test to maximum tolerance. Pedal frequency was maintained at 60 rpm.

Blood samples. Blood was sampled simultaneously from the pulmonary artery and brachial artery during rest and unloaded cycling and at each minute of increasing work rate exercise. Blood-gas samples were drawn over a 15- to 20-s period. The samples were collected in glass syringes that contained a small amount (mean 0.14 ml) of liquid heparin (1,000 U/ml). The blood samples were agitated to prevent clotting and were immediately placed in an ice slurry.

Blood analyses. Blood-gas analyses were performed by using an Instrumentation Laboratory 1306 blood-gas analyzer (Lexington, MA) for pH, PCO2, and PO2 and an Instrumentation Laboratory 482 CO-oximeter for Hb and SO2. The blood-gas analyzer was recalibrated every 20–30 min. Tonometryed blood samples were used to verify accuracy. The measured values were corrected for heparin dilution and the known consistent underestimation of blood PCO2 values >45 Torr (23, 51).

Calculation of CCO2, CvCO2, CaCO2, CO2, and Plasma [HCO3-]

Values of CCO2, CvCO2, CaCO2, CO2, and plasma [HCO3-]. The plasma CO2 (CCO2pl) (mM) and plasma [HCO3-] (mM) were calculated from the standard formula derived from the Henderson-Hasselbalch equation (13, 21, 34, 38)

\[ CCO2pl = PCO2 \times s \times [1 + 10^{(pH - pK'_{H})}] \] (1)

\[ \text{Plasma [HCO}_3^-] = CCO2pl - PCO2 \times s \] (2)

where s is the plasma solubility coefficient (in mM/Torr) of CO2 and pK' is the apparent dissociation constant of the CO2-HCO3 system. The variable s is 0.0307 in plasma at 37°C and pH 7.4 in normal human subjects (1). The variable pK' was calculated from Kelman’s equation (13, 28), assuming the temperature was stable at 37°C during short-period exercise (51)

\[ pK' = 6.086 + 0.042 \times (7.4 - pH) + 0.00472 \]
\[ + [0.00139 \times (7.4 - pH)] \] (3)

Total blood CCO2 (mM) was calculated from the equation of Douglas et al. (13), modified from Visser’s equation (34) after taking into account the effects of changing pH during exercise on pHk

\[ CCO2 = CCO2pl \times \frac{0.0289 \times [Hb]}{1 - \left(\frac{3.352 - 0.456 \times SO2}{8.142} - pH \right)} \] (4)

where [Hb] is Hb concentration.

Substituting CCO2pl of Eq. 1 into Eq. 4, we obtained Eq. 5

\[ CCO2 = s \times PCO2 \times [1 + 10^{pH - pK'_{H}}] \times \frac{0.0289 \times [Hb]}{1 - \left(\frac{3.352 - 0.456 \times SO2}{8.142} - pH \right)} \] (5)

After CacO2 and CvCO2 were calculated (Eq. 5), the CvCO2, CacO2, and concurrent CO2 were calculated, the latter by the Fick principle (16).

Default values of CCO2, CvCO2, CaCO2, and CO2. To compare the magnitude of error caused by the failure to acknowledge changes in pH, Hb, and SO2 from rest to exercise on the PCO2-CCO2 relationship, default (Def) values of C CO2 were calculated by using the resting values of pH, Hb, and/or SO2 for each individual. Def values are so named because they do not acknowledge the changes in one or more of the independent variables during exercise. For example, if the changing PCO2, Hb, and SO2 values were used to calculate CCO2 during each minute of exercise but the pH remained at its resting (or Def) value, the CCO2 would be identified as Def-pH CCO2. In the same way, we calculated Def-Hb, Def-SO2, and the combined Def-pH, Hb and SO2, the latter when changes in all three values were not taken in to account. The percent errors of the Def values from the actual values of CacO2, CvCO2, CacO2, CaCO2, and CO2 for each stage of exercise were calculated using the following formula: %error = 100 \times \left(\frac{\text{actual} - \text{actual}}{\text{actual}}\right)

Relative importance of multiple factors on the PCO2-CCO2 relationship during exercise. Besides physically dissolved CO2 ([CO2]), which depends only on PCO2 under isometric conditions, the two major factors influencing the PCO2-CCO2 relationship in the Douglas equation are the HCO3 factor (Fbic, i.e., the effect of pH on the quantity of [HCO3-] in both plasma and red blood cell) and the Hb factor (Fhb, i.e., the effect of Hb binding to CO2). The Fbic consists of three
subfactors, the Hb concentration ($F_{Hb,n}$), the SO$_2$ ($F_{Hb,o}$), and the pH ($F_{Hb,0}$) on Hb binding of CO$_2$. The formulas for the two major factors and the three subfactors are given in Appendix A. After calculation of actual values of each factor and subfactor using these equations, the relative importance of the changes in each factor and subfactor at each stage of exercise was calculated. Thus the influence of a specific factor or subfactor at any stage of exercise depends on how far its ratio differs from 1.00.

Estimation of Each Component of Blood CO$_2$ During Exercise

The CO$_2$ is transported as six components in human blood, [CO$_2$], [HCO$_3$], and carbamino concentration ([NH-CO$_2$]) in both the plasma and the red blood cell. To compare the relative importance and change of each component of CO$_2$ during exercise, we calculated each of these six components using the equations in Appendix B.

Data Analysis and Statistics

Unless otherwise specified, all data are expressed as means ± SD, with range values in parentheses. Data were analyzed predominantly by ANOVA; paired $t$-tests were used only when specified. The values of CO$_2$ calculated from Douglas’ equation and Appendix B were compared by using linear regression and Pearson product-moment correlation coefficients. A $P < 0.05$ was considered significant.

RESULTS

The subject’s physical characteristics and aerobic parameters were as follows: age, 25 ± 5 (20–34) yr; height, 179 ± 3 (173–183) cm; body weight, 73 ± 5 (68–80) kg; work rate at LAT, 126 ± 25 (98–154) W; maximum work rate, 302 ± 47 (225–360) W; V$_{O2}$ at LAT, 2.00 ± 0.31 (1.50–2.35) l/min; and maximal V$_{O2}$, 3.91 ± 0.61 (2.74–4.31) l/min. From rest to Max, V$_{O2}$ increased over 10-fold, from 0.37 ± 0.04 to 3.91 ± 0.61 l/min; V$_{CO2}$ increased over 16-fold, from 0.29 ± 0.04 to 4.84 ± 0.71 l/min; heart rate increased nearly threefold, from 63 ± 6 to 178 ± 12 beats/min; whereas cardiac output increased over threefold, from 7.06 ± 2.37 to 25.38 ± 3.90 l/min. The rate of increase in V$_{O2}$ as related to work rate increase was 10.03 ± 0.34 (9.50–10.68) ml·min$^{-1}$·W$^{-1}$, similar to that previously reported (24, 25, 52).

Actual Changes in Blood CO$_2$ Content

Both mixed venous and arterial Hb increased slightly but significantly above the LAT as Max was approached ($P < 0.05$ to $P < 0.01$) (Table 1). Mixed venous values for Hb are not shown because they differed minimally from arterial values (0.2 ± 0.3 g/dl). The mixed venous SO$_2$ progressively decreased from rest to Max ($P < 0.05$ to $P < 0.001$), whereas arterial SO$_2$ decreased slightly near Max ($P < 0.05$) (Table 1). Both mixed venous pH (pH$_{v}$) ($P < 0.05$ to $P < 0.001$) and arterial pH (pH$_{a}$) ($P < 0.05$) progressively decreased from rest to Max, with pH$_{a}$ decreasing more than the pH$_{v}$ ($P < 0.05$ to $P < 0.01$ by paired $t$-test) (Fig. 1 and Table 1). P$_{CO2}$ increases were marked, with progressively increasing values from rest to Max, particularly above the LAT ($P < 0.05$ to $P < 0.001$).
(Fig. 1 and Table 1). \( P_{\text{CO}_2} \) increased slightly from rest \((P < 0.05)\) to LAT, then stabilized, and then decreased moderately near Max \((P < 0.01 \text{ vs. LAT}; P < 0.05 \text{ vs. rest value})\) (Fig. 1). Thus mixed venous-arterial differences of \( O_2 \) concentration, \( C_{\text{vCO}_2} - C_{\text{aCO}_2} \) (Fig. 1), \( SO_2 \), \( pH \), and \( P_{\text{CO}_2} \) all progressively increased from rest to Max \((P < 0.05 \text{ to } P < 0.001)\) (Table 1).

It is impressive how little change took place in \( C_{\text{vCO}_2} \) from rest \((23 \text{ mM})\) to Max \((24 \text{ mM})\), despite large changes in \( P_{\text{vCO}_2} \) \((47–78 \text{ Torr})\) (see Figs. 1 and 2 and Table 2). Initially, as exercise became more intense, \( C_{\text{vCO}_2} \) increased more than \( C_{\text{aCO}_2} \), \( C_{\text{vCO}_2} \) then stabilized, whereas \( C_{\text{aCO}_2} \) began to decrease \((P < 0.05 \text{ or } P < 0.01)\) (Fig. 1). As exercise increased to maximum, the \( C_{\text{vCO}_2} \) decreased from its peak level (Table 2, at 5 min of incremental exercise) to slightly above its rest value in the case of \( C_{\text{vCO}_2} \) \((P < 0.01)\) and below the rest value in the case of mixed venous \( [HCO_3^-] \) (Fig. 2B). In contrast, \( C_{\text{aCO}_2} \) decreased to a much greater degree with little change in \( P_{\text{aCO}_2} \). Thus the progressive increase in \( C_{\text{vCO}_2} - C_{\text{aCO}_2} \) below LAT was due to an increasing \( C_{\text{vCO}_2} \), whereas above LAT it was primarily due to a decreasing \( C_{\text{aCO}_2} \) (Fig. 2).

**Influence of \( pH \) on the \( P_{\text{CO}_2}-C_{\text{CO}_2} \) and \( P_{\text{CO}_2}/[HCO_3^-] \) relationships during exercise.** As shown in Fig. 2A and Table 2, the \( P_{\text{CO}_2}-C_{\text{CO}_2} \) relationship was markedly influenced by \( pH \) changes during exercise. From rest to LAT, \( C_{\text{vCO}_2} \) increased relatively less than \( P_{\text{vCO}_2} \) because of a moderate decline in \( pH \) \((P < 0.001)\). Transiently, above LAT, \( C_{\text{vCO}_2} \) stabilized, despite further increases in \( P_{\text{vCO}_2} \) due to the start of a fall in \([HCO_3^-]\) reflected by the decrease in \( pH \). Eventually, despite the continuously increasing \( P_{\text{vCO}_2} \) \((P < 0.001)\), \( C_{\text{vCO}_2} \) decreased at Max \((P < 0.05, \text{ Max vs. LAT})\) due to the marked decrease in \( pH \) \((P < 0.001, \text{ Max vs. LAT})\).

Simultaneously, on the arterial blood side, \( C_{\text{aCO}_2} \) increased insignificantly at work rates below LAT \((P > 0.05)\), whereas \( P_{\text{aCO}_2} \) increased \((P < 0.05)\) and \( pH \) decreased slightly \((P < 0.05)\). Above LAT, \( C_{\text{aCO}_2} \) and \([HCO_3^-]\) decreased when lactic acid production increased, causing a significant decrease in \( pH \) \((P < 0.001)\) with only slight decreases in \( P_{\text{aCO}_2} \) (Fig. 2).

As shown in Fig. 2B, the pattern of changes in plasma \([HCO_3^-]\) was quite similar to the changes in \( C_{\text{CO}_2} \) as a function of \( P_{\text{CO}_2} \). This similarity reflects the dominant role of changes in \( pH \) and buffer base on the \( P_{\text{CO}_2}-C_{\text{CO}_2} \) relationship. It is clear from Figs. 1 and 2 that an increase in \( P_{\text{CO}_2} \) in venous blood does not necessarily predict an increase in \( HCO_3^- \) and therefore \( C_{\text{CO}_2} \). Thus there is no single \( P_{\text{CO}_2}-C_{\text{CO}_2} \) relationship that can be used to predict \( C_{\text{CO}_2} \) from \( P_{\text{CO}_2} \) during exercise.

**Errors in Def \( C_{\text{CO}_2} \) and \( CO_{\text{CO}_2} \) During Exercise**

Table 2 shows the absolute values and percent errors of Def-\( pH \) \( C_{\text{CO}_2} \) from actual \( C_{\text{CO}_2} \) values. Errors in \( C_{\text{vCO}_2}, C_{\text{aCO}_2}, \) and \( C_{\text{vCO}_2}-C_{\text{aCO}_2} \) increased consistently from actual values as exercise intensity increased, because changes in \( pH \) were not accounted for during exercise \((P < 0.05 \text{ below LAT}; P < 0.01 \text{ above LAT})\). Although errors in \( C_{\text{vCO}_2} \) and \( C_{\text{aCO}_2} \) were directionally the same, the absolute and percent errors of Def-\( pH \) \( C_{\text{CO}_2} \) were always larger than those of Def-\( pH \) \( C_{\text{aCO}_2} \) \((P < 0.001 \text{ by paired } t\text{-test})\), resulting in consistent overestimation of Def-\( pH \) \( C_{\text{vCO}_2}-C_{\text{aCO}_2} \). Although abso-
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Def-pH CaCO₂ (Table 2; *P < 0.001* by paired *t*-test). The overestimation of CaCO₂, CvCO₂, and VCO₂-CaCO₂ that results from failure to use pH in the calculation of CO₂ ranged from −0.2 to 27, 2 to 60, and 18 to 120%, respectively, even when correct, directly measured arterial and mixed venous PCO₂, Hb, and SO₂ are used in the calculation.

Figure 3 shows the percent errors of specific Def values of CvCO₂-CaCO₂, despite the use of correct measurements of PCO₂, when no change during exercise is assumed in Hb, SO₂, and/or pH. Defaulting changes in Hb during exercise results in trivial error (only −0.2 to 0.6%). Defaulting the change in SO₂ results in slight underestimation in CvCO₂-CaCO₂ over the entire exercise period (−6 to −3%; *P < 0.05*). In contrast, defaulting changes in either pH alone or pH, Hb, and SO₂ together cause large errors, i.e., a progressive overestimation in CvCO₂-CaCO₂ as exercise intensity increases, reaching −50% at LAT and 100% at Max.

As seen in Fig. 4, the percent errors in CO₂CO₂ that result from defaulting the influence of Hb, SO₂, and/or pH on the PCO₂-CO₂ relationship are always opposite to those on CvCO₂-CaCO₂. As with its effect on CvCO₂-CaCO₂, the change in Hb causes a trivial effect on CO₂CO₂ (only −0.6 to 0.2%), whereas the changes in SO₂ cause the estimate of CO₂CO₂ to be 3–6% higher than actual (*P < 0.05*). In contrast, defaulting on either pH alone or pH, Hb, and SO₂ together causes errors in CO₂CO₂ by >30% at LAT and >50% at Max.

Relative importance of multiple factors on the PCO₂-CO₂ relationship during exercise. The influence of each of several factors on the PCO₂-CO₂ relationship is shown in Table 3, with factor influence increasing as its value deviates from 1.0. Thus the deviations during exercise of Fbic (which are due to pH changes) far exceed those of FHb, both in mixed venous and arterial blood, during exercise (Table 3). Considering the subfactors of FHb, the deviations during exercise of FHbalt (due to the effect of pH changes on [NH-CO₂]) exceed those of FHbalt and FHbal, both in mixed venous and arterial blood. Thus the changes and influence of pH (Fbic and FHbalt) are quantitatively much more important in blood CO₂ transport during exercise, both in mixed venous and arterial blood, than are the changes and influences of change in Hb and SO₂. These findings contrast importantly with the conclusions of McHardy (34).

Changes in Blood Components of CO₂ During Exercise

The total CO₂ and each of its fractional components in mixed venous and arterial blood and the mixed venoarterial differences at rest and during exercise are shown in Fig. 5 and Table 4. These data were calculated from the equations described in APPENDIX B. Both CvCO₂ and CaCO₂ correlated well with the same variables calculated from the Douglas equation (*r = 0.9998, P < 0.0001*), with only very small deviations (−0.06 ± 0.14 mM) at rest and during exercise.
In Fig. 5, both mixed venous [CO$_3$] and [NH-CO$_2$] increased progressively from rest to Max ($P < 0.05$). Smaller percent but larger absolute changes in [HCO$_3$] dwarfed the impact of the larger percent but smaller absolute changes in mixed venous [CO$_3$] and [NH-CO$_2$]. Below LAT, trends in change in $C_v$ CO$_2$ were similar to those in [CO$_3$] or [NH-CO$_2$], but they differed markedly above LAT. The arterial [CO$_3$] and [NH-CO$_2$] increased only slightly from rest at LAT ($P < 0.05$) and then returned to near resting values at the highest work rate. In contrast to the relatively stable arterial [CO$_3$] and [NH-CO$_2$], large declines in arterial [HCO$_3$] above the LAT caused marked decreases in CaCO$_2$.
Thus changes in \( \mathrm{CvCO}_2 \) and \( \mathrm{CaCO}_2 \) conformed mainly to changes in \( [\mathrm{HCO}_3^-] \) with changes in \( [\mathrm{CO}_2] \) and \( [\mathrm{NH}_2\mathrm{CO}_2] \) having a relatively small effect.

Referring to Table 4 (selected exercise intensities), ratios of \( \mathrm{CvCO}_2 \) to \( [\mathrm{CO}_2] \) \( (\mathrm{CvCO}_2/[\mathrm{CO}_2]) \) were calculated by dividing the \( \mathrm{CvCO}_2 \) by the sum of plasma and red blood cell components of \( [\mathrm{CO}_2] \). Note that the \( \mathrm{CvCO}_2/[\mathrm{CO}_2] \) for mixed venous blood progressively decreased as the exercise intensity increased \( (P < 0.05) \), especially above \( \text{LAT} (P < 0.01) \). The \( \mathrm{CaCO}_2/[\mathrm{CO}_2] \) in arterial blood, which did not change significantly below \( \text{LAT} (P > 0.05) \), progressively decreased at and above \( \text{LAT} (P < 0.05) \), because of the reduction in \( [\mathrm{HCO}_3^-] \). Thus \( \mathrm{CaCO}_2/[\mathrm{CO}_2] \) is not constant but depends on the source of blood and the intensity of exercise.

It is also evident from Table 4 and Fig. 5 that plasma and red blood cell \( [\mathrm{HCO}_3^-] \) together comprise \( \sim 85\% \) (mixed venous) and 90% (arterial) of the \( \mathrm{CvCO}_2 \) at rest and during exercise. Even though the absolute values of mixed venous \( [\mathrm{HCO}_3^-] \) are always greater than those of the arterial \( [\mathrm{HCO}_3^-] \), the mixed venous \( [\mathrm{HCO}_3^-]/\mathrm{CvCO}_2 \) are always lower than the arterial \( [\mathrm{HCO}_3^-]/\mathrm{CaCO}_2 \) \( (P < 0.001) \) because of the greater amount of \( [\mathrm{CO}_2] \).

Relative contributions of the three forms of \( \mathrm{CO}_2 \)-to- \( \mathrm{CO}_2 \) exchange are shown for each level of exercise in Table 5. From rest to \( \text{Max} \), \( [\mathrm{HCO}_3^-] \) exchange remained large and quite constant at \( \sim 76-77\% \) of total \( \mathrm{CO}_2 \) excreted, i.e., \( \mathrm{CvCO}_2-\mathrm{CvCO}_2 \). In contrast, \( [\mathrm{CO}_2] \) and \( [\mathrm{NH}_2\mathrm{CO}_2] \) accounted for \( \sim 9 \) and 14%, respectively, of total \( \mathrm{CO}_2 \) excreted at rest. Above \( \text{LAT} \), the relative contribution to \( \mathrm{CO}_2 \) output of \( [\mathrm{CO}_2] \) progressively increased to \( 13\% \) \( (P < 0.05) \) and that of \( [\mathrm{NH}_2\mathrm{CO}_2] \) decreased to \( \sim 10\% \) \( (P < 0.01) \).

Finally, to support the validity of our calculated values for \( \mathrm{CaCO}_2, \mathrm{CvCO}_2 \), and \( \mathrm{CvCO}_2-\mathrm{CvCO}_2 \) at all levels of exercise, we compared the \( \mathrm{CvCO}_2 \) measured by the Fick principle with \( \mathrm{CO}_2 \) at rest and each level of exercise for the five subjects in this study (Table 6). At each level but \( \text{Max} \), the mean values are in good agreement. As noted in Table 6, in other quite similar exercise studies \( (51) \), the \( \mathrm{CO}_2 \) was insignificantly different from \( \mathrm{CO}_2 \) at peak and all other levels of exercise.

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**Table 3. Factors describing the magnitude by which \( \text{Hb}, \text{SO}_2, \) or \( \text{pH} \) changes affect the \( \text{PCO}_2-\text{CO}_2 \) relationship during exercise**

<table>
<thead>
<tr>
<th>Exercise Level</th>
<th>( F_{bic} )</th>
<th>( F_{Hb} )</th>
<th>( F_{bic} )</th>
<th>( F_{Hb} )</th>
<th>( F_{bic} )</th>
<th>( F_{Hb} )</th>
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<td>1.02±0.02</td>
<td>0.96±0.01</td>
<td>0.93±0.02</td>
<td>0.94±0.04†</td>
<td>1.01±0.01†</td>
<td>1.02±0.02†</td>
</tr>
<tr>
<td>Ex-5</td>
<td>0.84±0.03</td>
<td>1.03±0.01†</td>
<td>1.03±0.02</td>
<td>0.96±0.01</td>
<td>0.91±0.02</td>
<td>0.91±0.05†</td>
<td>1.01±0.01†</td>
<td>1.03±0.02†</td>
</tr>
<tr>
<td>Ex-6</td>
<td>0.80±0.04</td>
<td>1.03±0.01†</td>
<td>1.04±0.02</td>
<td>0.95±0.01</td>
<td>0.89±0.02</td>
<td>0.91±0.05†</td>
<td>1.01±0.01†</td>
<td>1.04±0.02†</td>
</tr>
<tr>
<td>Ex-7</td>
<td>0.75±0.04</td>
<td>1.04±0.01†</td>
<td>1.04±0.01</td>
<td>0.95±0.01</td>
<td>0.86±0.02</td>
<td>0.90±0.04†</td>
<td>1.01±0.01†</td>
<td>1.04±0.01†</td>
</tr>
<tr>
<td>Max</td>
<td>0.70±0.06†</td>
<td>1.04±0.01†</td>
<td>1.06±0.02</td>
<td>0.94±0.01</td>
<td>0.83±0.04‡</td>
<td>0.87±0.06†</td>
<td>1.01±0.01†</td>
<td>1.06±0.02‡</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \text{CO}_2 \), \( \text{CO}_2 \) concentration; \( F_{bic} \), pH factor of plasma and red cell bicarbonate; \( F_{Hb} \), \( \text{Hb} \) \( \text{CO}_2 \) binding factor; \( F_{bic} \), \( \text{Hb} \) subfactor of \( \text{Hb} \) \( \text{CO}_2 \) binding; \( F_{bic} \), pH subfactor of \( \text{Hb} \) \( \text{CO}_2 \) binding. Factors describe the relative values of variables in the Douglas equation (see Eq. 5 and APPENDIX A). In our subjects, resting factors values are \( F_{bic} = 18.69 \pm 0.54, F_{bic} = 0.81 \pm 0.01, F_{bic} = 0.45 \pm 0.01, F_{bic} = 0.33 \pm 0.003, \) and \( F_{bic} = 1.28 \pm 0.02 \) in mixed-venous blood and for arterial blood are \( F_{bic} = 20.06 \pm 0.48, F_{bic} = 0.79 \pm 0.01, F_{bic} = 0.45 \pm 0.01, F_{bic} = 0.34 \pm 0.000, \) and \( F_{bic} = 1.34 \pm 0.02 \). Values show the relative changes in factors from their resting values. The further the values differ from 1.00, the greater their influence on the \( \text{PCO}_2-\text{CO}_2 \) relationship. Significant difference vs. rest: *\( P < 0.05 \), †\( P < 0.01 \), ‡\( P < 0.001 \).
Table 5. Relative contribution to the CO2 exchange

<table>
<thead>
<tr>
<th>Stage</th>
<th>[CO2], %CvCO2-CaCO2</th>
<th>[NH-CO2], %CvCO2-CaCO2</th>
<th>[HCO3-], %CvCO2-CaCO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>9.3 ± 0.8</td>
<td>13.9 ± 1.3</td>
<td>76.9 ± 1.4</td>
</tr>
<tr>
<td>Unl</td>
<td>9.4 ± 1.4</td>
<td>14.5 ± 2.3</td>
<td>76.2 ± 2.4</td>
</tr>
<tr>
<td>Ex-1</td>
<td>9.6 ± 1.8</td>
<td>14.3 ± 2.5</td>
<td>76.3 ± 2.0</td>
</tr>
<tr>
<td>Ex-2</td>
<td>9.7 ± 1.5</td>
<td>14.6 ± 2.7</td>
<td>75.8 ± 2.5</td>
</tr>
<tr>
<td>Ex-3</td>
<td>10.0 ± 1.8</td>
<td>14.6 ± 2.3</td>
<td>75.4 ± 2.5</td>
</tr>
<tr>
<td>LAT</td>
<td>10.6 ± 1.3*</td>
<td>13.5 ± 1.6</td>
<td>75.9 ± 1.5</td>
</tr>
<tr>
<td>Ex-5</td>
<td>10.5 ± 0.7*</td>
<td>12.1 ± 0.8†</td>
<td>77.7 ± 1.0</td>
</tr>
<tr>
<td>Ex-6</td>
<td>11.4 ± 0.6†</td>
<td>12.1 ± 0.9†</td>
<td>76.5 ± 1.4</td>
</tr>
<tr>
<td>Ex-7</td>
<td>12.0 ± 0.8†</td>
<td>11.6 ± 1.3†</td>
<td>76.4 ± 0.7</td>
</tr>
<tr>
<td>Ex-8</td>
<td>12.6 ± 0.9†</td>
<td>11.2 ± 1.2†</td>
<td>76.2 ± 0.6</td>
</tr>
<tr>
<td>Max</td>
<td>13.0 ± 0.9†</td>
<td>9.9 ± 1.3†</td>
<td>77.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data were calculated from the equations in APPENDIX B. Significantly different vs. rest: *P < 0.05, †P < 0.01.
rate increases. The metabolic acidosis found in our subjects during high-intensity exercise causes plasma and red blood cell C\(_{\text{CO}_2}\) and [HCO\(_3^-\)] to decrease in both mixed venous and arterial blood, but especially in arterial blood.

As commonly graphed, the P\(_{\text{CO}_2}\)-C\(_{\text{CO}_2}\) relationship is depicted as nearly linear between P\(_{\text{CO}_2}\) values of \(~30–80\) Torr and C\(_{\text{CO}_2}\) values of \(~12–28\) mM but without reference to or depiction of the effect of a pH change. Changes in C\(_{\text{CO}_2}\) are sometimes fractionated into plasma and red blood cell components, and the differences between mixed venous and arterial blood due to Hb and SO\(_2\) are also considered, again with exclusion of the effect of pH. However, as seen in Figs. 1 and 2A and Tables 1 and 2, widely divergent P\(\text{V}_\text{CO}_2\) values (47–78 Torr) may occur with reasonably similar C\(_{\text{V}_\text{CO}_2}\) values (22.8–23.9 mM). This is due to relatively large decreases in the buffer base reflected in the change in pH (7.362–7.130) (Table 1 and Fig. 1). Furthermore, reasonably similar P\(\text{A}_\text{CO}_2\) values (41–38 Torr) may pair with widely different C\(_{\text{A}_\text{CO}_2}\) values (20.9–15.2 mM) because of relatively large differences in pH. Thus when the C\(_{\text{V}_\text{CO}_2}\) is calculated from P\(\text{V}_\text{CO}_2\), the change in blood pH during exercise must not be ignored.

During heavy exercise, C\(_{\text{CO}_2}\) is not linearly related to the P\(_{\text{CO}_2}\) in either arterial or mixed venous blood (see Fig. 2A). In fact, P\(_{\text{CO}_2}\) and C\(_{\text{CO}_2}\) change in opposite directions in mixed venous blood as metabolic acid is added to the blood by the muscles. In contrast to original reports and textbooks (8, 38, 56), Fig. 2 shows that C\(_{\text{V}_\text{CO}_2}\) does not increase as a function of P\(_{\text{V}_\text{CO}_2}\) during exercise above the LAT.

We suggest that the addition of pH (or [H\(^+\)]) isopleths to diagrams depicting the plasma [HCO\(_3^-\)]-P\(_{\text{CO}_2}\) and C\(_{\text{CO}_2}\)-P\(_{\text{CO}_2}\) relationships, such as shown in Fig. 2, conveys necessary and important information that is lacking in the present standard depictions of these relationships (8, 38, 56). Such isopleths clarify and reinforce the importance of the acid-base changes that can occur during exercise.

Total blood [HCO\(_3^-\)] comprises \(~85\)% (mixed venous) or \(~90\)% (arterial) of the C\(_{\text{CO}_2}\) (Table 4). Although the percent changes in mixed venous [CO\(_2\)] and [NH-CO\(_2\)] are appreciable (Tables 4 and 5 and Fig. 5), their impact is dwarfed by the magnitude of the large absolute changes in [HCO\(_3^-\)]. Additionally, Tables 2 and 3 and Fig. 3, which give Def values and factor ratios, analyze the relative importance of the components of C\(_{\text{CO}_2}\) in the Douglas equation and confirm the dominance of pH factors. In Table 3 and Fig. 3, it is shown that the pH change factor (F\(_{\text{bic}}\) from 1.00 at rest to 0.59 at Max) dominates over other factors. Within the red blood cell, the pH factor even dominates over the change in Hb concentration and SO\(_2\) factors (Table 3). Thus making adjustments for the influences of Hb and SO\(_2\) while ignoring pH to calculate C\(_{\text{V}_\text{CO}_2}\) or C\(_{\text{A}_\text{CO}_2}\) during exercise, as was done earlier (34), results in major errors.

Relevance of the C\(_{\text{CO}_2}\)-P\(_{\text{CO}_2}\) Relationship to the Accuracy and Precision of Estimation of C\(_{\text{V}_\text{CO}_2}\)-C\(_{\text{A}_\text{CO}_2}\) and C\(_{\text{CO}_2}\)

Our calculations of C\(_{\text{CO}_2}\) come from direct measurements of mixed venous and arterial P\(_{\text{CO}_2}\), pH, Hb, and SO\(_2\). As previously shown, the relationship between P\(_{\text{CO}_2}\) and C\(_{\text{CO}_2}\) is positively correlated between rest and LAT but then becomes negatively correlated as increasing metabolic acidosis develops. Thus a given P\(_{\text{CO}_2}\) value can be associated with widely divergent C\(_{\text{CO}_2}\) values.

It would be convenient if errors in measurement of C\(_{\text{V}_\text{CO}_2}\) and C\(_{\text{A}_\text{CO}_2}\) were to be offset in the calculation of C\(_{\text{V}_\text{CO}_2}\)-C\(_{\text{A}_\text{CO}_2}\), but this is not the case. It is evident from Table 2 that omitting the change in pH in calculating C\(_{\text{V}_\text{CO}_2}\)-C\(_{\text{A}_\text{CO}_2}\) causes a much larger error than when C\(_{\text{V}_\text{CO}_2}\) and C\(_{\text{A}_\text{CO}_2}\) are calculated individually. Figure 4 shows that, when these erroneous measurements are applied to calculate cardiac output, errors of large magnitude result. In contrast, ignoring the changes in [Hb] and SO\(_2\) during exercise results in relatively unimportant errors compared with ignoring changes in pH.

Possible Measurement and Calculation Errors in Our Data

The gas exchange rate of increase in V\(_{\text{O}_2}\) as related to work rate increase, and mixed venous and arterial blood values are unlikely to be significantly inaccurate or imprecise in this study, considering the similarity of these values to those of other studies in normal men and the quality control procedures used in our laboratory (3, 4, 21, 24, 25, 46–55). A limitation of this study is the absence of direct measurements of C\(_{\text{A}_\text{CO}_2}\) and C\(_{\text{V}_\text{CO}_2}\). Because all methods for such measurements require a minimum of 30 min for each blood sample, it was unrealistic to obtain the many measurements needed on so many samples (~24–28) for each study. However, the similar values obtained for CO\(_2\) and CO\(_{\text{CO}_2}\) at rest and all levels of exercise (Table 6) give credence to the reliability of the concurrent measurements of pH and P\(_{\text{CO}_2}\) and calculated mixed venous and arterial concentrations of CO\(_2\) and O\(_2\) at all levels of exercise.

We did not measure blood or body temperatures during exercise. From other studies using a similar exercise protocol, we estimated that the temperature increase from rest to peak exercise would be 0.2–0.8°C (51). We calculated that a temperature rise of 0.5°C during exercise would affect the values of C\(_{\text{A}_\text{CO}_2}\), C\(_{\text{V}_\text{CO}_2}\), and C\(_{\text{V}_\text{CO}_2}\)-C\(_{\text{A}_\text{CO}_2}\) by <1% at peak exercise (51). Therefore, this small temperature change would not significantly alter our findings.

Considering the diversity of sources and complexity of equations in APPENDIX B that were used to calculate the six components of C\(_{\text{CO}_2}\), the sum values for each blood sample from the six sources agree remarkably well with those calculated from the simpler Douglas equation. The C\(_{\text{CO}_2}\) values calculated from the Douglas equation have excellent linear correlation with the
total sum values at all levels of \(\text{CO}_2\) for both arterial and mixed venous blood \((r = 0.9998, \text{Table 4})\).

**Possible Errors Due to a Disequilibria of pH and CO\(_2\)**

There is no evidence that an alveolar-arterial \(\text{CO}_2\) disequilibrium occurs, except under conditions of high levels of carbonic anhydrase inhibition. The latter is a model of \(\text{CO}_2\) disequilibrium. In contrast, there are good arguments using physiological data against disequilibrium for \(\text{CO}_2\) at high work rates as follows.

1) End-tidal \(\text{PCO}_2\) (\(\text{PETCO}_2\)) exceeds \(\text{PaCO}_2\) during exercise with a maintained negative \(\text{PaCO}_2-\text{PETCO}_2\) difference of \(-4\) Torr, despite the increase in \(\text{VCO}_2\) to very high levels (54). If there were a disequilibrium, \(\text{PaCO}_2-\text{PETCO}_2\) difference and \(\text{PaCO}_2\) should increase relative to \(\text{PETCO}_2\) as \(\text{VCO}_2\) increases. However, the change in \(\text{PETCO}_2\) parallels the change in \(\text{PaCO}_2\), as \(\text{VCO}_2\) increases to maximum and remains above it (54).

2) If there were a disequilibrium, calculated dead space volume/tidal volume should increase as work rate increases, because \(\text{PaCO}_2\) would be increased relative to \(\text{PETCO}_2\). This happens when a right-to-left shunt opens during exercise but does not happen normally. Dead space volume/tidal volume decreases with exercise and remains decreased to similar levels or decreases further as work rate increases to the maximum in normal subjects.

3) If there were a disequilibrium, \(\text{VCO}_2\) would not increase appropriately as high-metabolic rates are achieved and \(\text{PaCO}_2\) would increase. Increasing \(\text{VCO}_2\) keeps pace with increasing \(\text{VO}_2\) even for very fit men and exceeds \(\text{VO}_2\) once lactic acidosis occurs. If metabolic \(\text{CO}_2\) plus \(\text{CO}_2\) released from buffer were retained because of an alveolar-capillary disequilibrium as blood passed from pulmonary artery to pulmonary vein, we might expect to see a higher \(\text{PaCO}_2\) and arterial [\(\text{HCO}_3\]) in fact, \(\text{PaCO}_2\) and arterial [\(\text{HCO}_3\)] decrease, without any evidence of \(\text{CO}_2\) retention.

4) The decrease in arterial [\(\text{HCO}_3\)] is approximately equal to the increase in arterial lactate during high levels of exercise. If there were a disequilibrium, [\(\text{HCO}_3\)] would be relatively high, because it would not dissociate adequately in its passage from pulmonary artery to systemic artery. In fact, despite the very high \(\text{PVCO}_2\) for work above the LAT (Fig. 1), the \(\text{PaCO}_2\) and [\(\text{HCO}_3\)] decrease more than that of the mixed venous blood. Our major finding is that the decrease in \(\text{Caco}_2\) is the primary explanation for the increase in \(\text{CvCO}_2\)-\(\text{CaCO}_2\) during exercise at high work rates above the LAT, not the increase in \(\text{CvCO}_2\). This contradicts the changes we would see if there were a disequilibrium of significance.

**Relative Contributions of Components of Blood \(\text{CO}_2\)-to-\(\text{CO}_2\) Exchange Across the Lung**

Table 5 illustrates that the contribution of [\(\text{HCO}_3\)] to \(\text{CO}_2\) exchange is relatively constant as a percentage of \(\text{CVCO}_2\)-\(\text{CaCO}_2\). In contrast, the contribution of [\(\text{CO}_2\)] increases and [\(\text{NH-CO}_2\)] decreases as exercise intensity increases. This is because [\(\text{CO}_2\)] is influenced only by \(\text{PCO}_2\), whereas [\(\text{NH-CO}_2\)] is also influenced by \(\text{SO}_2\) and pH.

The traditional dissociation curve for \(\text{CO}_2\) over the range of 30 to 70–80 Torr depicted in textbooks (8, 38, 56) suggests that a large part of the \(\text{CvCO}_2\)-\(\text{CaCO}_2\) is dependent on the \(\text{SO}_2\) difference between mixed venous and arterial blood. Considering the relative contributions of all components to \(\text{CO}_2\) exchange that account for the increase in \(\text{CvCO}_2\)-\(\text{CaCO}_2\) during exercise, our study shows that [\(\text{HCO}_3\)] accounts for over three-fourths of the total difference at rest and at all levels of exercise, whereas [\(\text{CO}_2\)] and [\(\text{NH-CO}_2\)], in combination, account for less than one-fourth of the total difference. Of this, approximately three-fifths come from [\(\text{NH-CO}_2\)] and two-fifths come from [\(\text{CO}_2\)] below LAT. This relationship reverses as work rate increases above LAT.

The difference in \(\text{CvCO}_2\) caused by changing the state of oxygenation of the blood at the same \(\text{PCO}_2\) is fully attributable to the change in red blood cell [\(\text{NH-CO}_2\)]. Our calculations of [\(\text{NH-CO}_2\)] in \(\text{CVCO}_2\) and in \(\text{CaCO}_2\) at rest are not in agreement with earlier reports (38, 44), which estimated that one-third of \(\text{CO}_2\) exchange was attributable to oxygen-induced changes in [\(\text{NH-CO}_2\)]. These earlier studies used blood devoid of 2,3-diphospho-d-glycerate (DPG). Later studies in blood that considered the effect of DPG (2, 30) found that the [\(\text{NH-CO}_2\)] contribution to \(\text{CO}_2\) exchange at rest is only between 10 and 15%, in close agreement with our finding of 14%. If we had ignored the DPG effect, our \(\text{CvCO}_2\) values calculated from APPENDIX B would have deviated further from the Douglas equation \(\text{CvCO}_2\) values.

It is clear that the dissociation of [\(\text{HCO}_3\)] plays the dominant role in \(\text{CO}_2\) exchange at the lung, whereas [\(\text{CO}_2\)] and [\(\text{NH-CO}_2\)] play smaller roles in total \(\text{CO}_2\) exchange. Although \(\text{PCO}_2\) differences account for the transfer of \(\text{CO}_2\) out of blood, >75% of the quantity transferred comes from the dissociation of mixed venous [\(\text{HCO}_3\)]. We have shown that \(\text{CVCO}_2\) in blood actually decreases during exercise above the LAT, despite increasing \(\text{PCO}_2\). The major reason for this is that the \(\text{CO}_2\) dissociation curve is shifted downward when lactic acid is generated during exercise. Simultaneously, the \(\text{PVCO}_2\) increases as additional \(\text{CO}_2\) over that produced from metabolism is released from the \(\text{HCO}_3\) buffering of lactic acid. Because of changing [\(\text{HCO}_3\)] and pH during exercise, it is inappropriate to determine \(\text{CvCO}_2\) from extrapolations that assume a near-linear \(\text{CvCO}_2\)-\(\text{PCO}_2\) relationship.

**APPENDIX A**

**Calculating the Factors Influencing the \(\text{Pco}_2\)-\(\text{Cvo}_2\) Relationship**

The following formulas for the two main factors (\(F_{bic}\) and \(F_{Hb}\)) and the three subfactors of \(F_{Hb}\) that influence the \(\text{Pco}_2\)-\(\text{Cvo}_2\) relationship were derived from Douglas’ equation (13) shown in METHODS

\[
F_{bic} = 1 + 10^{(pH - pK)} \tag{A1}
\]
19

Estimating the CO₂ Components in Blood

Estimating plasma [CO₂] (in mM/l blood).

\[
[CO₂]ₚ = PCO₂ × s × (1 - Hct)
\]

(A1)

Estimating red blood cell [CO₂] (in mM/l blood).

\[
[CO₂]ᵣ = PCO₂ × s × 0.93 × Hct × 0.717
\]

(B2)

where \([CO₂]ₚ\) is plasma [CO₂], and hematocrit (Hct) is the ratio of red blood cell volume to blood volume calculated from Hb and the mean corpuscular Hb concentration of 34.4 g/dl red blood cell (57, 59).

Estimating the plasma [HCO₃⁻] (in mM/l blood). From the Henderson-Hasselbalch equation (13, 21, 34, 38, 41)

\[
[HCO₃⁻]ₚ = PCO₂ × s × 10^{[pH - pKₗ]} × (1 - Hct)
\]

(B3)

where \([HCO₃⁻]ₚ\) is plasma [HCO₃⁻].

Estimating the plasma [NH₄⁺] (in mM/l blood). From the Henderson-Hasselbalch equation (13, 21, 34, 38, 41)

\[
[HCO₃⁻]ₚ = PCO₂ × s × 10^{[pH - pKₗ]} × (1 - Hct)
\]

(B4)

where \([HCO₃⁻]ₚ\) is plasma [HCO₃⁻].

Estimating the red blood cell \([HCO₃⁻]ₚ\) (in mM/l blood).

\[
r = r₀² × S₂ + r₀² × (1 - S₂)
\]

(B5)

where \([HCO₃⁻]ₚ\) is red blood cell [HCO₃⁻], \(r\) is the Donnan relationship for \([HCO₃⁻]ₚ\) / \([HCO₃⁻]ₚ\) (17), \(r₀²\) is for oxygenated blood, and \(r₀²\) is for deoxygenated blood, assuming a linear relationship between \(r\) and SO₂.

Estimating the plasma \([NH₄⁺]ₚ\) (in mM/l blood). See Ref. 19

\[
[NH₄⁺]ₚ = \frac{n₁ × [prot] × [CO₂]ₚ}{[CO₂]ₚ + \frac{[H⁺]}{pKₗ₁} + \frac{[H⁺]^2}{pKₗ₁ × pKₗ₂}} + \frac{n₂ × [prot] × [CO₂]ₚ}{[CO₂]ₚ + \frac{[H⁺]}{pKₗ₂} + \frac{[H⁺]^2}{pKₗ₂ × pKₗ₃}}
\]

(B9)

where \([prot]\) is plasma protein concentration of g/dl blood, \([H⁺]\) is calculated from plasma pH, \(n\) is the number of amino CO₂ binding sites (based on an assumed molecular weight of 69,000 for plasma protein), pKₗ is \(\log_{10}\) values of the amino CO₂ binding equilibrium (association/dissociation) constants, and pK₂ is \(\log_{10}\) values of the ionization constants of the terminal amino groups. Type 1 (C1, Z1) may be interpreted as \(α-NH₂\) groups, and type 2 (C2, Z2) may be interpreted as \(ε-NH₂\) groups. The values of these constants are shown in the following table (19)

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>pKₗ₁</th>
<th>pKₗ₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.55</td>
<td>4.20</td>
<td>7.00</td>
</tr>
<tr>
<td>2</td>
<td>5.92</td>
<td>4.26</td>
<td>9.03</td>
</tr>
</tbody>
</table>

Estimating the red blood cell \([NH₄⁺]ₚ\) (in mM/l blood). The red blood cell \([NH₄⁺]ₚ\) \((NH₄⁺)ₚ\) was calculated using the equations of Perrella et al. (39, 40), assuming a linear relationship between \([NH₄⁺]ₚ\) and SO₂ (14) as follows

\[
[NH₄⁺]ₚ = ([Hbₐ-CO₂] + [Hbₐ-CO₂] × SO₂ + ([Hbₐ-CO₂] + [Hbₐ-CO₂])) × (1 - SO₂) + HB + 16.2
\]

where \([Hbₐ-CO₂]\) is CO₂ binding to \(ε-NH₂\) groups of the \(α\)-chain of oxyhemoglobin, \([Hbₐ-CO₂]\) is CO₂ binding to \(α-NH₂\) groups of the \(β\)-chain of oxyhemoglobin, \([Hbₐ-CO₂]\) is CO₂ binding to \(α-NH₂\) groups of the \(β\)-chain of deoxyhemoglobin, and 16.2 converts g/dl Hb to mM.

The \([Hbₐ-CO₂]\) (mM/M Hb) was calculated from the following equation (39)

\[
[Hbₐ-CO₂] = 2 × λₐ × [CO₂]ₚ × (1 + λₐ × [CO₂]ₚ) (B10)
\]

where \(λₐ\) is the pH-dependent association constant of oxyhemoglobin binding to the \(α-NH₂\) group of \(α\)-chain.

The \([Hbₐ-CO₂]\) (mM/M Hb) was also calculated with Eq. B11, replacing \(λₐ\) with \(λₐ^{SO₂}\), the pH-dependent association constant of deoxyhemoglobin CO₂ binding on the \(α-NH₂\) group of \(α\)-chain.

Considering the influence of DPG (5, 22), the \([Hbₐ-CO₂]\) (mM/M Hb) was calculated from the following equation (40)

\[
\]

where \(Kp\) is the association constant for DPG binding to the amino groups of \(β\)-chain Hb in absence of CO₂, \(Kp^{}\) and \(Kp^{}\) are the DPG association constants when one or two CO₂ molecules, respectively, are bound, and \(K_p = 5,000 ~M^{-1}, K_p^{} = 1,700 ~M^{-1}\), and \(K_p^{} = 500 ~M^{-1}\). [DPG] is the DPG concentration in red blood cells and 0.088 M/M Hb in normal subjects (58); \(λₐ\) is the pH-dependent association constant of oxyhemoglobin CO₂ binding on the \(α-NH₂\) group of \(β\)-chain.

The \([Hbₐ-CO₂]\) (mM/M Hb) was also calculated with Eq. B12, replacing \(λₐ\) with \(λₐ^{SO₂}\), the pH-dependent association constant of deoxyhemoglobin CO₂ binding on the \(α-NH₂\) group of \(β\)-chain.

The \(λ\) is the pH-dependent association constant of Hb CO₂ binding. At pH 7.4 and 37°C, the constant values of \(λ\) are \(λ = 92 ~M^{-1}, λₐ = 100 ~M^{-1}\), \(λₐ = 120 ~M^{-1}\), \(λₐ = 190 ~M^{-1}\), and \(λₐ^{SO₂} = 579 ~M^{-1}\) (39, 40). Because the \(λ\) values are pH dependent, the values used in Eqs. B11 and B12 were calculated from the following equations (39) by replacing the red blood cell \([H⁺]\) \((H⁺)ₚ\) at red blood cell pH 7.4 (not plasma pH) with the calculated \([H⁺]\) \((H⁺)ₚ\)
\[ \lambda = \frac{K_C \times K_S}{K_2 \times [H^+]_o + [H^+]_{rc}} \]  
\[ R = R_{O^2} \times SO_2 + R_{CO} \times (1 - SO_2) \]  
\[ R_{O^2} = 3.883 - 0.440 \times pH \]  
\[ R_{CO} = 3.338 - 0.364 \times pH \]  
\[ [H^+]_{rc} = 10^{-pH} \]

where \( R_{O^2} \) is for oxygenated blood and \( R_{CO} \) is for deoxygenated blood.

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