Arterial O₂ content and tension in regulation of cardiac output and leg blood flow during exercise in humans

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Roach, Robert C., Maria D. Koskolou, José A. L. Calbet, and Bengt Saltin. Arterial O₂ content and tension in regulation of cardiac output and leg blood flow during exercise in humans. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H438–H445, 1999.—A universal O₂ sensor presumes that compensation for impaired O₂ delivery is triggered by low O₂ tension, but in humans, comparisons of compensatory responses to altered arterial O₂ content (CaO₂) or tension (PaO₂) have not been reported. To directly compare cardiac output (Q̇TOT) and leg blood flow (LBF) responses to a range of CaO₂ and PaO₂, seven healthy young men were studied during two-legged knee extension exercise with control hemoglobin concentration ([Hb] = 144.4 ± 4.6 g/l) and at least 1 wk later after isovolemic hemodilution ([Hb] = 315 ± 2 g/l). On each study day, subjects exercised twice at 30 W and on to voluntary exhaustion with an FIO₂ of 0.21 or 0.11. The interventions resulted in two conditions with matched CaO₂, but markedly different PaO₂ (hypoxia and anemia) and two conditions with matched PaO₂ and different CaO₂ (hypoxia and anemia + hypoxia). PaO₂ varied from 46 ± 3 Torr in hypoxia to 95 ± 3 Torr (range 37 to >100) in anemia (P < 0.001), yet LBF at exercise was nearly identical. However, as CaO₂ dropped from 190 ± 5 ml/l in control to 132 ± 2 ml/l in anemia + hypoxia (P < 0.001), Q̇TOT and LBF at 30 W rose to 12.8 ± 0.8 and 7.2 ± 0.3 l/min, respectively, values 23 and 47% above control (P < 0.01). Thus regulation of Q̇TOT, LBF, and arterial O₂ delivery to contracting intact human skeletal muscle is dependent for signaling primarily on CaO₂, not PaO₂. This finding suggests that factors related to CaO₂ or [Hb] may play an important role in the regulation of blood flow during exercise in humans.

vasodilatation; red blood cell; hemoglobin; anemia; hypoxia; nitric oxide

HYPOXIA IS THE main stimulus for ventilatory and cardiovascular compensation for diminished arterial O₂ content (CaO₂) (9, 20). However, there are reports suggesting a role for hemoglobin-induced variations in CaO₂ to play a role as well (14). This relates primarily to systemic and limb blood flow being altered to maintain O₂ delivery. Thus, in chronic anemia, Sproule et al. (18) demonstrated cardiac output to be elevated both at rest and during exercise in severely anemic patients. In addition, a lower hemoglobin concentration ([Hb]) accounts for the higher cardiac output in women compared with men at a given submaximal work load (2). On the regional level, blood flow has been shown to vary in healthy people with varying [Hb] levels (14), a finding recently shown also to occur with acute anemia (10). The question then arises to what extent CaO₂, independent of arterial O₂ tension (Pao₂), can affect the compensatory regulation taking place when the human body is challenged by hypoxemia. To directly compare the effects of PaO₂ and CaO₂ on ventilatory and cardiovascular responses to exercise, a wide range of CaO₂ was studied in subjects during hypoxia, acute isovolemic anemia, and combined hypoxia and acute anemia (anemia + hypoxia). The contributions of low CaO₂ and low PaO₂ were further elucidated by comparing responses to hypoxia with normal [Hb] (hypoxia) to responses seen in low [Hb] with normoxia (anemia), thus allowing contrast of two situations with identical CaO₂. Another comparison is made between hypoxia and anemia + hypoxia, two conditions with near-identical levels of PaO₂ but markedly different CaO₂. Moreover, the anemia + hypoxia condition caused a very extreme arterial hypoxemia. We have previously reported some of these data, and they solely focused on comparing normoxia with hypoxia (9), or normal with low [Hb] (10).

METHODS

Subjects. Seven young men (age 24 ± 1 yr) participated in the study. Their mean height and weight were 183 ± 3 cm and 85.1 ± 4.6 kg, respectively. Their maximal pulmonary O₂ consumption (V̇O₂), determined by cycle ergometry, was 55 ± 5 ml·kg⁻¹·min⁻¹ (range 41–70), and maximal cardiac output (Q̇TOT) was 26 ± 0.8 l/min (range 23–28). Additional description of anthropometric and muscle characteristics of these seven subjects as well as more details on methods and study design are available in a previous publication (10). All subjects were informed about the procedures and risks of the study before giving written informed consent to participate as approved by the Copenhagen Fredriksberg Ethical Committee.

Experimental protocol. Subjects were studied on two occasions: once with their normal [Hb] and at least 1 wk later after blood withdrawal with low [Hb]. The afternoon before the low [Hb] experiment, 1–1.5 l of whole blood (average 10%). The contributions of low CaO₂ and low PaO₂ were further elucidated by comparing responses to hypoxia with normal [Hb] (hypoxia) to responses seen in low [Hb] with normoxia (anemia), thus allowing contrast of two situations with identical CaO₂. Another comparison is made between hypoxia and anemia + hypoxia, two conditions with near-identical levels of PaO₂ but markedly different CaO₂. Moreover, the anemia + hypoxia condition caused a very extreme arterial hypoxemia. We have previously reported some of these data, and they solely focused on comparing normoxia with hypoxia (9), or normal with low [Hb] (10).

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The femoral artery and vein were cannulated distal to the inguinal ligament for blood sampling, detection of Cardio-green (arterial), and determination of limb blood flow (venous). An additional catheter was placed in a vein in the left upper arm for the injection of the Cardio-green dye. After the catheters were placed, the subjects sat on the knee-extension ergometer and breathed through a two-way valve inspirings the pertinent gas mixture, starting 5 min before resting measurements. Dynamic contractions of the knee-extensor muscles of the two legs were performed at a rate of 1 Hz starting at 30 W for ~5 min. Subjects then completed a similar exercise bout at 50% of their predicted peak work load. Those data are not reported here. After an ~10-min rest (while breathing normoxic air and only during the last 2–3 min returning to gas mixture inhalation), the exercise was resumed starting at 50% of the peak work load and continuing to 75 and 90% of peak work load for 2 min at each work load. From there on, 5-W increments were applied until the subjects achieved their maximal attainable work load (peak effort). At 30 W and peak effort, data collection started with blood sampling and measurements of leg blood flow (LBF) and QTOT. At peak effort, the measurements were made within ~1 min of exhaustion. When possible, duplicate measurements of LBF and femoral arteriovenous O2 differences were made during the brief period of peak exercise. Heart rate (HR), arterial blood pressure, pulmonary Vo2, CO2 production (VCO2), and expired minute ventilation (Ve) were measured at the same time as LBF and QTOT.

Measurements. LBF was measured in the femoral vein by constant-infusion thermodilution as described in detail elsewhere (1). Limb Vo2 of the knee extensors was calculated by the Fick principle (LBF times femoral arteriovenous O2 difference). Pulmonary Vo2, VCO2, and Ve were measured with an on-line system (Medical Graphics CPX) while the subjects breathed through a low-resistance breathing valve. Gases with known O2 and CO2 concentration (micro-Scholander) were used for gas analyzer calibration.

QTOT was measured by dye-dilution using indocyanine green dye (Cardio-green, Becton Dickinson, Cockeyesville, MD). Cardio-green (4–8 mg, depending on the exercise intensity) was injected in a peripheral vein, and femoral arterial blood was drawn through a photodensitometer (Waters, CO) at a constant rate of 22 ml/min by a withdrawal pump (Harvard, 2202A). The withdrawn blood (~20 ml) was reinjected after each determination. Arterial blood pressure was continuously monitored by a transducer (Gould Electronics, P23) placed at the femoral level (mean distance below the heart 57 cm). HR was obtained either from the pressure curve or from the continuously recorded electrocardiogram signal.

Blood analysis. Blood volume was determined after the subjects were supine for at least 45 min, at 10, 20, and 30 min after the injection of the tracer (131I-RISA, ~250 kBq). [Hb] and O2 saturation (SO2) were measured with a co-oximeter (ABL 912 Co-Oxylite). P02, Pco2, and P02 were determined by standard techniques (ABL Compact 2) and corrected for measured body temperature. Hematocrit was determined by microcentrifugation on triplicate samples and corrected for trapped plasma (1.5%). Blood O2 content (CaO2 and CvO2) was computed from the saturation and [Hb] (i.e., (1.34[Hb] × SO2) − (0.003 × P02)). From blood gas and hemoglobin data at peak effort, O2 conductance into the muscle cell (DO2) was estimated by a numerical integration procedure (17, 22). Mean transit time (MTT) was estimated as previously described (14) from data in a companion paper on these subjects (see Ref. 9, Table 1). Plasma K+ was measured with an ion-sensitive electrode (AVL 983-S). Whole blood lactate concentrations were measured with Triton X-100 as an erythrocyte-lysing agent (YSI 2300 Stat Plus). Leg lactate release was calculated as the product of LBF and the venous-arterial lactate difference. Plasma norepinephrine (NE) and epinephrine concentrations were measured by HPLC with electrochemical detection (6). NE spillover into plasma was calculated using the following equation (16): NE spillover = [(C1 − C2) + C3(A6/LPF)], where C1 and C2 are plasma concentrations in the femoral vein and artery, respectively; A6 is the fractional extraction of epinephrine; and LPF is the leg plasma flow calculated from LBF and hematocrit. NE extraction, determined from the fractional extraction of [3H]NE, has been shown to be ~68% of A6 under steady-state conditions in three subjects (r = 0.88; Ref. 16).

Statistical analysis. Differences in the measured variables among conditions and exercise levels were analyzed with two-way ANOVA for repeated measures, with condition and work load as within-subjects factors. The Newman-Keuls post hoc test was used to assign specific differences in the ANOVA when F was significant. Simple linear regression analyses were performed to determine the relation between variables. Significance was accepted at P < 0.05. Data are reported as means ± SE.

RESULTS

Interventions. The CaO2, in anemia and hypoxia (FiO2 = 0.11) ranged from 150.8 ± 7.2 to 163.9 ± 6.8 ml/l (P < 0.01; see matched content in Fig. 1A). Combining anemia with hypoxia resulted in a further drop in CaO2 from control of 58 to 76 ml/l (P < 0.001). The PaO2 in these conditions was on average 41.9 ± 1.5 Torr (See matched PaO2 in Fig. 1B). The lowest PaO2 values were reached in anemia + hypoxia, with a mean value of 40.0 ± 1.2 Torr at peak effort, and individual values as low as 32.5 Torr.

Whole body responses. Pulmonary Vo2 rose linearly with increasing power output from rest to peak effort (slope = 0.02 l O2 min−1 W−1), and this rise was independent of hypoxia or anemia. At rest and 30 W, pulmonary Vo2, VCO2, and Ve/Vo2 were nearly the same among conditions (Table 1). The matching of CaO2 between hypoxia and anemia resulted in similar peak power outputs and pulmonary Vo2, values ~19% lower than control (P < 0.01) but ~25% higher than in anemia + hypoxia (Table 1). Thus, although PaO2 was matched between hypoxia and anemia + hypoxia, peak power output was ~25% lower in anemia + hypoxia (P < 0.01). Taking power output into account revealed similar pulmonary Vo2 per watt at peak effort in all conditions.

At peak effort, Ve/Vo2 was markedly higher with hypoxia or anemia + hypoxia compared with control or anemia (P < 0.05; Table 1). In anemia + hypoxia, Ve/Vo2 was ~27% higher than in control and rose an additional 15% in hypoxia, suggesting a slight blunting of hypoxic exercise ventilation by anemia. Such blunting of Ve was also revealed when CaO2 was matched at peak effort, as hypoxia resulted in a 30% higher Ve/Vo2 than in anemia (P < 0.05). Reflecting the larger ventilation with hypoxia was a drop in PaCO2 to 29.6 ± 0.7 Torr in anemia + hypoxia at peak effort, a value close to the 28.2 ± 1.3 Torr observed in hypoxia, but lower than the 35.1 ± 0.5 Torr reached in anemia (P < 0.01).
Table 1. Pulmonary $\dot{V}O_2$, $\dot{V}E/\dot{V}O_2$, and two-leg $\dot{V}O_2$ and femoral venous lactate concentration, lactate release, and pH during two-legged knee extensor exercise in conditions with varied oxygen and hemoglobin levels

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary $\dot{V}O_2$, l/min</th>
<th>$\dot{V}E/\dot{V}O_2$</th>
<th>Two-Leg $\dot{V}O_2$, l/min</th>
<th>Lactate, mM</th>
<th>Leg Lactate Release, mmol/min</th>
<th>pH</th>
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<tbody>
<tr>
<td>Anemia + hypoxia</td>
<td></td>
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<tr>
<td>Rest</td>
<td>0.38 ± 0.03</td>
<td>54.2 ± 7.0</td>
<td>0.03 ± 0.01</td>
<td>0.9 ± 0.1</td>
<td>0.01 ± 0.02</td>
<td>7.37 ± 0.01</td>
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<tr>
<td>30 W</td>
<td>0.91 ± 0.03</td>
<td>37.6 ± 3.1</td>
<td>0.60 ± 0.03</td>
<td>1.8 ± 0.5</td>
<td>0.9 ± 0.4</td>
<td>7.36 ± 0.01</td>
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<td>85 ± 7 W</td>
<td>1.70 ± 0.10</td>
<td>51.5 ± 3.2</td>
<td>1.27 ± 0.10</td>
<td>5.0 ± 0.6</td>
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<tr>
<td>Rest</td>
<td>0.40 ± 0.03</td>
<td>31.2 ± 1.5</td>
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<td>30 W</td>
<td>0.88 ± 0.07</td>
<td>28.7 ± 2.1</td>
<td>0.62 ± 0.02</td>
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<td>118 ± 11 W</td>
<td>2.31 ± 0.13</td>
<td>43.2 ± 2.3</td>
<td>1.60 ± 0.13</td>
<td>6.7 ± 1.1</td>
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<td>Rest</td>
<td>0.30 ± 0.01</td>
<td>44.1 ± 1.2</td>
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<tr>
<td>30 W</td>
<td>0.88 ± 0.04</td>
<td>40.5 ± 3.6</td>
<td>0.62 ± 0.02</td>
<td>1.5 ± 0.3</td>
<td>-0.1 ± 0.2</td>
<td>7.35 ± 0.02</td>
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<td>114 ± 10 W</td>
<td>2.11 ± 0.14</td>
<td>59.3 ± 3.2</td>
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<td>7.7 ± 0.7</td>
<td>9.4 ± 1.1</td>
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<tr>
<td>Rest</td>
<td>0.35 ± 0.02</td>
<td>35.6 ± 3.5</td>
<td>0.04 ± 0.01</td>
<td>0.9 ± 0.1</td>
<td>0.01 ± 0.01</td>
<td>7.34 ± 0.01</td>
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<td>30 W</td>
<td>0.84 ± 0.02</td>
<td>31.0 ± 3.1</td>
<td>0.61 ± 0.02</td>
<td>0.8 ± 0.1</td>
<td>-0.1 ± 0.1</td>
<td>7.31 ± 0.01</td>
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<tr>
<td>143 ± 11 W</td>
<td>2.76 ± 0.14</td>
<td>40.7 ± 3.7</td>
<td>1.95 ± 0.14</td>
<td>6.0 ± 0.3</td>
<td>7.2 ± 0.9</td>
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Values are means ± SE. Arterial oxygen content was matched between anemia and hypoxia. Arterial oxygen tension was matched between anemia + hypoxia and hypoxia. $\dot{V}O_2$, O2 consumption; $\dot{V}E$, expired minute ventilation. Statistical significance is represented by the following symbols: *P < 0.01 vs. hypoxia; †P < 0.01 vs. anemia; ‡P < 0.01 vs. anemia + hypoxia; §P < 0.01 vs. all other conditions; §§P < 0.001 compared with all other conditions.
Mean arterial pressure (MAP) rose from rest to peak effort in all groups (P < 0.001) with no separate effect of hypoxia or anemia. Average values for MAP in control, hypoxia, and anemia were 84.1 ± 1.5 mmHg at rest and 87.9 ± 2.7 mmHg at 30 W, increasing to 117.0 ± 2.3 mmHg at peak effort. In anemia + hypoxia, MAP at peak effort was at 104.5 ± 5.8 mmHg, or ~10% lower than in control, hypoxia, or anemia (P < 0.01).

Similar QTOT (l/min) responses were observed across conditions at rest, despite a marked elevation of resting HR from 76 ± 5 beats/min in control to 92 ± 8 beats/min in anemia + hypoxia (P < 0.001 compared with all conditions). At 30 W and peak effort, QTOT was measured in five of the seven subjects in all conditions. At 30 W, QTOT was higher in anemia and anemia + hypoxia compared with control (P < 0.05; Fig. 2A). The increase in QTOT at 30 W was in anemia due to a rise in stroke volume (SV) above control, hypoxia, or anemia + hypoxia (average SV of 115.2 ± 11 ml; P < 0.05). In contrast, in anemia + hypoxia, the higher QTOT values at 30 W can be accounted for by a rise in HR (P < 0.01), with no elevation of SV compared with control or hypoxia. At peak effort, QTOT (l/min) was similar among control, anemia, and anemia + hypoxia, but slightly lower in hypoxia (compared with all conditions, P < 0.05; Fig. 2A). The lower QTOT in hypoxia was due entirely to an 18% fall in SV from the average values for peak SV of 146.9 ± 14.4 ml in control, anemia, and anemia + hypoxia (P < 0.05). At peak effort, HR was similar among conditions, reaching an average value of 154 ± 8 beats/min. It is of note that at peak effort, QTOT in anemia + hypoxia was higher by ~4 l/min per l/minVO2 compared with control (P < 0.01). Furthermore, the higher QTOT in anemia + hypoxia at peak effort (as a function of VO2 or W) was related to the fall in CaO2 from control to anemia + hypoxia (r = 0.4, P < 0.01).

Systemic O2 delivery was at rest and 30 W maintained in all interventions compared with control. In contrast, at peak effort, systemic O2 delivery ranged from a mean of 4.5 ± 0.2 l/min in control to 2.6 ± 0.2 ml/min in anemia + hypoxia, a drop of 38%. The drop in pulmonary VO2 matched the fall in systemic O2 delivery.

Leg responses. LBF (l/min) rose in all subjects in all conditions above control values during 30-W exercise (P < 0.001; Fig. 2B). The rise was related to the fall in CaO2 across conditions (r = 0.99, P < 0.01), not PaO2 (Fig. 3, B and D). At peak effort, LBF expressed as a function of work load was also higher than control (P < 0.01) in all subjects in anemia, in six of seven in hypoxia, and in all subjects in anemia + hypoxia. The trend of changes in LBF at peak effort also followed CaO2, not PaO2 (r = 0.78, P < 0.001; Fig. 3, A and C). The increase in LBF above control in all experimental conditions was sufficient to maintain leg O2 delivery at rest and 30 W. At peak effort, leg O2 delivery both in hypoxia and anemia fell 23% from control values and a further 15% in anemia + hypoxia (P < 0.001 compared with all other conditions). O2 delivery to the muscle in relation to power output (or O2 consumed) appears nearly constant (1, 13), a relationship that was unchanged by hypoxia or anemia. The 38% lower leg O2 delivery observed at peak effort in anemia + hypoxia compared with control accounted for 92% of the 0.68 l/min decrement in leg VO2 from control to anemia + hypoxia. Leg VO2 was similar in all conditions at rest and 30 W, and at peak effort when CaO2 was matched between hypoxia and anemia (Table 1). The relationship of leg VO2 to power output from rest through peak exercise in anemia + hypoxia was 13.2 ml O2·min⁻¹·W⁻¹, a value similar to previous reports from subjects breathing normoxic air and having normal [Hb] (12) (see Fig. 3D in Ref. 9 and Fig. 3A in Ref. 10). Leg O2 extraction was at rest 52% and similar among conditions. At 30 W, leg O2 extraction rose in anemia + hypoxia to 73 ± 2%, a value slightly higher than observed in any other condition (P < 0.003; Fig. 4A). At peak effort, leg O2 extraction in hypoxia reached 79 ± 2% (Fig. 4A). The femoral arteriovenous O2 difference

![Figure 2](http://ajpheart.physiology.org/)

**Fig. 2.** A: at 30 W (open bars), cardiac output was slightly higher in anemia and anemia + hypoxia than control values (††P < 0.05). At peak effort (solid bars), cardiac output was slightly lower in hypoxia compared with all conditions (††P < 0.05). B: leg blood flow (l/min) is shown in all 4 conditions at 30 W and peak effort. †P < 0.01 vs. control; ††P < 0.01 vs. all conditions. See text and Fig. 3 regarding relationship of rise in leg blood flow and CaO2.
was, as expected, similar when $CaO_2$ was matched between hypoxia and anemia and lower than control (and higher than anemia + hypoxia) from rest to peak effort ($P < 0.01$; Fig. 4B). In anemia + hypoxia at rest, femoral arteriovenous $O_2$ difference was $71 \pm 4$ ml/l, increasing at 30 W to $84 \pm 2$ ml/l and reaching $98 \pm 3$ ml/l at peak effort ($P < 0.001$ vs. all conditions from rest to peak effort; Fig. 4B). Estimated leg $DO_2$ at peak effort reached $23.5 \pm 2.2$ and $25 \pm 2.6$ ml·min$^{-1}$·Torr$^{-1}$ in control and hypoxia, respectively, and dropped as [Hb] fell in anemia and anemia + hypoxia to $19.8 \pm 2.1$ and $21 \pm 2.5$ ml·min$^{-1}$·Torr$^{-1}$ ($P < 0.05$ for both conditions compared with control and hypoxia), respectively. At peak effort, estimated MTT was $529 \pm 31$ ms in control, a value that rose to $565 \pm 29$ ms ($P < 0.05$) in hypoxia, and was similar to values seen in anemia ($525 \pm 28$ ms) and anemia + hypoxia ($533 \pm 31$ ms).

Catecholamine responses. At peak effort, arterial NE reached $8.4 \pm 1.1$ nM in anemia + hypoxia, a value lower than in control ($10.3 \pm 1.0$ nM; $P < 0.05$), but similar to hypoxia ($9.3 \pm 0.9$ nM) or anemia ($9.2 \pm 1.7$ nM). NE spillover was elevated above baseline only at peak effort, reaching $4.6 \pm 1.0$ nM/min in all conditions ($P < 0.01$ vs. baseline), with no differences due to anemia or hypoxia. Epinephrine was only higher than baseline at peak effort in all conditions ($P < 0.05$ vs. rest, average values of $0.8 \pm 0.2$ nM at 30 W and $2.5 \pm 0.2$ nM at peak effort), also with no notable effects of anemia or hypoxia.

Metabolic responses. With hypoxia (both hypoxia alone and anemia + hypoxia), venous pH was higher at 30 W and peak effort compared with values in anemia or control ($P < 0.01$; Table 1). Also at peak effort, similar femoral venous lactate values were reached in hypoxia and anemia, largely because of the nearly identical peak power outputs in these conditions (Table 1). In contrast, as peak power output in anemia + hypoxia only reached 59% of the control values, lactate at peak effort was also lower, reaching only $5.0 \pm 0.5$ mM ($P < 0.001$ vs. all conditions, Table 1). Expression of lactate at peak effort relative to peak power output reveals that in anemia + hypoxia, lactate per watt was matched to all other conditions. Leg lactate release was higher at peak effort in hypoxia compared with all conditions ($P < 0.05$; Table 1). Venous $K^+$ rose with
increasing work in all conditions \((r = 0.7, P < 0.001)\), but the increase was not greater with hypoxia or anemia.

DISCUSSION

The major new finding of this study is the key role that \(\text{CaO}_2\) plays in the regulation of muscle blood flow during exercise, which is likely due to vasodilatation as it occurs in the face of an unchanging MAP, and hence invariant perfusion pressure. On the other hand, the effects of \(\text{PaO}_2\) seem limited during exercise to carotid body-linked stimulation of pulmonary ventilation. Evidence that a low \(\text{PaO}_2\) alone does not cause vasodilatation comes from the observation of similar limb blood flows in the two conditions with nearly identical \(\text{CaO}_2\) but widely different \(\text{PaO}_2\). Moreover, despite using the knee extensor exercise model and thus not taxing fully the capacity of the cardiovascular system at the highest work loads, we found that blood flow (both cardiac output and limb blood flow) rose to an upper level beyond which no further elevation was achieved regardless of intervention. The similarity of responses between hypoxia and anemia illustrates the dependence of power output on \(O_2\) delivery (blood flow \(\times \text{CaO}_2\)).

The apparent difference between \(\text{CaO}_2\) and \(\text{PaO}_2\) in the regulation of the LBF response to hypoxemia suggests that factors related to \([\text{Hb}]\) play a role in the vasodilatation. Recent findings have proposed an important role for the hemoglobin molecule in the regulation of peripheral vasodilatation in the face of altered \(O_2\) concentrations. Stamler et al. (19) have shown that hemoglobin acts as a nitric oxide scavenger in vitro and in vivo in an \(O_2\)-dependent manner, resulting in more nitric oxide available for local vasodilatation when fewer \(O_2\) binding sites are occupied, as happens with a lowering of \(\text{CaO}_2\), but less so when only \(\text{PaO}_2\) was lowered. Another, although also speculative, possible similar mechanism proposed by Ellsworth et al. (4) is \(O_2\)-dependent ATP release. Arguing against a role for anemia- or hypoxia-mediated \([\text{K}^+]\) release playing a dominant role in the regulation of vasodilatation was the similarity in all conditions in \([\text{K}^+]\) response both in arterial and femoral venous blood as well as release from active muscles. Low \([\text{Hb}]\) or hypoxia or a combination does not further elevate the highest attained blood flow in two-legged kicking. This is surprising because the LBF only amounts to a fraction of the attainable maximal cardiac output. Blood pressure is not further enhanced either. There is also some indication of a maintained perfusion of the splanchnic region despite the exhaustive efforts and HR in the range of 150–160 beats/min (9, 10). Moreover, at peak effort, \(Q_{\text{TOT}}\) reached 18–20 l/min as compared with \(Q_{\text{TOT}}\) in ordinary cycle exercise of \(\approx 26\) l/min. Thus, despite substantial \(O_2\) uptake, \(Q_{\text{TOT}}\) rose no further. In this connection, the comparison with the classic study of Sproule et al. (18) is worth mentioning. \(Q_{\text{TOT}}\) was up to 23 l/min during maximal treadmill exercise. This, however, resulted in only 1.8 l/min \(V_{O_2}\) or an \(O_2\) uptake in the same range as in the present subjects exercising only with the knee extensors of the two legs. In whole body exercise, it is easy to understand the lack of a further increase in \(Q_{\text{TOT}}\) at peak effort when \(\text{CaO}_2\) is reduced, since the upper limit in the pump capacity of the heart is reached already in normoxia. The reason for this lack of compensation in the present study where an additional 3–4 l/min in \(Q_{\text{TOT}}\) would have been sufficient is unknown. The explanation most likely lies in the size of the muscle mass involved in the exercise. Dynamic knee-extensor exercise inhibits the parasympathetic activity to the heart but has limited effect on the sympathetic drive to the heart and vascular beds of noncontracting tissue (15).

A lowering of blood viscosity has previously been reported to be a cause for an increase in \(Q_{\text{TOT}}\) with low \(\text{CaO}_2\) (11), although \(Q_{\text{TOT}}\) was not studied when \(\text{CaO}_2\) was held constant and viscosity altered. Matching \(\text{CaO}_2\) in the present study between hypoxia and anemia
allows comparison of the effects of viscosity and CaO2, and reveals a close coupling of blood flow to CaO2, independent of viscosity within the range studied. In support of this are observations made by Jones et al. (8) on cerebral blood flow over a wide range of CaO2, PaO2, and hematocrit in sheep. As CaO2 fell, there was a reciprocal rise in cerebral blood flow, independent of PaO2 or hematocrit. Thus, although viscosity may play a role in vasodilatation in extreme hypovolemia, or in the polycythemia of chronic altitude exposure, it is unlikely that viscosity contributes to the regulation of blood flow in acute, isovolemic anemia. Furthermore, the findings of Jones et al. (8) suggest that the close coupling of CaO2 to regulation of blood flow to exercising skeletal muscle as observed in the present study also may be a widespread mechanism of O2 sensing in other vascular beds.

A remarkable finding in the present study is that the subjects could tolerate the very marked acute drop in CaO2 to 126.6 ± 2.9 ml/l and PaO2 to 40.0 ± 1.2 Torr that occurred in the anemia + hypoxia intervention. Other situations exist with such low CaO2 and PaO2 values as seen in the present intervention. In chronic anemia (3, 18) but not in chronic hypoxia (21) an elevated blood flow contributes in maintaining O2 delivery. O2 extraction, expressed as a percentage of arterially transported O2, is enlarged in both conditions as a result of a right shift in the O2 dissociation curve. In acute anemia or hypoxia (or the combination of the two, see Fig. 4A), blood flow is up both on the systemic and regional level, but the systemic O2 extraction is some 10% lower than in chronic exposure (~65 vs. 70–80%). Of note is, however, that these high O2 extractions occur at much lower power outputs.

Recently, Ferretti et al. (5) have further developed the idea that CaO2 and blood flow are regulated to maintain a fixed femoral CVO2 or mixed venous CVO2 at a given exercise level. In the present study, with cardiac output measured by dye dilution and direct measurement of femoral CVO2, we were not able to verify a constancy of QTOT(CaO2) is 1.0 and 33.4 ± 2.1 ml/l in anemia + hypoxia and hypoxia, respectively. A too short transit time for full O2 off-loading could be one explanation for the higher femoral PvO2 or CVO2. This was not the case, since MTT shows no relationship to femoral PvO2 or CVO2. Thus factors affecting off-loading of O2, or its further transport to and utilization by the mitochondria, must explain the observed high residual femoral venous tension and content. A low pH is one factor that would alter the O2 dissociation curve to favor an increase in end-capillary off-loading of O2. This may have been the case in the present study because a linear drop was noted in femoral PVO2 as pH rose at peak effort in anemia + hypoxia. The higher pH values with hypoxia were due largely to the respiratory stimulation that resulted in a marked drop in PaO2. Furthermore, O2 conductance into the muscle cell estimated by numerical integration (17, 22) shows a close coupling of the predicted DO2 values and the resulting femoral venous PO2, but not the femoral CVO2. Combining pH and DO2 into a regression equation to predict femoral venous PO2 reveals that ~60% of the variance in femoral PV02 was accounted for by pH and DO2 (R² = 0.59, F = 20.3, P < 0.0001).

In conclusion, cardiac output and LBF rise as CaO2 falls, suggesting that O2 delivery, rather than the regulation of capillary PO2, is the main regulatory goal of the vasodilatation. The mechanisms necessary to adjust blood flow according to local demands for O2 delivery are likely situated in the tissue. Several compounds await further investigation as likely regulators of blood flow according to changes in CaO2 or [Hb]. These include red blood cell ATP release (4), arachidonic acid metabolites released in response to changes in O2 levels (7), and a [Hb] specific effect on scavenging of nitric oxide to effect vasodilatation in the face of lowered [Hb] (19).

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