

## Evidence that diffusion limitation determines oxygen uptake kinetics during exercise in humans.

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### Research Article

To determine the role of arterial O<sub>2</sub> content on the mechanism of muscle O<sub>2</sub> utilization, we studied the effect of 2, 11, and 20% carboxyhemoglobin (COHb) on O<sub>2</sub> uptake (VO<sub>2</sub>), and CO<sub>2</sub> output (VCO<sub>2</sub>) kinetics in response to 6 min of constant moderate- and heavy-intensity cycle exercise in 10 subjects. Increased COHb did not affect resting heart rate, VO<sub>2</sub> or VCO<sub>2</sub>. Also, the COHb did not affect the asymptotic VO<sub>2</sub> in response to exercise. However, VO<sub>2</sub> and VCO<sub>2</sub> kinetics were affected differently. The time constant (TC) of VO<sub>2</sub> significantly increased with increased COHb for both moderate and heavy work intensities. VO<sub>2</sub> TC was positively correlated with blood lactate. In contrast, VCO<sub>2</sub> TC was negatively correlated with increased COHb for the moderate but unchanged for the heavy work intensity. The gas exchange ratio reflected a smaller increase in CO<sub>2</sub> stores and faster VCO<sub>2</sub> kinetics relative to VO<sub>2</sub> with increased COHb. These changes can be explained by compensatory cardiac output (heart rate) increase in response to reduced arterial O<sub>2</sub> content. The selective slowing of VO<sub>2</sub> kinetics, with decreased blood O<sub>2</sub> content and increased cardiac output, suggests that O<sub>2</sub> is diffusion limited at the levels of exercise studied.

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# Evidence That Diffusion Limitation Determines Oxygen Uptake Kinetics during Exercise in Humans

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## Abstract

To determine the role of arterial  $O_2$  content on the mechanism of muscle  $O_2$  utilization, we studied the effect of 2, 11, and 20% carboxyhemoglobin (COHb) on  $O_2$  uptake ( $\dot{V}O_2$ ), and  $CO_2$  output ( $\dot{V}CO_2$ ) kinetics in response to 6 min of constant moderate- and heavy-intensity cycle exercise in 10 subjects. Increased COHb did not affect resting heart rate,  $\dot{V}O_2$  or  $\dot{V}CO_2$ . Also, the COHb did not affect the asymptotic  $\dot{V}O_2$  in response to exercise. However,  $\dot{V}O_2$  and  $\dot{V}CO_2$  kinetics were affected differently. The time constant (TC) of  $\dot{V}O_2$  significantly increased with increased COHb for both moderate and heavy work intensities.  $\dot{V}O_2$  TC was positively correlated with blood lactate. In contrast,  $\dot{V}CO_2$  TC was negatively correlated with increased COHb for the moderate but unchanged for the heavy work intensity. The gas exchange ratio reflected a smaller increase in  $CO_2$  stores and faster  $\dot{V}CO_2$  kinetics relative to  $\dot{V}O_2$  with increased COHb. These changes can be explained by compensatory cardiac output (heart rate) increase in response to reduced arterial  $O_2$  content. The selective slowing of  $\dot{V}O_2$  kinetics, with decreased blood  $O_2$  content and increased cardiac output, suggests that  $O_2$  is diffusion limited at the levels of exercise studied. (*J. Clin. Invest.* 1990. 86:1698–1706.) Key words: anaerobic threshold • carbon monoxide • carboxyhemoglobin • heart rate kinetics • lactate

## Introduction

$O_2$  uptake ( $\dot{V}O_2$ )<sup>1</sup> by the lungs reflects  $\dot{V}O_2$  by the muscles. The  $\dot{V}O_2$  differs from the  $O_2$  requirement only when the  $O_2$  delivery to the muscles is inadequate to maintain an  $O_2$  partial pressure ( $PO_2$ ) difference between muscle capillaries and muscle mitochondria to meet the immediate bioenergetic  $O_2$  requirement. Whereas a primary determinant of  $\dot{V}O_2$  kinetics in response to exercise must be muscle bioenergetics (1), the ade-

quacy of the  $O_2$  supply to the muscles must also determine the kinetics. Reducing the arterial  $O_2$  content, without changing arterial  $PO_2$  and without an anticipatory increase in blood flow, would result in a more rapid rate of fall in capillary  $PO_2$  than normal. This would affect the pattern of  $\dot{V}O_2$  by the muscles, only if  $O_2$  transport from muscle capillaries to mitochondria was diffusion limited.

Inhaling low concentrations of carbon monoxide (2–5) can affect  $O_2$  content of the arterial blood without affecting the diffusion equilibrium between  $O_2$  in the alveolar gas and the pulmonary capillary blood. At the tissue level, capillary  $PO_2$  would fall more rapidly than normal because of the reduced blood  $O_2$  content and the leftward shift of the oxyhemoglobin dissociation curve caused by the increased carboxyhemoglobin (COHb) (6). The partial pressure of carbon monoxide (PCO) required to increase COHb to 20% ( $\sim 0.15$  mm Hg) does not significantly affect oxymyoglobin (binding of carbon monoxide to myoglobin is only 10% of that to hemoglobin) and mitochondrial cytochromes (7). Any increase in blood flow caused by the reduced arterial  $O_2$  content would be compensatory and incomplete, resulting in a reduced mean and end capillary  $PO_2$  (4), and therefore reduced  $O_2$  availability to the exercising muscles. Thus if  $O_2$  uptake by the muscle mitochondria were limited by diffusion, rather than muscle bioenergetics alone,  $\dot{V}O_2$  kinetics in response to a given work rate will be slowed when capillary  $PO_2$  was decreased. In order to determine the role of diffusion in  $O_2$  utilization during exercise, we measured  $\dot{V}O_2$ ,  $CO_2$  output ( $\dot{V}CO_2$ ) and heart rate kinetics and end-exercise lactate while COHb was systematically controlled at 11% (level of heavy cigarette smoker) and 20%, during constant work rate exercise.

## Methods

**Subjects.** 10 normal nonsmoking subjects without cardiac or pulmonary disease ranging in age from 18 to 45 yr were studied (Table I). The nature and purpose of the study and the risks involved were explained. Each subject voluntarily consented to participate in the study. The protocol and procedures for this study were reviewed and approved by the Institution's Human Subjects Committee.

**Carbon monoxide loading.** Carbon monoxide loading was accomplished using a procedure modified from Vogel and Gleser (4). This technique involved establishing a titration curve by monitoring venous COHb levels after breathing successive levels of 5–10 liters of 1% carbon monoxide in air using a co-oximeter (Instrument Laboratory, Inc., Lexington, MA) to measure COHb. This titration was used to estimate the volume of 1% carbon monoxide that the subject needed to breathe in order to achieve 11% or 20% COHb levels on the study days. No subject experienced symptoms from breathing the gas, and resting heart rate and minute ventilation were not changed.

**Exercise protocol.** Exercise tests were employed using an upright, electromagnetically braked cycle ergometer (Gould Godhart BV, Bilthoven, The Netherlands). Each subject performed two levels of exercise during three sessions on different days in randomized order as follows: one session without added carbon monoxide (control), one

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1. Abbreviations used in this paper: COHb, carboxyhemoglobin;  $\dot{V}CO_2$ ,  $CO_2$  output;  $\dot{V}O_2$ ,  $O_2$  uptake.

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Table I. Physical Characteristics and Work Rates of Moderate and Heavy Intensity Studied for Each Subject

| Subject | Age      | Sex | Height    | Weight   | Work      |            |
|---------|----------|-----|-----------|----------|-----------|------------|
|         |          |     |           |          | Moderate  | Heavy      |
|         | yr       |     | cm        | kg       | W         |            |
| 1       | 45       | M   | 180       | 79       | 120       | 200        |
| 2       | 31       | M   | 168       | 59       | 55        | 110        |
| 3       | 40       | M   | 168       | 70       | 50        | 110        |
| 4       | 32       | M   | 175       | 67       | 145       | 260        |
| 5       | 29       | F   | 168       | 60       | 100       | 200        |
| 6       | 39       | M   | 168       | 71       | 60        | 130        |
| 7       | 31       | M   | 186       | 86       | 130       | 260        |
| 8       | 18       | M   | 165       | 62       | 80        | 190        |
| 9       | 28       | M   | 175       | 69       | 80        | 200        |
| 10      | 35       | M   | 173       | 74       | 90        | 205        |
| Mean±SD | 32.8±7.1 |     | 172.6±6.2 | 69.7±8.1 | 91.0±30.9 | 186.5±51.6 |

session with ~ 11% COHb, one session with ~ 20% COHb. During each session, moderate and heavy work intensity exercise of 6 min each starting from rest were performed. Three repetitions of both moderate and heavy work intensity exercise were completed during each session whenever possible. The subjects rested at least 30 min between repetitions. The moderate and heavy work intensity exercise levels were determined by finding the work rates during air breathing corresponding to the  $\dot{V}O_2$  at 80% of the anaerobic threshold (moderate exercise intensity), and the work rate at a  $\dot{V}O_2$  which was 40% of the difference between the anaerobic threshold and maximal  $\dot{V}O_2$  (heavy exercise intensity). The actual work rates performed at the moderate and heavy work intensities for each subject are shown in Table I. The anaerobic threshold and maximal  $\dot{V}O_2$  were previously determined for each subject from an incremental cycle ergometer exercise test while breathing room air without added carbon monoxide. The same protocol was used for all three levels of COHb.

To obviate the energetic effect of inertia during acceleration of the flywheel at the start of exercise, an electric motor was used to drive the flywheel at 60 rpm during the rest periods. The motor was switched off when the subject began pedaling. The start of exercise was signaled by the change of a light from red to green within the subject's view. To avoid startle responses, no verbal command was given.

To maintain the COHb at the 11% or 20% level, subjects breathed 0.023% carbon monoxide in air during exercise.

**Measurements.** Heart rate was continuously monitored by a cardiometer, and superficial forearm vein blood was obtained before and 2 min after the exercise for measurement of lactate (enzymatic method [8]). COHb level was also measured before and at the end of exercise.

Subjects breathed through a mouthpiece attached to a turbine volume transducer (Alpha Technologies, Hayward, CA) for measurements of ventilatory volumes during the test. Dead space of the system was 170 ml. Inspired gases were sampled continuously from a site at the mouthpiece for analysis of  $O_2$ ,  $CO_2$ , and  $N_2$  by mass spectrometry (Perkin-Elmer Corp., Norwalk, CT). Breath-by-breath calculations of alveolar  $\dot{V}O_2$  and  $\dot{V}CO_2$  were performed as previously described (9).

**Data analysis.** To enhance the signal to noise ratio, three repetitions of each test were performed and superimposed for both moderate and heavy work intensity exercise in six subjects. Two repetitions were performed and superimposed for only moderate work intensity exercise in two subjects. For the remaining two subjects with less noisy breathing, a single transition of each test was performed and analyzed. The repetitions were more important for low work rate exercise in subjects with noisy breathing.

The breath-by-breath data from each of the three repetitions for each 6-min test were interpolated to give values second by second. These values were time-aligned to a mark at the start of exercise, and superimposed to average random noise and enhance the underlying response patterns for each test with each COHb level for each subject (10). These averaged 60 responses (two work rates, three COHb levels, and 10 subjects) were then used for data analysis.  $O_2$  pulse was calculated every second by dividing  $\dot{V}O_2$  by heart rate.

To determine the kinetics of  $\dot{V}O_2$ , the time constant was determined for all the data from the start of exercise, assuming exponential kinetics, using least square nonlinear regression through the data. The asymptotic  $\dot{V}O_2$  was determined by fitting the data to the sum of two exponentials, as previously described by Linnarsson (11). The first exponential term described the kinetics to 180 s and the second exponential term described the kinetics for the data between 180 and 360 s. The overall dynamics were then expressed as a single exponential time constant through all the data from the resting  $\dot{V}O_2$  to the asymptotic  $\dot{V}O_2$ , when  $\dot{V}O_2$  did not reach steady state by 6 min, or actual steady-state  $\dot{V}O_2$  if steady state was established before 6 min. The same analysis was used for  $\dot{V}CO_2$ , heart rate, and  $O_2$  pulse. While  $\dot{V}O_2$  kinetics may be more complex than a single exponential increase for heavy exercise (12–14), this approach is a useful model, as previously reported (15).

The increase in  $\dot{V}O_2$  at 6 min as compared to 3 min of constant work rate tests [ $\Delta\dot{V}O_2$  (6–3)], a measurement which was described by Roston et al. (16) to be highly correlated with the increase in blood lactate, was also calculated.

COHb levels for a given test were determined from the average of the values before and after the test.

**Statistical methods.** Differences in the parameters without added carbon monoxide (control), with ~ 11% COHb and 20% COHb were determined by analysis of variance for repeated measures. When the *F* test was significant, individual comparisons were made by Newman-Keuls' multiple-range test. Variations about the mean are expressed as  $\pm 1$  SD and differences were considered significant at the  $P < 0.05$  level.

## Results

The physical characteristics of the subjects are shown in Table I. Venous lactate concentration at rest before exercise averaged  $1.0 \pm 0.4$  mM/liter for control studies,  $1.2 \pm 0.5$  mM/liter for 11% COHb studies, and  $1.2 \pm 0.7$  mM/liter for 20% COHb studies. The differences were not significant.

Table II. COHb Level of Each Exercise Test

| Subject | Moderate intensity tests |          |          | Heavy intensity tests |          |          |
|---------|--------------------------|----------|----------|-----------------------|----------|----------|
|         | Control                  | 11% COHb | 20% COHb | Control               | 11% COHb | 19% COHb |
| 1       | 2.0                      | 13.5     | 19.6     | 2.0                   | 13.7     | 18.5     |
| 2       | 2.5                      | 11.3     | 19.4     | 2.5                   | 12.6     | 19.4     |
| 3       | 2.5                      | 11.4     | 19.4     | 2.5                   | 11.8     | 19.7     |
| 4       | 2.0                      | 11.6     | 21.2     | 2.0                   | 11.8     | 20.2     |
| 5       | 1.1                      | 12.4     | 20.5     | 1.1                   | 11.2     | 19.3     |
| 6       | 3.0                      | 12.1     | 19.0     | 3.0                   | 13.1     | 18.9     |
| 7       | 0.8                      | 9.6      | 20.1     | 0.8                   | 9.7      | 18.6     |
| 8       | 0.9                      | 10.2     | 23.3     | 0.7                   | 10.7     | 20.4     |
| 9       | 0.8                      | 9.2      | 21.6     | 0.8                   | 10.0     | 19.4     |
| 10      | 0.6                      | 11.3     | 19.4     | 0.6                   | 11.1     | 17.8     |
| Mean±SD | 1.6±0.8                  | 11.2±1.2 | 20.3±1.3 | 1.6±0.9               | 11.5±1.2 | 19.2±0.7 |

The mean COHb level of each test during exercise is given in Table II. The actual COHb level without added carbon monoxide (control tests), and with added carbon monoxide during exercise were  $1.6\pm0.8\%$ ,  $11.2\pm1.2\%$ , and  $20.3\pm1.3\%$ , for the moderate work intensity tests, and  $1.6\pm0.9\%$ ,  $11.5\pm1.2\%$ , and  $19.2\pm0.7\%$  for heavy work intensity tests, respectively. These increased COHb levels were kept almost constant during exercise by breathing 0.023% carbon monoxide.

Superficial forearm venous lactate concentration of the control (air breathing) study obtained 2 min after the moder-

ate work intensity exercise was  $1.1\pm0.6$  mM/liter and increased to a small but significant amount at the 20% COHb level (Table III, Fig. 1). For the heavy work intensity exercise, lactate concentration was  $5.0\pm2.0$  mM/liter for the control study and increased to  $6.7\pm1.8$  and  $9.4\pm1.1$  mM/liter, for the 11.5% and 19.2% COHb levels, respectively. These increases were significant at each level of COHb (Table III, Fig. 1).

Fig. 2 shows the responses of  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and gas exchange ratio in one representative subject (subject 4 in Table I) during both moderate and heavy work intensity tests without added carbon monoxide and with  $\sim 20\%$  COHb. The  $\dot{V}O_2$  of

Table III.  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , Heart Rate, and  $O_2$ -Pulse Responses to Moderate and Heavy Work Rate Tests and Lactate Concentration Obtained 2 min after Exercise

|                                      | Moderate intensity tests |          |          |         | Heavy intensity tests |          |          |         |
|--------------------------------------|--------------------------|----------|----------|---------|-----------------------|----------|----------|---------|
|                                      | Control                  | 11% COHb | 20% COHb | P value | Control               | 11% COHb | 19% COHb | P value |
| Time constant of $\dot{V}O_2$ (s)    | 28.8                     | 30.5     | 33.6     | <0.0001 | 51.4                  | 59.5     | 67.5     | 0.0001  |
| ±SD                                  | 4.1                      | 5.5      | 5.5      |         | 11.7                  | 12.8     | 16.5     |         |
| $\dot{V}O_2$ at 6 min (liter/min)    | 1.69                     | 1.70     | 1.71     | NS      | 2.88                  | 2.90     | 2.87     | NS      |
| ±SD                                  | 0.37                     | 0.38     | 0.39     |         | 0.67                  | 0.67     | 0.60     |         |
| $\dot{V}O_2$ asymptote (liter/min)   | 1.71                     | 1.69     | 1.74     | NS      | 2.95                  | 3.00     | 2.97     | NS      |
| ±SD                                  | 0.36                     | 0.38     | 0.40     |         | 0.67                  | 0.68     | 0.61     |         |
| $\Delta\dot{V}O_2$ (6-3) (liter/min) | 0.030                    | 0.036    | 0.041    | NS      | 0.145                 | 0.209    | 0.265    | 0.003   |
| ±SD                                  | 0.047                    | 0.045    | 0.062    |         | 0.051                 | 0.073    | 0.089    |         |
| Time constant of $\dot{V}CO_2$ (s)   | 68.3                     | 57.5     | 53.9     | 0.007   | 70.0                  | 73.1     | 75.4     | NS      |
| ±SD                                  | 10.2                     | 12.6     | 12.8     |         | 17.5                  | 15.9     | 22.4     |         |
| $\dot{V}CO_2$ asymptote (liter/min)  | 1.54                     | 1.51     | 1.57     | NS      | 2.81                  | 2.98     | 3.07     | <0.02   |
| ±SD                                  | 0.35                     | 0.36     | 0.36     |         | 0.65                  | 0.64     | 0.59     |         |
| HR asymptote (beats/min)             | 115.0                    | 118.9    | 131.7    | <0.0001 | 157.4                 | 166.6    | 173.6    | 0.0002  |
| ±SD                                  | 8.3                      | 12.8     | 11.4     |         | 6.8                   | 5.1      | 10.6     |         |
| $O_2$ -pulse asymptote (ml/beat)     | 14.8                     | 14.0     | 13.1     | <0.0001 | 18.9                  | 18.0     | 16.9     | 0.0002  |
| ±SD                                  | 2.8                      | 3.1      | 2.6      |         | 3.9                   | 4.1      | 3.4      |         |
| Lactate (mM/liter)                   | 1.1                      | 1.5      | 1.9      | <0.02   | 5.0                   | 6.7      | 9.4      | <0.0001 |
| ±SD                                  | 0.6                      | 0.8      | 0.6      |         | 2.0                   | 1.8      | 1.1      |         |

$\Delta\dot{V}O_2$  (6-3) = the increase in  $O_2$  uptake at 6 min as compared to 3 min of constant work rate tests. P value was determined by analysis of variance for repeated measures. HR, heart rate.

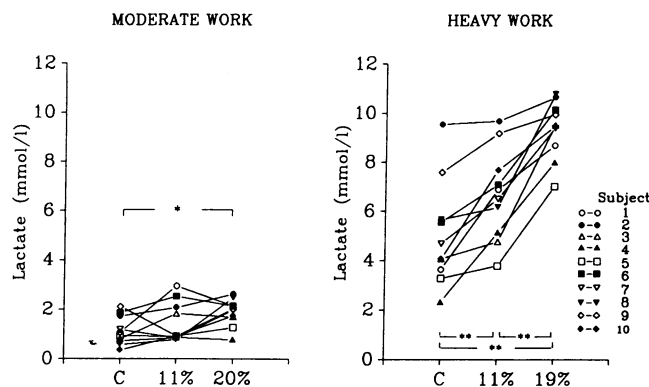


Figure 1. Venous lactate concentration of each test obtained 2 min after exercise. The symbols for each subject are indicated on the figure, and the subject numbers correspond to those in Tables I and II. \* $P < 0.05$ , \*\* $P < 0.01$  by Newman-Keuls' multiple-range test. C, control studies; 11%, studies with 11.2% COHb for moderate work intensity and 11.5% COHb for heavy work intensity exercise; 19%, studies with 19.2% COHb; 20%, studies with 20.3% COHb. For moderate-intensity exercise, the two levels of COHb averaged 11.2% and 20.3%. For heavy work intensity exercise, the two levels of COHb averaged 11.5% and 19.2%.

the moderate work intensity test of the control study reached a steady state within  $\sim 1$  min and remained constant until the end of exercise.

Mean  $\dot{V}O_2$  at 6 min of the control, 11% and 20% COHb test were  $1.69 \pm 0.37$ ,  $1.70 \pm 0.38$ , and  $1.71 \pm 0.39$  liter/min for the moderate work intensity tests and  $2.88 \pm 0.67$ ,  $2.90 \pm 0.67$ , and  $2.87 \pm 0.60$  liter/min for the heavy work intensity tests, respectively (Table III). There was no difference in  $\dot{V}O_2$  at 6 min as related to COHb level. For both moderate and heavy work intensity tests, the  $\dot{V}O_2$  asymptote, calculated by a single exponential curve fit to the  $\dot{V}O_2$  response, did not change with increased COHb levels (Table III). However, the time constant for  $\dot{V}O_2$  kinetics were significantly increased with increased COHb levels (Fig. 3). The time constants of the heavy work intensity tests were higher than those of the moderate work intensity tests, and increased to a greater degree with increased COHb levels (Fig. 3).

Fig. 4 shows the relationship between the time constant of  $\dot{V}O_2$  and the lactate concentration. The time constant of  $\dot{V}O_2$  increased with the level of lactate, showing a strong statistical correlation ( $r = 0.869$ ,  $P < 0.0001$ ).

Fig. 5 shows  $\Delta\dot{V}O_2$  (6-3) of each test. Although there was no significant difference in  $\Delta\dot{V}O_2$  (6-3) for moderate work intensity tests,  $\Delta\dot{V}O_2$  (6-3) of heavy work intensity tests significantly increased with increased COHb. Despite the fact that the time constant is dominated by data during the first 3 min of testing, whereas  $\Delta\dot{V}O_2$  (6-3) is determined by data between 3 and 6 min of exercise,  $\Delta\dot{V}O_2$  (6-3) correlated well with lactate concentration ( $r = 0.784$ ,  $P < 0.0001$ ) (Fig. 6).

The  $\dot{V}CO_2$  asymptote did not change for moderate work

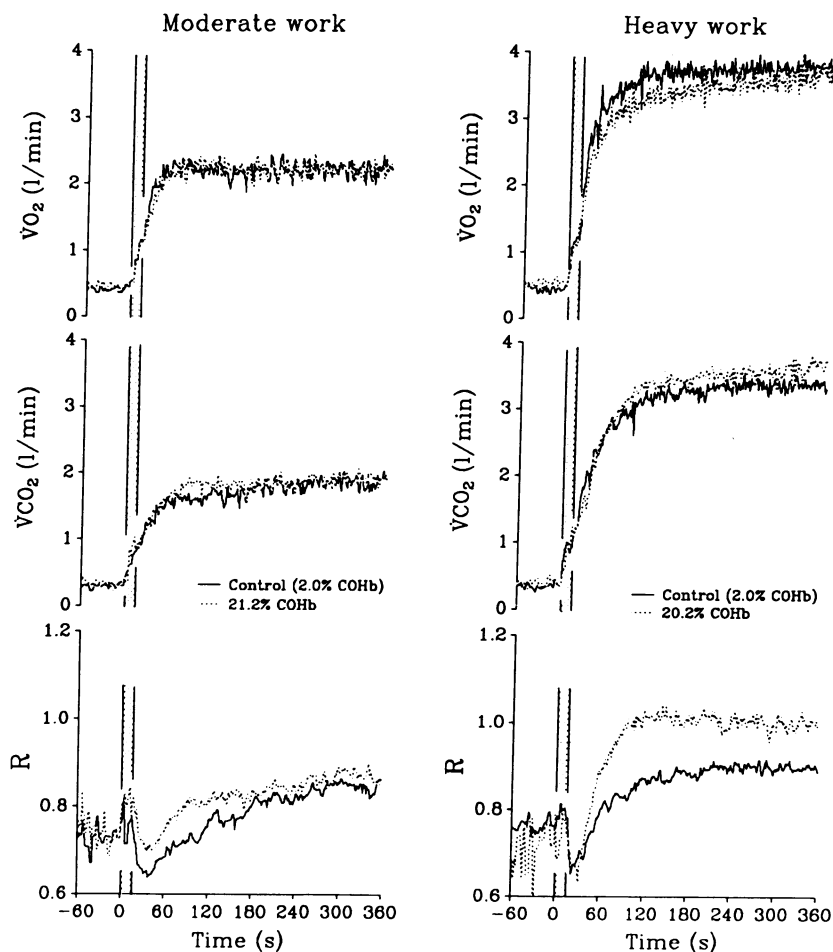
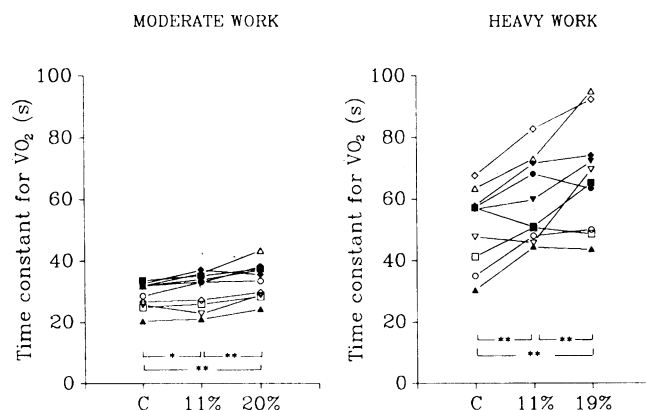


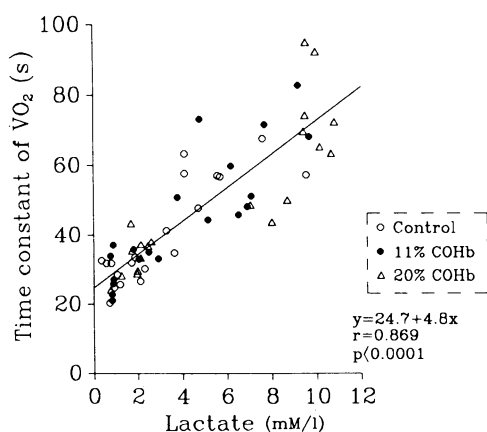
Figure 2. Effects of increased COHb on  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , and gas exchange ratio (R) during moderate (145 W) and heavy (260 W) work intensity exercise without added carbon monoxide and with  $\sim 20\%$  COHb in one subject (subject 4 in Table I). The values to the left of time "0" are measured at rest. The shaded area denotes cardiodynamic phase (15 s after the onset of exercise).



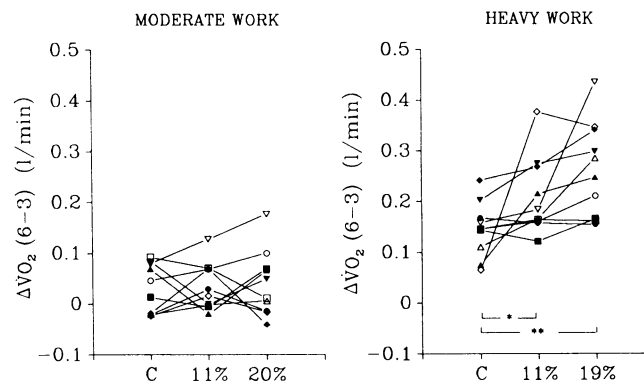
**Figure 3.** The time constant of  $\dot{V}O_2$  in response to moderate and heavy work intensity exercise for 10 subjects. Time constant of  $\dot{V}O_2$  for both moderate and heavy work intensity tests was significantly increased with increased COHb levels. \* $P < 0.05$ , \*\* $P < 0.01$  by Newman-Keuls' multiple-range test. C, control studies; 11%, studies with 11.2% COHb for moderate work intensity and 11.5% COHb for heavy work intensity exercise; 19%, studies with 19.2% COHb; 20%, studies with 20.3% COHb.

intensity tests, but was significantly higher for the heavy work intensity 19% COHb studies ( $3.07 \pm 0.59$  liter/min) than the control studies ( $2.81 \pm 0.65$  liter/min) (Table III). The time constant of  $\dot{V}CO_2$  of the moderate work intensity tests was  $68.3 \pm 10.2$  s for the control study,  $57.5 \pm 12.6$  s for the 11% COHb study, and  $53.9 \pm 12.8$  s for the 20% COHb study, showing a significant decrease with increased COHb levels ( $P < 0.05$  between the control and 11% COHb study, and  $P < 0.01$  between the control and 20% COHb study) in contrast to the  $\dot{V}O_2$  changes (Table III). There was no significant difference in the  $\dot{V}CO_2$  time constant for heavy work intensity tests.

Fig. 7 relates the ratio of the  $\dot{V}CO_2$  and  $\dot{V}O_2$  time constants to blood lactate. The ratio, while averaging 2.4 for the air-



**Figure 4.** Relationship between the time constant of  $\dot{V}O_2$  and superficial forearm vein lactate concentration obtained 2 min after exercise. Data are for both moderate and heavy work intensity exercise at each COHb level; symbols for 20% COHb studies in this figure include 20% COHb studies of moderate work intensity exercise and 19% COHb studies of heavy work intensity exercise.

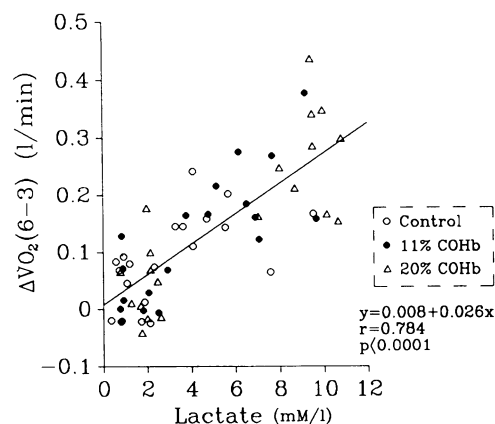


**Figure 5.** The increase in  $O_2$  uptake at 6 min as compared to 3 min of constant work rate tests [ $\Delta\dot{V}O_2$  (6-3)] for each test.  $\Delta\dot{V}O_2$  (6-3) of heavy work intensity tests significantly increased with increased COHb. \* $P < 0.05$ , \*\* $P < 0.01$  by Newman-Keuls' multiple-range test. C, control studies; 11%, studies with 11.2% COHb for moderate work intensity and 11.5% COHb for heavy work intensity exercise; 19%, studies with 19.2% COHb; 20%, studies with 20.3% COHb.

breathing moderate work intensity tests, decreased strikingly and approached 1 as lactate increased. This ratio significantly decreased with increased COHb for both moderate and heavy work intensity exercise, as well as between moderate and heavy exercise at each COHb level. This decrease was primarily due to slowed  $\dot{V}O_2$  kinetics with faster or unchanged  $\dot{V}CO_2$  kinetics.

Fig. 8 shows heart rate responses during both moderate and heavy work intensity exercise in one subject (the same subject as shown in Fig. 2). There was no difference in heart rate at rest but the rate of rise and peak heart rate response was greater the higher the COHb level for both moderate and heavy work intensity. The asymptotic values were significantly increased with increased COHb for the group as a whole (Table III).

Fig. 9 shows the kinetics of  $O_2$  pulse during moderate and heavy work intensity exercise tests in one subject (the same subject as shown in Fig. 2). Peak values are seen by 1 min for



**Figure 6.** Relationship between the increase in  $O_2$  uptake at 6 min as compared to 3 min of constant work rate tests [ $\Delta\dot{V}O_2$  (6-3)] and superficial forearm vein lactate concentration obtained 2 min after exercise. Symbols are same as those noted in Fig. 4.

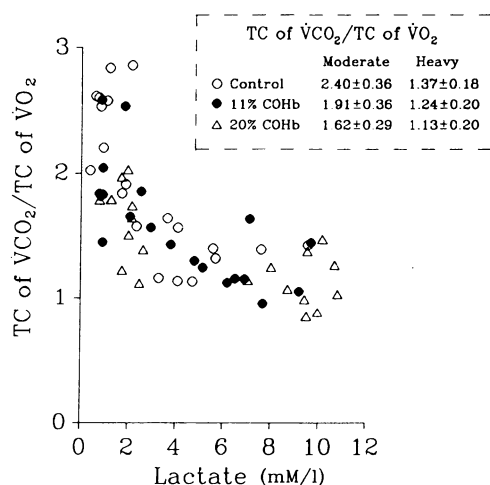


Figure 7. Relationship between the ratio of the time constant (TC) of  $\dot{V}O_2$  and that of  $\dot{V}O_2$  and forearm vein lactate concentration obtained 2 min after exercise. Each symbol includes the data of both moderate and heavy work intensity exercise of each COHb level, as described in Fig. 4. The inset relates TC of  $\dot{V}O_2$ /TC of  $\dot{V}O_2$  to the intensity of work rate and the COHb level.

the moderate work intensity. For the heavy work intensity with increased COHb,  $O_2$  pulse increased further after the 1st-min increase. In all instances  $O_2$  pulse abruptly increased at the start of exercise and then oscillated for 15 s before increasing to its asymptote. The asymptotic values were significantly decreased with increased COHb for both work rate tests (Table III). Since  $O_2$  pulse is equal to stroke volume  $\times$  arterial-venous  $O_2$  difference, and stroke volume is known not to change (4), the decrease likely reflects the expected reduction in arterial-venous  $O_2$  difference.

For both moderate and heavy work intensity tests, the gas exchange ratio decreased during the first minute, after a 15-s delay (Fig. 2). The near-constant gas exchange ratio at resting levels during the first 15 s of exercise, before the gas exchange ratio decreases, is postulated to be due to the proportional increase in  $\dot{V}O_2$  and  $\dot{V}O_2$  caused by increase in pulmonary blood flow at the start of exercise with the arterial-venous difference being that of the before-exercise resting state (17). The subsequent decrease in the gas exchange ratio is postulated to be due to arrival of blood at the lungs from exercising muscle. Because of  $CO_2$  solubility in tissues, part of the early metabolic  $CO_2$  is retained as tissue  $PCO_2$  increases consequent to the smaller increase in cardiac output relative to metabolic rate, i.e., increase in arterial-venous  $O_2$  difference is greater than increase in venous-arterial  $CO_2$  difference during this non-steady-state period.

With increased COHb, the cardiac output response is increased, even for moderate intensity work, as evidenced by the increased heart rate response (Fig. 8 and Table III). This would result in a reduced arterial-venous  $O_2$  (lower  $O_2$  pulse, Fig. 9) and  $CO_2$  concentration difference and therefore less  $CO_2$  retention in the tissues. Thus  $CO_2$  output kinetics would be expected to be faster (approach that of  $O_2$ ) even for moderate work intensity exercise (Table III), and the decrease in the gas exchange ratio during the first minute would be expected to be attenuated as found in this study (Fig. 2).

## Discussion

Constant work rate exercise does not require a subject's maximum effort if the work rate is not unreasonably high and if exercise duration is not too long. Using a constant work rate test, the patterns of  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , heart rate, and  $O_2$ -pulse increase in response to an exercise stimulus can be quantified and be used as descriptors of cardiopulmonary adaptations to exercise (10, 18–21). The binding of hemoglobin by carbon monoxide reversibly decreases the  $O_2$  carrying capacity, producing a useful model of the effect of a modest acute reduction in  $O_2$  transport. Thus this model was applied in this study to determine the sensitivity of  $\dot{V}O_2$  kinetics to detect small

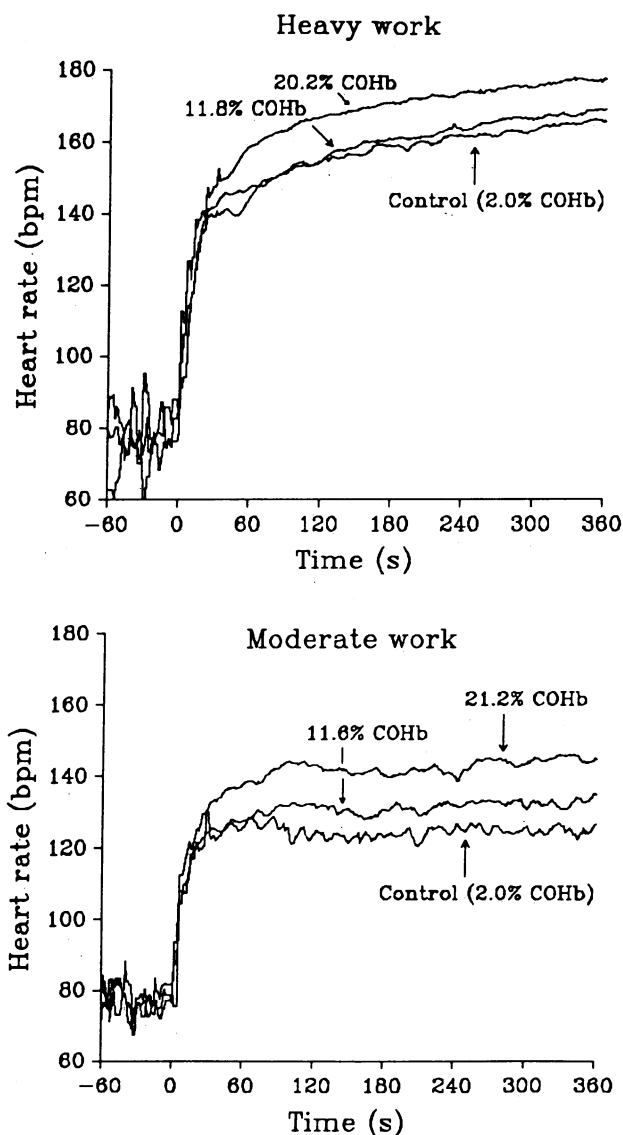


Figure 8. The effect of increased COHb on heart rate response during moderate (145 W) and heavy (260 W) work intensity exercise in one subject (subject 4 in Table I). There was no difference in heart rate at rest but the rate of rise and peak heart rate response during exercise was greater the higher the COHb level for both moderate and heavy work intensity.

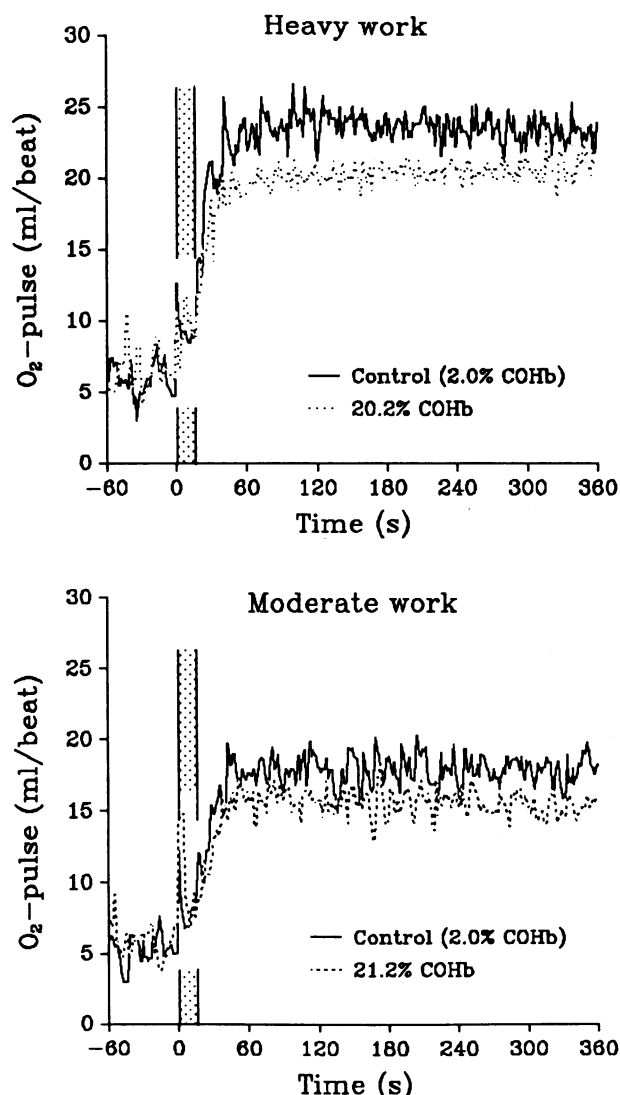


Figure 9. The effect of increased COHb on  $O_2$  pulse as related to time during moderate (145 W) and heavy (260 W) work intensity exercise without added carbon monoxide and with  $\sim 20\%$  COHb in one subject (subject 4 in Table I). For the heavy work intensity with increased COHb,  $O_2$  pulse increased slightly after the rapid rise within the 1st min.

changes in arterial  $O_2$  content and transport without a change in  $PO_2$ .

Carbon monoxide has the effect of reducing  $O_2$  content and shifting the oxyhemoglobin dissociation curve to the left requiring a lower capillary  $PO_2$  for  $O_2$  unloading from hemoglobin. It has been described by Root (22) that the concentration to which COHb can be increased, which is compatible with life, does not poison the cells of the body. While there are only limited studies on the level of  $P_{CO}$  that starts to affect resting cell redox state or electron transport, it appears to be considerably higher than that required to raise COHb to 20% according to the studies of Wittenberg and Wittenberg (7) on cardiac myocytes and our lactate measurements.

To maintain the level of COHb during exercise, our subjects breathed a concentration of 0.023% carbon monoxide in

air. During the moderate and heavy intensity exercise the COHb slightly increased for the 11% COHb ( $11.0 \pm 1.2$  to  $11.8 \pm 1.4\%$ ) and was unchanged or decreased for the 20% COHb level study ( $19.9 \pm 1.5$  to  $19.6 \pm 1.3\%$ ). Thus the blood and therefore tissue  $PCO$  must have been in the range of 0.1–0.2 mmHg, in agreement with the predicted  $PCO$  for 11 and 20% COHb from the COHb dissociation curve in air (6). This is well below the  $PCO$  that has a demonstrable effect on oxymyoglobin (affinity of carbon monoxide to myoglobin is only 10% of that to hemoglobin) and levels of  $PCO$  that affect the mitochondrial cytochromes (7). Further evidence that the low levels of  $PCO$  used in this study did not cause tissue toxicity is that  $\dot{V}O_2$  and ventilation (sensitive markers of acid-base balance and cell redox state) at rest were not affected. Also, resting lactate concentration was not affected by these levels of  $PCO$ . Furthermore, we found no difference in the 6-min  $\dot{V}O_2$  and  $\dot{V}O_2$  asymptote for both moderate and heavy work intensity tests as related to COHb level.

It had been reported that the time constant of  $\dot{V}O_2$  is prolonged in patients with obstructive pulmonary disease (19) and patients with heart disease (10) compared with normal subjects. It had also been reported by Hughson and Smyth (20) and Petersen et al. (23) that beta-blockade slows the  $\dot{V}O_2$  increase during submaximal exercise in normal subjects. In contrast to the latter studies, which slowed  $\dot{V}O_2$  kinetics by attenuating the cardiac output increase, this study slowed  $\dot{V}O_2$  kinetics by effectively reducing capillary  $PO_2$ . Heart rate (cardiac output) was increased (Fig. 8), presumably as compensation for the impaired  $O_2$  delivery.

In this study, the time constant for  $\dot{V}O_2$  significantly increased with increasing levels of COHb (Fig. 3), even if the work intensity was moderate and lactate concentration was  $< 2$  mM/liter at the end of exercise. Since the heart rate increase suggests that cardiac output is actually increased under these circumstances (4), slowing of  $\dot{V}O_2$  kinetics must be attributed to the reduced blood  $O_2$  content and capillary–mitochondrial  $PO_2$  difference. For higher-intensity work, the effect of reducing  $O_2$  content by increased COHb was more marked (Fig. 3) and  $\dot{V}O_2$  kinetics were slower, the higher the blood lactate (Fig. 4). These studies show that both lactate concentration and the time constant for  $\dot{V}O_2$  are increased in response to a relatively small reduction in  $O_2$  transport.

$\dot{V}O_2$  continues to increase slowly beyond 3 min at work rates associated with increased blood lactate (16, 24). Roston et al. (16) measured the increase in  $\dot{V}O_2$  at 6 min as compared to 3 min [ $\Delta\dot{V}O_2$  (6-3)], during constant work rate exercise of different intensities in normal men and showed a good correlation with the increase in blood lactate. In this study, we also showed a good correlation between  $\Delta\dot{V}O_2$  (6-3) and lactate concentration (Fig. 6).

At rest, gas exchange at the lungs is equal to gas exchange at the cells. Thus, the gas exchange ratio at rest reflects the metabolic respiratory quotient that is determined by the mixture of substrate used for energy (24, 25). After the onset of exercise, the immediate increase in pulmonary blood flow (resulting from increased heart rate and stroke volume) causes an abrupt increase in both  $\dot{V}O_2$  and  $\dot{V}CO_2$  (25), during which the gas exchange ratio changes little for the first 15 s (Fig. 2) (17). The gas exchange ratio then decreases, i.e.,  $\dot{V}CO_2$  rise lags the increase in  $\dot{V}O_2$ , because  $CO_2$  is more soluble in tissues and



blood than  $O_2$ . Thus the time constant of  $\dot{V}CO_2$  is longer than that of  $\dot{V}O_2$  for work rates below the anaerobic threshold (19, 24–27). However, at work rates accompanying lactic acidosis (Fig. 7), the rate of increase in  $\dot{V}CO_2$  approaches and sometimes exceeds that of  $\dot{V}O_2$  as the latter slows and the former remains the same or becomes faster. Additional  $CO_2$  generated from  $HCO_3^-$ , as it buffers the increase in lactic acid, is undoubtedly responsible for the disparate changes in  $\dot{V}CO_2$  and  $\dot{V}O_2$  kinetics observed in this study. Consistent with these findings, Springer et al. (28, 29) observed slowing of  $\dot{V}O_2$  kinetics and speeding of  $\dot{V}CO_2$  kinetics in response to hypoxia.

Although heart rate at rest did not differ for different COHb levels, heart rate during exercise became significantly higher with increased COHb levels. This is presumably due to a compensatory stimulation of heart rate to maintain adequate  $O_2$  delivery to the working muscles when blood  $O_2$  content is reduced. Increasing the cardiac output relative to metabolic rate, without a reduction in  $O_2$  content, should increase capillary  $PO_2$ . The increase in cardiac output (heart rate) (Fig. 8), and the rightward shift in the oxyhemoglobin dissociation curve consequent to increased lactic acidosis, are apparently the mechanisms by which it was possible for  $\dot{V}O_2$  to increase to the same steady state (moderate intensity) or 6-min  $\dot{V}O_2$  (heavy intensity) in the control and increased COHb studies.

$O_2$  pulse, which did not differ at rest, significantly decreased during both moderate and heavy work intensity exercise with increased COHb levels (Fig. 9). As  $O_2$  pulse is mathematically equal to the product of stroke volume and the arterial–venous  $O_2$  difference, decreased  $O_2$  content of the arterial blood and decreased arterial–venous  $O_2$  difference must be responsible for the decreased  $O_2$  pulse during exercise observed with increased COHb. This is similar to the effect of anemia.

Fick's law of diffusion states that the mass transfer ( $D$ ) of a substance, such as  $O_2$ , is directly proportional to the partial pressure difference between the high pressure point in the capillary ( $P_c$ ) to the low pressure point in the mitochondria ( $P_m$ ) and the surface area ( $A$ ) (degree of capillary hyperemia), and inversely related to the diffusion distance ( $L$ ) (capillary to mitochondria), that is,  $DO_2 = k(P_c - P_m) * A/L$ . The proportionality constant ( $k$ ) is a function of the diffusibility and solubility of  $O_2$  in the tissue substance.

Increasing the COHb should have no effect on arterial  $PO_2$  (4, 30). However, as with anemia (31), the capillary and venous  $PO_2$  should be decreased at submaximal work rates (4) since the increase in blood flow is compensatory and does not completely adjust for the reduced  $O_2$  flow caused by the increased COHb. Increased COHb also results in a further lowering of capillary  $PO_2$  because it causes leftward shift in the oxyhemoglobin dissociation curve. Whereas the effect of the increased COHb is to reduce the capillary to mitochondrial  $PO_2$  difference, any increase in capillary surface area resulting from increased blood flow, would facilitate diffusion. Also, capillary recruitment with increased blood flow should reduce diffusion distance and speed  $\dot{V}O_2$  kinetics.

In this study, we experimentally reduced the capillary–mitochondrial  $O_2$  diffusion gradient without affecting the steady-state or asymptotic  $\dot{V}O_2$  and thus presumably mitochondrial function. We conclude that the slowing of  $\dot{V}O_2$  kinetics found in this study is most likely explained by diffusion limited  $O_2$  transport to the contracting muscle mitochondria at moderate as well as heavy work intensities.

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