Arterial O_2 content and tension in regulation of cardiac output and leg blood flow during exercise in humans

ROBERT C. ROACH, MARIA D. KOSKOLOU, JOSÉ A. L. CALBET, AND BENGT SALTIN
The Copenhagen Muscle Research Centre, Rigshospitalet, DK-2200 Copenhagen, Denmark

Roach, Robert C., Maria D. Koskolou, José A. L. Calbet, and Bengt Saltin. Arterial O_2 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 O_2 sensor presumes that compensation for impaired O_2 delivery is triggered by low O_2 tension, but in humans, comparisons of compensatory responses to altered arterial O_2 content (Ca_{O_2}) or tension (Pa_{O_2}) have not been reported. To directly compare cardiac output ($\dot{Q}_{TOT}$) and leg blood flow (LBF) responses to a range of Ca_{O_2} and Pa_{O_2}, seven healthy young men were studied during two-legged knee extension exercise with control hemoglobin concentration ([Hb] = 144.4 ± 4 g/l) and at least 1 wk later after isovolemic hemodilution ([Hb] = 115 ± 2 g/l). On each study day, subjects exercised twice at 30 W and on to voluntary exhaustion with an FI_{O_2} of 0.21 or 0.11. The interventions resulted in two conditions with matched Ca_{O_2} but markedly different Pa_{O_2} (hypoxia and anemia) and two conditions with matched Pa_{O_2} and different Ca_{O_2} (hypoxia and anemia + hypoxia). Pa_{O_2} 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 Ca_{O_2} dropped from 190 ± 5 ml/l in control to 132 ± 2 ml/l in anemia + hypoxia ($P < 0.001$), $\dot{Q}_{TOT}$ 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 $\dot{Q}_{TOT}$, LBF, and arterial O_2 delivery to contracting intact human skeletal muscle is dependent for signaling primarily on Ca_{O_2}, not Pa_{O_2}. This finding suggests that factors related to Ca_{O_2} 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 O_2 content (Ca_{O_2}) (9, 20). However, there are reports suggesting a role for hemoglobin-induced variations in Ca_{O_2} to play a role as well (14). This relates primarily to systemic and limb blood flow being altered to maintain O_2 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 Ca_{O_2}, independent of arterial O_2 tension (Pa_{O_2}), can affect the compensatory regulation taking place when the human body is challenged by hypoxemia. To directly compare the effects of Pa_{O_2} and Ca_{O_2} on ventilatory and cardiovascular responses to exercise, a wide range of Ca_{O_2} was studied in subjects during hypoxia, acute isovolemic anemia, and combined hypoxia and acute anemia (anemia + hypoxia). The contributions of low Ca_{O_2} and low Pa_{O_2} 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 Ca_{O_2}. Another comparison is made between hypoxia and anemia + hypoxia, two conditions with near-identical levels of Pa_{O_2} but markedly different Ca_{O_2}. 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 O_2 consumption ($\dot{V}O_2$), determined by cycle ergometry, was 55 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$ (range 41–70), and maximal cardiac output ($\dot{Q}_{TOT}$) 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 1.3 ± 0.05 l, ~20% of subject's blood volume) were withdrawn from each subject and replaced by an equal volume of human serum albumin (5% albumin). After normovolemic hemodilution, the blood volume was maintained (7.06 ± 0.46 to 6.93 ± 0.48 l, pre-post blood removal, $P > 0.8$), and [Hb] and hematocrit dropped ~20%, to 114.7 ± 1.9 g/l and 34.4 ± 0.4%, respectively (10). At the end of the low [Hb] experiment, the previously removed whole blood was reinfused to the subject. During the normal and low [Hb] experiments, the subjects inspired 0.21 and 0.11 FI_{O_2} in N_2 administered in random order. These tests were separated by at least 1 h of rest in the semirecumbent position while subjects breathed room air. In those subjects who breathed hypoxic gas first, in some cases the subsequent resting measurements for lactate were above baseline, although no effects of order of hypoxia were observed during exercise.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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 inspiring 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 $\dot{Q}_{TOT}$. At peak effort, the measurements were made within ~1 min of exhaustion. When possible, duplicate measurements of LBF and femoral arteriovenous O_2 differences were made during the brief period of peak exercise. Heart rate (HR), arterial blood pressure, pulmonary $\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$), and expired minute ventilation ($\dot{V}E$) were measured at the same time as LBF and $\dot{Q}_{TOT}$.

Measurements. LBF was measured in the femoral vein by constant-infusion thermodilution as described in detail elsewhere (1). Limb $\dot{V}O_2$ of the knee extensors was calculated by the Fick principle (LBF times femoral arteriovenous O_2 difference). Pulmonary $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ were measured with an on-line system (Medical Graphics CPX) while the subjects breathed through a low-resistance breathing valve. Gases with known O_2 and CO_2 concentration (micro-Scholander) were used for gas analyzer calibration.

$\dot{Q}_{TOT}$ was measured by dye-dilution using indocyanine green dye (Cardio-green, Becton Dickinson, Cockeysville, 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-10) at a constant rate of 22 ml/min by a withdrawal pump (Harvard, 2202A). The withdrawn blood (~20 ml) was reinfused 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 (^{131}I-RISA, ~250 kBq). [Hb] and O_2 saturation (So_2) were measured with a co-oximeter (AVL 912 Co-Oxylite). Po_2, Pco_2, and pH were determined by standard techniques (AVL Compact 2) and corrected for measured body temperature. Hematocrit was determined by microcentrifugation on triplicate samples and corrected for trapped plasma (1.5%). Blood O_2 content (Ca_{O_2} and Cv_{O_2}) was computed from the saturation and [Hb] [i.e., $(1.34[Hb] \times So_2) - (0.003 \times Po_2)$]. From blood gas and hemoglobin data at peak effort, O_2 conductance into the muscle cell (Do_2) 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 = $[(C_v - C_a) + C_a(A_e)]LPF$, where C_v and C_a are plasma concentrations in the femoral vein and artery, respectively; A_e 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 [^{3}H]NE, has been shown to be ~68% of A_e 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 Ca_{O_2} in anemia and hypoxia ($F_{I_{O_2}} = 0.11$) ranged from 150.8 ± 7.2 to 163.9 ± 6.8 ml/l ($P < 0.01$; see matched content in Fig. 1*A*). Combining anemia + hypoxia resulted in a further drop in Ca_{O_2} from control of 58 to 76 ml/l ($P < 0.01$). The Pa_{O_2} in these conditions was on average 41.9 ± 1.5 Torr (see matched Pa_{O_2} in Fig. 1*B*). The lowest Pa_{O_2} 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 $\dot{V}O_2$ rose linearly with increasing power output from rest to peak effort (slope = 0.02 l $O_2 \cdot min^{-1} \cdot W^{-1}$), and this rise was independent of hypoxia or anemia. At rest and 30 W, pulmonary $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E/\dot{V}O_2$ were nearly the same among conditions (Table 1). The matching of Ca_{O_2} between hypoxia and anemia resulted in similar peak power outputs and pulmonary $\dot{V}O_2$, values ~19% lower than control ($P < 0.01$) but ~25% higher than in anemia + hypoxia (Table 1). Thus, although Pa_{O_2} 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 $\dot{V}O_2$ per watt at peak effort in all conditions.

At peak effort, $\dot{V}E/\dot{V}O_2$ was markedly higher with hypoxia or anemia + hypoxia compared with control or anemia ($P < 0.05$; Table 1). In anemia + hypoxia, $\dot{V}E/\dot{V}O_2$ 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 $\dot{V}E$ was also revealed when Ca_{O_2} was matched at peak effort, as hypoxia resulted in a 30% higher $\dot{V}E/\dot{V}O_2$ than in anemia ($P < 0.05$). Reflecting the larger ventilation with hypoxia was a drop in Pa_{CO_2} 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 ($P < 0.01$).

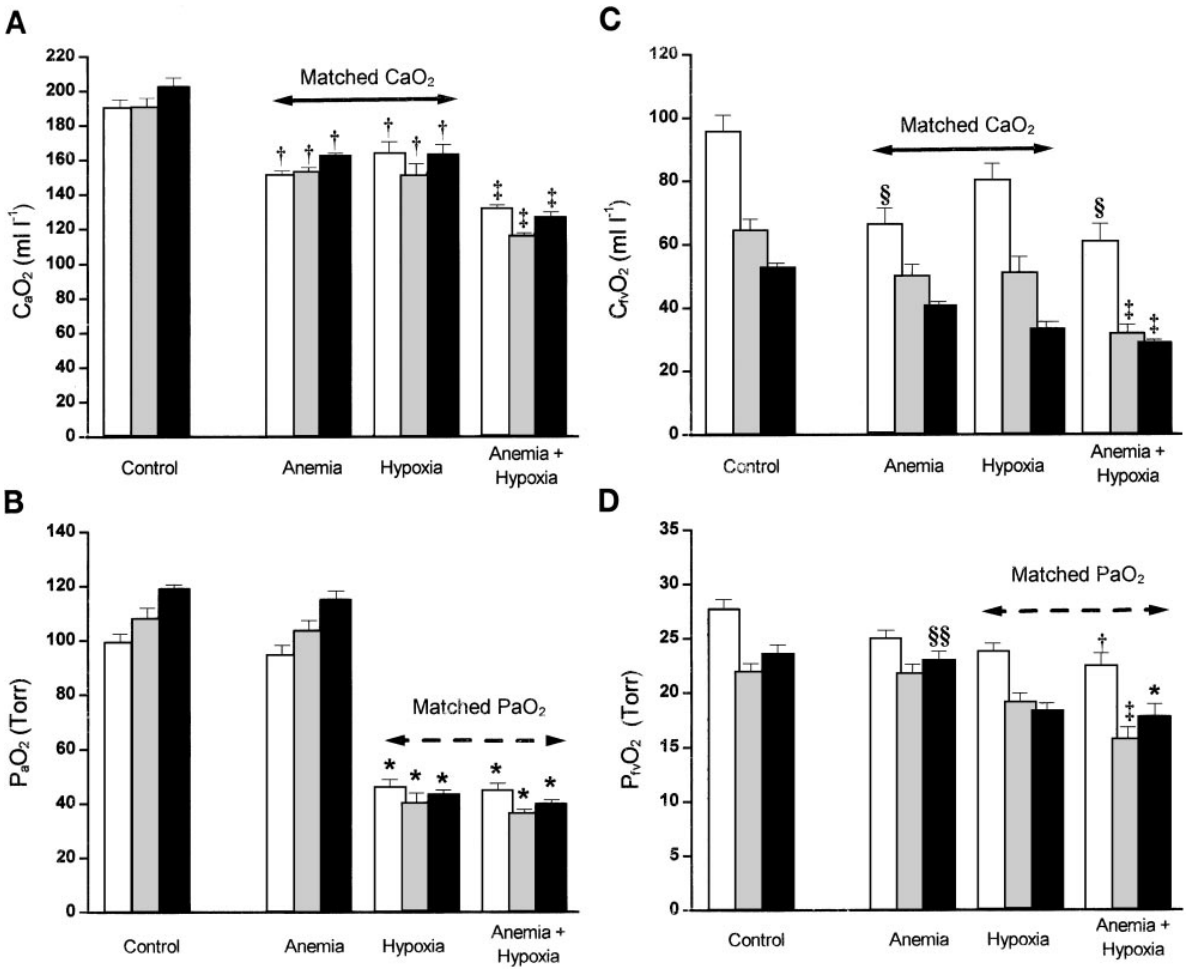

Fig. 1. Indexes of arterial and femoral venous oxygenation in control, anemia, hypoxia, and anemia + hypoxia at rest (open bars), 30 W (gray bars), and peak (solid bars) dynamic knee extensor exercise. Ca_{O_2}, arterial O_2 content; Pa_{O_2}, arterial O_2 tension; Cfv_{O_2}, femoral venous O_2 content; Pfv_{O_2}, femoral venous O_2 tension. †$P < 0.01$ vs. control; ‡$P < 0.01$ vs. all conditions; *$P < 0.01$ vs. control and anemia; §$P < 0.01$ vs. control and hypoxia; §§$P < 0.01$ vs. hypoxia alone.

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*

	Pulmonary $\dot{V}O_2$, l/min	$\dot{V}E/\dot{V}O_2$	Two-Leg $\dot{V}O_2$, l/min	Lactate, mM	Leg Lactate Release, mmol/min	pH
Anemia + hypoxia						
Rest	0.38 ± 0.03	54.2 ± 7.0	0.03 ± 0.01	0.9 ± 0.1	0.01 ± 0.02	7.37 ± 0.01
30 W	0.91 ± 0.03	37.6 ± 3.1	0.60 ± 0.03	1.8 ± 0.5	0.9 ± 0.4	7.36 ± 0.01
85 ± 7 W	1.70 ± 0.10	51.5 ± 3.2	1.27 ± 0.10	5.0 ± 0.5[e]	6.60 ± 1.0	7.26 ± 0.01[e]
Anemia						
Rest	0.40 ± 0.03	31.2 ± 1.5	0.04 ± 0.01	0.9 ± 0.1	0.01 ± 0.01	7.34 ± 0.01
30 W	0.88 ± 0.07	28.7 ± 2.1	0.62 ± 0.02	1.2 ± 0.3	−0.1 ± 0.3	7.31 ± 0.01[a,c]
118 ± 11 W	2.31 ± 0.13	43.2 ± 2.3	1.60 ± 0.13	6.7 ± 1.1[a,c]	6.5 ± 1.0	7.16 ± 0.02[a]
Hypoxia						
Rest	0.30 ± 0.01	44.1 ± 1.2	0.05 ± 0.01	1.2 ± 0.2	0.07 ± 0.05	7.36 ± 0.01
30 W	0.88 ± 0.04	40.5 ± 3.6	0.62 ± 0.02	1.5 ± 0.3	−0.1 ± 0.2	7.35 ± 0.02[b]
114 ± 10 W	2.11 ± 0.14	59.3 ± 3.2	1.58 ± 0.12	7.7 ± 0.7[e]	9.4 ± 1.1[d]	7.20 ± 0.02
Control						
Rest	0.35 ± 0.02	35.6 ± 3.5	0.04 ± 0.01	0.9 ± 0.1	0.01 ± 0.01	7.34 ± 0.01
30 W	0.84 ± 0.02	31.0 ± 3.1	0.61 ± 0.02	0.8 ± 0.1	−0.1 ± 0.1	7.31 ± 0.01[a,c]
143 ± 11 W	2.76 ± 0.14	40.7 ± 3.7	1.95 ± 0.14	6.0 ± 0.3	7.2 ± 0.9	7.17 ± 0.01[a]

Values are means ± SE. Arterial oxygen content was matched between anemia and hypoxia. Arterial oxygen tension was matched between anemia + hypoxia and hypoxia. $\dot{V}O_2$, O_2 consumption; $\dot{V}E$, expired minute ventilation. Statistical significance is represented by the following symbols: [a]$P < 0.01$ vs. hypoxia; [b]$P < 0.01$ vs. anemia; [c]$P < 0.01$ vs. anemia + hypoxia; [d]$P < 0.05$ vs. all other conditions; [e]$P < 0.001$ 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 $\dot{Q}_{TOT}$ (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, $\dot{Q}_{TOT}$ was measured in five of the seven subjects in all conditions. At 30 W, $\dot{Q}_{TOT}$ was higher in anemia and anemia + hypoxia compared with control ($P < 0.05$; Fig. 2*A*). The increase in $\dot{Q}_{TOT}$ 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 $\dot{Q}_{TOT}$ 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, $\dot{Q}_{TOT}$ (l/min) was similar among control, anemia, and anemia + hypoxia, but slightly lower in hypoxia (compared with all conditions, $P < 0.05$; Fig. 2*A*). The lower $\dot{Q}_{TOT}$ 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, $\dot{Q}_{TOT}$ in anemia + hypoxia was higher by ~4 l/min per l/min $\dot{V}O_2$ compared with control ($P < 0.01$). Furthermore, the higher $\dot{Q}_{TOT}$ in anemia + hypoxia at peak effort (as a function of $\dot{V}O_2$ or W) was related to the fall in Ca_{O_2} from control to anemia + hypoxia ($r = 0.4$, $P < 0.01$).

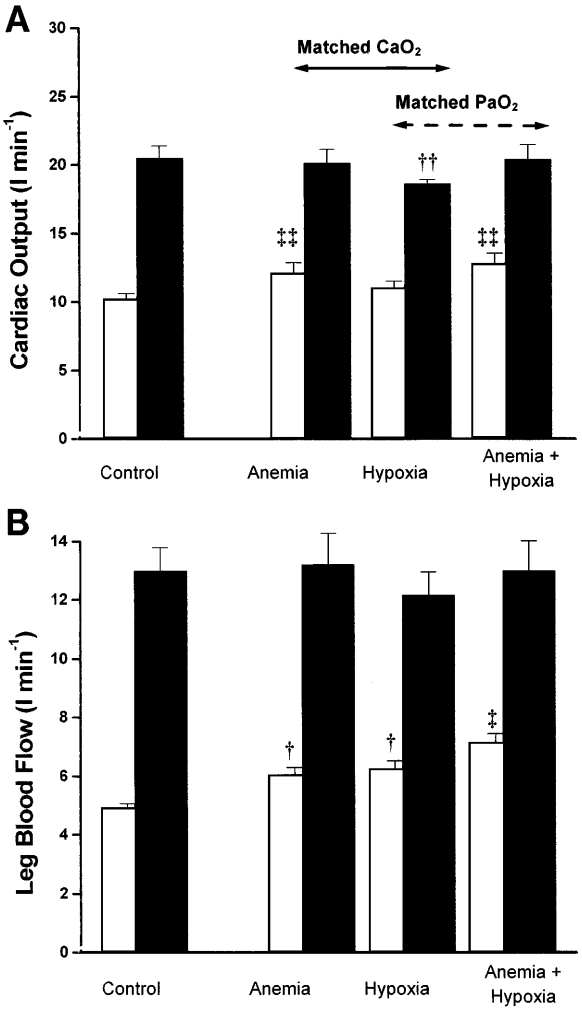

Fig. 2. *A*: at 30 W (open bars), cardiac output was slightly higher in anemia and anemia + hypoxia than control values (‡‡$P < 0.05$). At peak effort (solid bars), cardiac output was slightly lower in hypoxia compared with all conditions (††$P < 0.05$). *B*: leg blood flow (l/min) is shown in all 4 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 Ca_{O_2}.

Systemic O_2 delivery was at rest and 30 W maintained in all interventions compared with control. In contrast, at peak effort, systemic O_2 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 $\dot{V}O_2$ matched the fall in systemic O_2 delivery.

Leg responses. LBF (l/min) rose in all subjects in all conditions above control values during 30-W exercise ($P < 0.001$; Fig. 2*B*). The rise was related to the fall in Ca_{O_2} across conditions ($r = 0.99$, $P < 0.01$), not Pa_{O_2} (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 Ca_{O_2}, not Pa_{O_2} ($r = 0.78$, $P < 0.001$; Fig. 3, *A* and *C*). The increase in LBF above control in all experimental conditions was sufficient to maintain leg O_2 delivery at rest and 30 W. At peak effort, leg O_2 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). O_2 delivery to the muscle in relation to power output (or O_2 consumed) appears nearly constant (1, 13), a relationship that was unchanged by hypoxia or anemia. The 38% lower leg O_2 delivery observed at peak effort in anemia + hypoxia compared with control accounted for 92% of the 0.68 l/min decrement in leg $\dot{V}O_2$ from control to anemia + hypoxia. Leg $\dot{V}O_2$ was similar in all conditions at rest and 30 W, and at peak effort when Ca_{O_2} was matched between hypoxia and anemia (Table 1). The relationship of leg $\dot{V}O_2$ to power output from rest through peak exercise in anemia + hypoxia was 13.2 ml $O_2 \cdot min^{-1} \cdot W^{-1}$, a value similar to previous reports from subjects breathing normoxic air and having normal [Hb] (12) (see Fig. 3*D* in Ref. 9 and Fig. 3*A* in Ref. 10). Leg O_2 extraction was at rest 52% and similar among conditions. At 30 W, leg O_2 extraction rose in anemia + hypoxia to 73 ± 2%, a value slightly higher than observed in any other condition ($P < 0.001$; Fig. 4*A*). At peak effort, leg O_2 extraction in hypoxia reached 79 ± 2% (Fig. 4*A*). The femoral arteriovenous O_2 difference

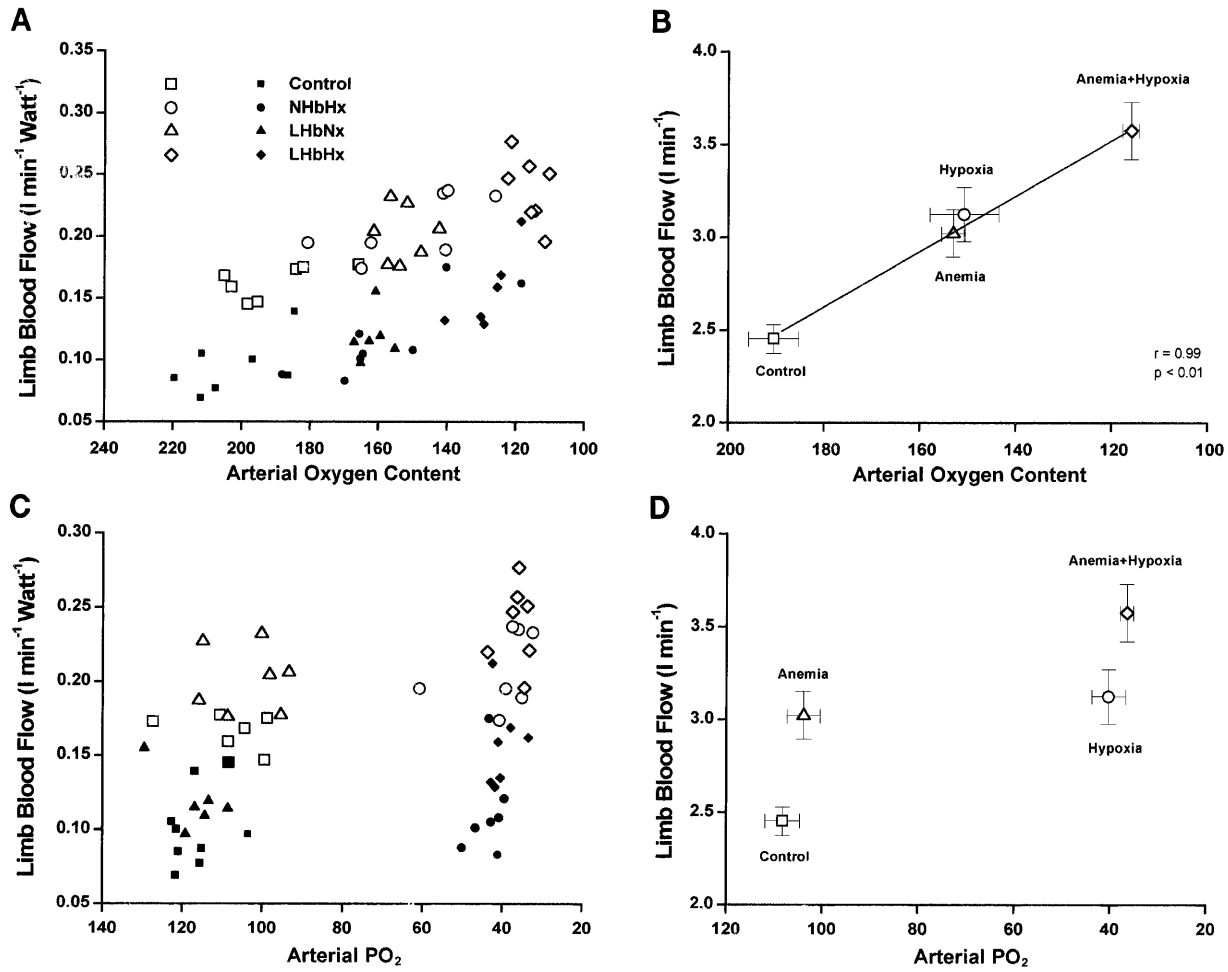

Fig. 3. Individual data for limb blood flow ($l \cdot min^{-1} \cdot W^{-1}$) responses to varied Ca_{O_2} for 30 W and peak effort (*A*). Each condition has a large symbol for 30 W and a small symbol for peak effort. In *B*, rise in limb blood flow (l/min) with falling Ca_{O_2} is shown at 1 work load (30 W). In *C*, leg blood flow (l/min)-to-P_{O_2} relationship is shown for 30 W and peak effort. In *D*, lack of relationship of limb blood flow to Pa_{O_2} at 30 W is shown.

was, as expected, similar when Ca_{O_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. 4*B*). In anemia + hypoxia at rest, femoral arteriovenous O_2 difference was 71 ± 4 ml/l, increasing at 30 W to 84 ± 2 ml/l and reaching 98 ± 3 ml/l at peak effort ($P < 0.001$ vs. all conditions from rest to peak effort; Fig. 4*B*). Estimated leg D_{O_2} at peak effort reached 23.5 ± 2.2 and 25 ± 2.6 $ml \cdot min^{-1} \cdot Torr^{-1}$ in control and hypoxia, respectively, and dropped as [Hb] fell in anemia and anemia + hypoxia to 19.8 ± 2.1 and 21 ± 2.5 $ml \cdot min^{-1} \cdot Torr^{-1}$ ($P < 0.05$ for both conditions compared with control and hypoxia), respectively. At peak effort, estimated MTT was 529 ± 31 ms in control, a value that rose to 565 ± 29 ms ($P < 0.05$) in hypoxia, and was similar to values seen in anemia (525 ± 28 ms) and anemia + hypoxia (533 ± 31 ms).

Catecholamine responses. At peak effort, arterial NE reached 8.4 ± 1.1 nM in anemia + hypoxia, a value lower than in control (10.3 ± 1.0 nM; $P < 0.05$), but similar to hypoxia (9.3 ± 0.9 nM) or anemia (9.2 ± 1.7 nM). NE spillover was elevated above baseline only at peak effort, reaching 4.6 ± 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 ± 0.2 nM at 30 W and 2.5 ± 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 ± 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

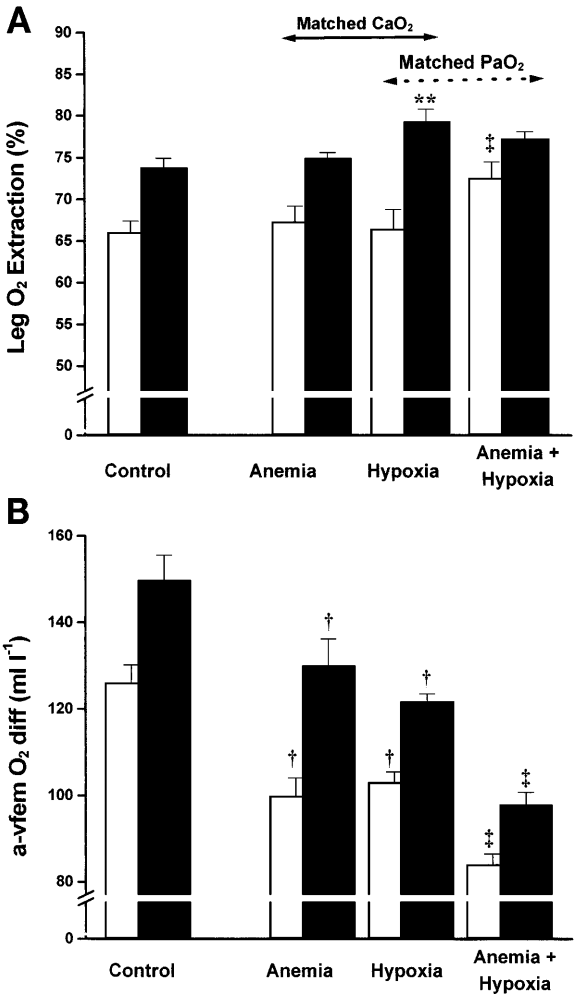

Fig. 4. Leg O_2 extraction (*A*) was higher at 30 W in anemia + hypoxia compared with all other conditions (‡$P < 0.01$). At peak effort (solid bars), leg O_2 extraction in hypoxia rose above control and anemia (**$P < 0.05$). In *B* drop from control in femoral arteriovenous O_2 difference (a-vfem O_2 diff; in ml/l) with exercise in all experimental conditions (†$P < 0.01$ vs. control; ‡$P < 0.01$ vs. all conditions) is shown. At peak effort (solid bars), femoral arteriovenous O_2 difference (ml/l) in anemia + hypoxia reaches values near those observed when exercising at only 30 W (open bars) in anemia or hypoxia.

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 Ca_{O_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 Pa_{O_2} seem limited during exercise to carotid body-linked stimulation of pulmonary ventilation. Evidence that a low Pa_{O_2} alone does not cause vasodilation comes from the observation of similar limb blood flows in the two conditions with nearly identical Ca_{O_2} but widely different Pa_{O_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 $\times$ Ca_{O_2}).

The apparent difference between Ca_{O_2} and Pa_{O_2} in the regulation of the LBF response to hypoxemia suggests that factors related to [Hb] play a role in the vasodilatation. Recently, such an important role has been proposed 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 Ca_{O_2}, but less so when only Pa_{O_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 an anemia- or hypoxia-mediated [K^+] release playing a dominant role in the regulation of vasodilatation was the similarity in all conditions in [K^+] response both in arterial and femoral venous blood as well as release from active muscles.

Low [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 effort and HR in the range of 150–160 beats/min (9, 10). Moreover, at peak effort, $\dot{Q}_{TOT}$ reached 18–20 l/min as compared with $\dot{Q}_{TOT}$ in ordinary cycle exercise of ~26 l/min. Thus, despite substantial reserve capacity, $\dot{Q}_{TOT}$ rose no further. In this connection, the comparison with the classic study of Sproule et al. (18) is worth mentioning. $\dot{Q}_{TOT}$ was up to 23 l/min during maximal treadmill exercise. This, however, resulted in only 1.8 l/min $\dot{V}O_2$ 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 $\dot{Q}_{TOT}$ at peak effort when Ca_{O_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 $\dot{Q}_{TOT}$ 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 $\dot{Q}_{TOT}$ with low Ca_{O_2} (11), although $\dot{Q}_{TOT}$ was not studied when Ca_{O_2} was held constant and viscosity altered. Matching Ca_{O_2} in the present study between hypoxia and anemia

allows comparison of the effects of viscosity and Ca_{O_2} and reveals a close coupling of blood flow to Ca_{O_2}, 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 Ca_{O_2}, Pa_{O_2}, and hematocrit in sheep. As Ca_{O_2} fell, there was a reciprocal rise in cerebral blood flow, independent of Pa_{O_2} 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 Ca_{O_2} to regulation of blood flow to exercising skeletal muscle as observed in the present study also may be a widespread mechanism of O_2 sensing in other vascular beds.

A remarkable finding in the present study is that the subjects could tolerate the very marked acute drop in Ca_{O_2} to 126.6 ± 2.9 ml/l and Pa_{O_2} to 40.0 ± 1.2 Torr that occurred in the anemia + hypoxia intervention. Other situations exist with such low Ca_{O_2} and Pa_{O_2} 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 O_2 delivery. O_2 extraction, expressed as a percentage of arterially transported O_2, is enlarged in both conditions as a result of a right shift in the O_2 dissociation curve. In acute anemia or hypoxia (or the combination of the two, see Fig. 4*A*), blood flow is up both on the systemic and regional level, but the systemic O_2 extraction is some 10% lower than in chronic exposure (~65 vs. 70–80%). Of note is, however, that these high O_2 extractions occur at much lower power outputs.

Recently, Ferretti et al. (5) have further developed the idea that Ca_{O_2} and blood flow are regulated to maintain a fixed femoral Cv_{O_2} or mixed venous Cv_{O_2} at a given exercise level. In the present study, with cardiac output measured by dye dilution and direct measurement of femoral Cv_{O_2}, we were not able to verify a constancy of $\dot{Q}_{TOT} \times Cv_{O_2}$. The explanation for this discrepancy from the report of Ferretti et al. (5) is likely that the slope of $\dot{V}O_2 \times \dot{Q}_{TOT}(Ca_{O_2})$ is <1, which violates a key assumption in their theoretical analysis. Furthermore, the lower slope of the $\dot{V}O_2 \times \dot{Q}_{TOT}(Ca_{O_2})$ relationship (values ranged from 0.6 to 0.8) is explained to a large degree by the much wider range in [Hb] and FI_{O_2} in the present study compared with the conditions studied by Ferretti et al. (5).

A large amount of O_2 was left in the femoral vein in all interventions also at peak effort. In the present study, hypoxia resulted at peak effort in femoral Cv_{O_2} of 28.8 ± 1.0 and 33.4 ± 2.1 ml/l in anemia + hypoxia and hypoxia, respectively. A too short transit time for full O_2 off-loading could be one explanation for the higher femoral Pv_{O_2} or Cv_{O_2}. This was not the case, since MTT shows no relationship to femoral Pv_{O_2} or Cv_{O_2}. Thus factors affecting off-loading of O_2, 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 O_2 dissociation curve to favor an increase in end-capillary off-loading of O_2. This may have been the case in the present study because a linear drop was noted in femoral Pv_{O_2} 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 Pa_{CO_2}. Furthermore, O_2 conductance into the muscle cell estimated by numerical integration (17, 22) shows a close coupling of the predicted D_{O_2} values and the resulting femoral venous P_{O_2}, but not the femoral Cv_{O_2}. Combining pH and D_{O_2} into a regression equation to predict femoral venous P_{O_2} reveals that ~60% of the variance in femoral Pv_{O_2} was accounted for by pH and D_{O_2} ($R^2 = 0.59$, $F = 20.3$, $P < 0.0001$).

In conclusion, cardiac output and LBF rise as Ca_{O_2} falls, suggesting that O_2 delivery, rather than the regulation of capillary P_{O_2}, is the main regulatory goal of the vasodilatation. The mechanisms necessary to adjust blood flow according to local demands for O_2 delivery are likely situated in the tissue. Several compounds await further investigation as likely regulators of blood flow according to changes in Ca_{O_2} or [Hb]. These include red blood cell ATP release (4), arachidonic acid metabolites released in response to changes in O_2 levels (7), and a [Hb] specific effect on scavenging of nitric oxide to effect vasodilatation in the face of lowered [Hb] (19).

Thanks are due to the subjects for their enthusiastic participation and to Dr. Peter Wagner for performing the numerical integration calculations and providing the estimates of D_{O_2}.

This study was made possible by Danish National Research Foundation Grant 501 14.

Present addresses: M. D. Koskolou, Dept. of Physical Education and Sport Science, Univ. of Athens, Athens, Greece; and J. A. L. Calbet, Dept. of Physical Education, Univ. of Las Palmas de Gran Canaria, Canary Islands, Spain.

Address for reprint requests: R. Roach, Life Sciences, New Mexico Highlands University, Las Vegas, NM 87701.

Received 22 January 1998; accepted in final form 16 September 1998.

REFERENCES

1. **Andersen, P., and B. Saltin.** Maximal perfusion of skeletal muscle in man. *J. Physiol. (Lond.)* 366: 233–249, 1985.
2. **Åstrand, P. O., T. E. Cuddy, B. Saltin, and J. Stenberg.** Cardiac output during submaximal and maximal work. *J. Appl. Physiol.* 19: 268–274, 1964.
3. **Celsing, F., J. Svendenhag, P. Pihlstedt, and B. Ekblom.** Effects of anemia and stepwise induced polycythemia on maximal aerobic power in individuals with high and low hemoglobin concentrations. *Acta Physiol. Scand.* 129: 46–54, 1987.
4. **Ellsworth, M. L., T. Forrester, C. G. Ellis, and H. H. Dietrich.** The erythrocyte as a regulator of vascular tone. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H2155–H2161, 1995.
5. **Ferretti, G., B. Kayser, F. Schena, D. L. Turner, and H. Hoppeler.** Regulation of perfusive O_2 transport during exercise in humans: effects of changes in haemoglobin concentration. *J. Physiol. (Lond.)* 455: 679–688, 1992.
6. **Hallman, H., L. O. Farnebo, B. Hamberger, and G. Jonsson.** A sensitive method for the determination of plasma catecholamines using liquid chromatography with electrochemical detection. *Life Sci.* 23: 1049–1055, 1978.
7. **Harder, D. R., J. Narayanan, E. K. Birks, J. F. Liard, J. D. Imig, J. H. Lombard, A. R. Lange, and R. J. Roman.** Identification of a putative microvascular oxygen sensor. *Circ. Res.* 79: 54–61, 1996.

8. **Jones, M. D., R. J. Traystman, M. A. Simmons, and R. A. Molteni.** Effects of changes in arterial O_2 content on cerebral blood flow in the lamb. *Am. J. Physiol.* 240 (*Heart Circ. Physiol.* 9): H209–H215, 1981.
9. **Koskolou, M., J. A. L. Calbet, G. Rådegran, and R. C. Roach.** Hypoxia and the cardiovascular response to dynamic knee-extensor exercise. *Am. J. Physiol.* 272 (*Heart Circ. Physiol.* 41): H2655–H2663, 1997.
10. **Koskolou, M., R. C. Roach, J. A. L. Calbet, G. Rådegran, and B. Saltin.** Cardiovascular responses to dynamic exercise with acute anemia in humans. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H1787–H1793, 1997.
11. **Murray, J. F., and E. Escobar.** Circulatory effect of blood viscosity: comparison of methemoglobinemia and anemia. *J. Appl. Physiol.* 25: 594–599, 1968.
12. **Richardson, R. S., D. S. Knight, D. C. Poole, S. S. Kurdak, M. C. Hogan, B. Grassi, and P. D. Wagner.** Determinants of maximal exercise $\dot{V}O_2$ during single leg knee-extensor exercise in humans. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1453–H1461, 1995.
13. **Rowell, L. B., B. Saltin, B. Kiens, and N. J. Christensen.** Is peak quadriceps blood flow in humans even higher during exercise with hypoxemia? *Am. J. Physiol.* 251 (*Heart Circ. Physiol.* 20): H1038–H1044, 1986.
14. **Saltin, B., B. Kiens, G. Savard, and P. K. Pedersen.** Role of haemoglobin and capillarization for oxygen delivery and extraction in muscular exercise. *Acta Physiol. Scand.* 128: 21–32, 1986.
15. **Saltin, B., G. Rådegran, M. D. Koskolou, and R. C. Roach.** Skeletal muscle blood flow in humans and its regulation during exercise. *Acta Physiol. Scand.* 162: 421–436, 1998.
16. **Savard, G. K., E. A. Richter, S. Strange, B. Kiens, N. J. Christensen, and B. Saltin.** Norepinephrine spillover from skeletal muscle during exercise in humans: role of muscle mass. *Am. J. Physiol.* 257 (*Heart Circ. Physiol.* 26): H1812–H1818, 1989.
17. **Schaffartzik, W., E. D. Barton, D. C. Poole, K. Tsukimoto, M. C. Hogan, D. E. Bebout, and P. D. Wagner.** Effect of reduced haemoglobin concentration on leg oxygen uptake during maximal exercise in humans. *J. Appl. Physiol.* 75: 491–498, 1993.
18. **Sproule, B. J., J. H. Mitchell, and W. F. Miller.** Cardiopulmonary physiological responses to heavy exercise in patients with anemia. *J. Clin. Invest.* 39: 378–388, 1960.
19. **Stamler, J. S., L. Jia, J. P. Eu, T. J. McMahon, I. T. Demchenko, J. Bonaventura, K. Gernert, and C. A. Piantadosi.** Blood flow regulation by *S*-nitrosohemoglobin in the physiological oxygen gradient. *Science* 276: 2034–2037, 1997.
20. **Stenberg, J., B. Ekblom, and R. Messin.** Hemodynamic response to work at simulated altitude, 4000 m. *J. Appl. Physiol.* 21: 1589–1594, 1966.
21. **Sutton, J. R., J. T. Reeves, P. D. Wagner, B. M. Groves, A. Cymerman, M. K. Malconian, P. B. Rock, P. M. Young, S. D. Walter, and C. S. Houston.** Operation Everest II. Oxygen transport during exercise at extreme simulated altitude. *J. Appl. Physiol.* 64: 1309–1321, 1988.
22. **Wagner, P. D.** An integrated view of the determinants of maximum oxygen uptake. In: *Oxygen Transfer From Atmosphere to Tissue*, edited by N. C. Gonzalez and M. R. Fedde. New York: Plenum, 1988, p. 245–256.