J Appl Physiol
89: 29–37, 2000.

Operation Everest III: role of plasma volume expansion on $\dot{V}O_{2max}$ during prolonged high-altitude exposure

PAUL ROBACH,[1,2] MICHÈLE DÉCHAUX,[3] SÉBASTIEN JARROT,[2] JENNY VAYSSE,[4] JEAN-CHRISTOPHE SCHNEIDER,[2] NICHOLAS P. MASON,[2] JEAN-PIERRE HERRY,[1,2] BERNARD GARDETTE,[5] AND JEAN-PAUL RICHALET[2]

[1]*Ecole Nationale de Ski et d'alpinisme, 74401 Chamonix;* [2]*Association pour la Recherche en Physiologie de l'Environnement, 93017 Bobigny;* [3]*Laboratoire de Physiologie, hôpital Necker, 75015 Paris;* [4]*Laboratoire de Biochimie, hôpital Jean Verdier, 93140 Bondy; and* [5]*COMEX S.A., 13275 Marseille, France*

Received 28 December 1998; accepted in final form 17 February 2000

Robach, Paul, Michèle Déchaux, Sébastien Jarrot, Jenny Vaysse, Jean-Christophe Schneider, Nicholas P. Mason, Jean-Pierre Herry, Bernard Gardette, and Jean-Paul Richalet. Operation Everest III: role of plasma volume expansion on $\dot{V}O_{2max}$ during prolonged high-altitude exposure. *J Appl Physiol* 89: 29–37, 2000.—We hypothesize that plasma volume decrease (ΔPV) induced by high-altitude (HA) exposure and intense exercise is involved in the limitation of maximal O_2 uptake ($\dot{V}O_{2max}$) at HA. Eight male subjects were decompressed for 31 days in a hypobaric chamber to the barometric equivalent of Mt. Everest (8,848 m). Maximal exercise was performed with and without plasma volume expansion (PVX, 219–292 ml) during exercise, at sea level (SL), at HA (370 mmHg, equivalent to 6,000 m after 10–12 days) and after return to SL (RSL, 1–3 days). Plasma volume (PV) was determined at rest at SL, HA, and RSL by Evans blue dilution. PV was decreased by 26% ($P < 0.01$) at HA and was 10% higher at RSL than at SL. Exercise-induced ΔPV was reduced both by PVX and HA ($P < 0.05$). Compared with SL, $\dot{V}O_{2max}$ was decreased by 58 and 11% at HA and RSL, respectively. $\dot{V}O_{2max}$ was enhanced by PVX at HA (+9%, $P < 0.05$) but not at SL or RSL. The more PV was decreased at HA, the more $\dot{V}O_{2max}$ was improved by PVX ($P < 0.05$). At exhaustion, plasma renin and aldosterone were not modified at HA compared with SL but were higher at RSL, whereas plasma atrial natriuretic factor was lower at HA. The present results suggest that PV contributes to the limitation of $\dot{V}O_{2max}$ during acclimatization to HA. RSL-induced PVX, which may be due to increased activity of the renin-aldosterone system, could also influence the recovery of $\dot{V}O_{2max}$.

hypoxia; blood volume; plasma lactate; gas exchange

ACUTE EXPOSURE TO HYPOXIA decreases maximal O_2 uptake ($\dot{V}O_{2max}$). This phenomenon is related to several limiting factors, both central and peripheral, that impair convective and/or diffusive O_2 delivery to exercising muscles (23, 29). Acclimatization to high altitude (HA) does not improve $\dot{V}O_{2max}$ (23, 24, 27) even though the rise in red cell mass enhances blood O_2-carrying capacity. Indeed, other acclimatization-related processes such as loss of muscle mass (22) or decrease in maximal heart rate (17, 24) may alter some components of O_2 transport. Acclimatization to hypoxia also induces a decrease in plasma volume (PV) (9, 16, 19). This process could have multiple causes, including plasma protein loss (26), increase in capillary permeability (7), and dehydration or increased diuresis (6). Severe muscular work provokes a loss in PV that is due to fluid transfer from the vascular bed into the interstitium and active muscles, resulting from an increase in both muscle osmotic pressure and capillary hydrostatic pressure (13). These two mechanisms suggest that PV during maximal exercise in prolonged hypoxia could be decreased in an additive manner. However, it is not known whether such a reduction in the circulating volume associated with an elevated blood viscosity plays a significant role on maximal O_2 transport at high altitude.

The present study therefore tested the hypothesis that the decrease in PV (ΔPV) related to prolonged hypoxia and/or maximal exercise contributes to the impairment of $\dot{V}O_{2max}$ at HA. The role of PV on $\dot{V}O_{2max}$ was examined by means of plasma expansion during exercise at sea level (SL), at the simulated HA of 6,000 m, and after HA exposure. On return to sea level (RSL), $\dot{V}O_{2max}$ is known to remain depressed (3), but the underlying mechanisms are not well understood. Undocumented PV alterations after extreme altitude exposure could also influence $\dot{V}O_{2max}$ recovery. To further examine the control of PV shifts, the fluid- and sodium-regulating hormones renin, aldosterone, and atrial natriuretic factor (ANF) were also measured.

METHODS

Operation Everest III was a simulated ascent of Mt. Everest in a hypobaric chamber at COMEX S.A. in Marseille, France. Complete details of this study have been described previously, including the selection and characteristics of the

Address for reprint requests and other correspondence: P. Robach, ENSA, BP 24, 74401 Chamonix, France (E-mail: med@ensa.jeunesse-sports.fr).

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.

subjects, a description of the hypobaric chambers, the ascent profile, and the various hypotheses investigated (20).

Subjects. Eight male subjects participated in the experiment. Each subject underwent a medical examination and gave written, informed consent. The study was approved by the Ethics Committee of the University Hospital of Marseille, France. The subjects were not acclimatized to altitude before the study. Their mean ± SD age, height, and body mass were 27 ± 4 yr, 180 ± 6 cm, and 74.4 ± 6.7 kg, respectively.

Procedures. All experiments were conducted in the hypobaric chamber at COMEX S.A., Marseille, France. The ascent profile is presented in Fig. 1, as described elsewhere (20). Briefly, after a 10-day period of baseline investigations at SL (760 mmHg), the subjects were transported by helicopter to Observatoire Vallot (4,350-m altitude, 452 mmHg) where they were preacclimatized for 7 days, without performance of any scientific protocol. The subjects then descended to SL and were transported to Marseille, where decompression from 422 to 253 mmHg (8,848 m) started within 24 h and lasted 31 days. The HA studies were performed 10–12 days after the beginning of decompression during a 4-day period at 370 mmHg (6,000 m). The ambient pressure of 253 mmHg, which corresponds to the summit of Everest, was reached on *days 29–30*. RSL studies were performed on *days 1–3* after the end of HA exposure. Pure O_2 was breathed by the investigators, using a sealed helmet system, for a few minutes before decompression and during hypobaria to facilitate denitrogenation and to avoid nitrogen bubble formation. Expired gas was evacuated by a vacuum pump so that O_2 concentration in the chamber remained constant at 21%. During studies, temperature and hygrometry in the chamber were controlled between 18 and 24°C and 30 and 60%, respectively (20).

Progressive maximal exercise was performed on a cycle ergometer (Monark 864). After a 4-min warmup at 60 W at SL and RSL and at 45 W at HA, power output was increased by 30 W every 2 min (at HA, 15 W for the first 2 min, then 30 W every 2 min) until the subjects could not keep up the fixed pedaling rate (60 rpm). Verbal encouragement was given throughout the test. The incremental test was repeated at each of the three altitudes, without (Ctl) and with plasma volume expansion (PVX). Before exercise, an 18-gauge polyethylene catheter was inserted into an antecubital vein of one arm in Ctl for blood samples and in both arms in PVX for PV measurement at rest and for fluid infusion and blood samples during exercise. With this procedure, neither the subjects nor investigators were blinded to the experimental condition (Ctl or PVX). However, subjects were not aware of the main objective of the study, (i.e., the role of PVX in aerobic performance), to minimize any placebo effect. For each altitude, the time interval between Ctl and PVX experiments was 48 h. The order between Ctl and PVX was randomly assigned among the subjects and remained the same in SL, HA, and RSL.

Experiments with PVX. To minimize exercise-induced ΔPV, a plasma expander (Hesteril 6%, 6% hydroxyethyl starch, Fresenius) was infused in an antecubital vein from the beginning of exercise until cessation. A special recommendation from the ethical committee was to restrict the infused volume to 300 ml for all subjects. According to the mean body mass of our subjects, this limitation corresponded to a volume of ~4 ml/kg. Our objective was to infuse this volume of plasma expander over the entire exercise period. The duration of incremental exercise was therefore determined individually during a preliminary testing session, and infusion flow was adjusted for each subject using a multiple electric-syringe driver (Infusion Station; Vial Medical/Fresenius). This system provided a constant infusion rate that was not altered by the increase in arm venous pressure related to arm contractions during cycling exercise. The individual infusion flow, determined at SL, remained the same at HA and RSL. The volume of plasma expander infused during exercise was 292 ± 24 ml at SL, 219 ± 22 ml at HA ($P < 0.05$ vs. SL), and 290 ± 38 ml at RSL.

Gas exchange. Gas exchange was measured breath by breath at rest and during incremental exercise by using an integrated computer system (CPX/D cardiopulmonary exercise system; Medical Graphics, Minneapolis, MN). Minute ventilation was measured by a symmetrically disposed Pitot tube flowmeter. O_2 concentration was measured by a galvanic fuel cell and CO_2 by an infrared analyzer. The characteristics of this device have been described previously (21). The gas exchange analyzer, located in the hypobaric chamber during all studies, was modified to ensure a correct measurement of gas exchange in hypobaria. Hypobaric hypoxia was associated with an increase in the expired fraction of CO_2 (but a decrease in the partial pressure of CO_2) that exceeded the normal calibration range for the CO_2 analyzer. The CO_2 gain was therefore amplified, and the original CPX/D software was modified. Preliminary tests using a gas exchange simulator (10) showed that the measurements of O_2 uptake ($\dot{V}O_2$) taken at rest and during exercise ($\dot{V}O_2$ ~2.7 l/min) with the modified gas analyzer were reliable within 300–760

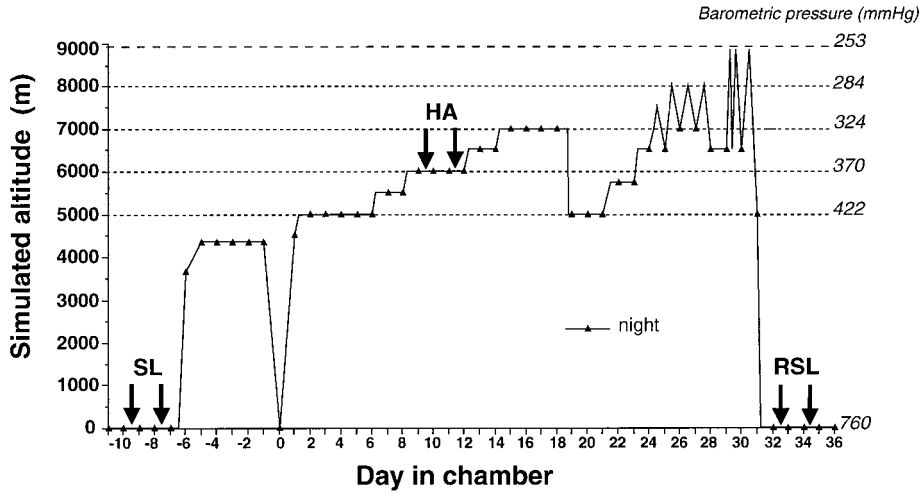

Fig. 1. Simulated ascent profile of Operation Everest III. Studies on plasma volume expansion (PVX) during maximal exercise were repeated twice (control and PVX experiments, separated by 48 h) at sea level (SL), at simulated high altitude (HA, 6,000 m), and on return to sea level (RSL). Arrows indicate days of studies.

mmHg. $\dot{V}O_{2max}$ corresponded to the highest value of $\dot{V}O_2$ averaged over a 30-s time interval. Heart rate was measured continuously, as was as arterial O_2 saturation, by a pulse oximeter (Biox II, Ohmeda).

Blood analyses. Resting plasma volume was determined by Evans blue dilution (T-1824). After a 30-min resting period in the sitting position, 5 ml of T-1824 were injected into one arm. Blood was sampled from the opposite arm at 15-, 20-, and 25-min postinjection (19). Resting PV (PV_{rest}) was determined before the infusion of hydroxyethyl starch, three times in each subject, at SL, HA, and RSL. Hemoglobin concentration ([Hb]) was determined by spectrophotometry (CO oximeter, model 270, Ciba Corning) and the hematocrit (Hct) by micromethod, at rest and every 4 min, from the end of the 120-W exercise period until exhaustion. Resting blood volume (BV) and red cell volume (RCV) were calculated using the formulas BV = PV/(1 − Hct) and RCV = BV − PV, respectively, and the appropriate correction for trapped plasma and peripheral blood sampling (8) was also used. The relative exercise-induced ΔPV (%) was calculated from [Hb] and Hct with the following formula [Hct not corrected for F_{cell} ratio (overall hematocrit/peripheral hematocrit)] (8)

$$\Delta PV\% = [([Hb]_{pre}/[Hb]_{post}) \times (100 - Hct_{post})/(100 - Hct_{pre}) - 1] \times 100 \quad (1)$$

The absolute exercise-induced ΔPV (ml) was calculated as

$$\Delta PV = \Delta PV\% \times PV_{rest}/100 \quad (2)$$

and PV during maximal exercise (PV_{atmax}) was calculated as

$$PV_{atmax} = PV_{rest} - \Delta PV \quad (3)$$

Forearm venous blood samples were collected at rest and at cessation of exercise and were immediately centrifuged. The separated plasma was immediately stored at −80°C for further analysis. Plasma protein concentration was determined by end-point colorimetry. Plasma albumin concentration ([Alb]) was measured by immunonephelometry, and concentration of lactate was determined after perchloric deproteinization by the end-point enzymatic ultraviolet method (Cobas Fara-Roche).

Plasma renin ([Ren]) and aldosterone ([Aldo]) concentrations were measured with an immunoradiometric assay (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) and a radioimmunoassay (Diagnostic Products, Los Angeles, CA), respectively. Plasma ANF concentration ([ANF]) was measured with a radioimmunoassay following an extraction step. Briefly, blood was collected on a tube containing EDTA and trasylol (a protease inhibitor), and plasma was separated and stored at −80°C until assay. One milliliter of plasma was then acidified with 3 ml of a 4% acetic acid solution, followed by deposition of 4 ml of acidified plasma on a C_{18} Sep Pak column (Waters Millford) that had been previously activated with methanol (5 ml). The column was rinsed with 5 ml of distilled water. Elution was performed with 3 ml of the following solution: 60% acetonitril and 0.1% trifluoroacetic acid, in distilled water. Eluate was dried under nitrogen and assayed afterward with a radioimmunoassay kit (Amersham International).

Statistics. Data are presented as means ± SD. A one-way ANOVA with repeated measures was used to compare the effect of altitude on resting parameters. A two-way ANOVA with repeated measures was performed to analyze *1*) the effects of altitude and exercise in the control condition and *2*) the effects of altitude and infusion during maximal exercise. A Dunnett's test was used for multiple comparisons. Relationships between two variables were evaluated by linear regression. Differences were considered significant at $P < 0.05$.

RESULTS

Simulated HA effects. Body mass decreased from 74.1 ± 6.5 kg at SL to 71.5 ± 6.1 kg ($P < 0.01$) at HA and to 71.2 ± 6.0 kg ($P < 0.01$) after 31 days of hypobaric exposure. PV_{rest} decreased 26% from 3.68 ± 0.51 liters at SL to 2.73 ± 0.63 liters at HA ($P < 0.01$). After HA exposure, PV_{rest} tended to be higher than at SL (4.06 ± 1.01 liters; +10%, $P = 0.15$). Individual PV values show that, of eight subjects, seven experienced PVX after hypobaric exposure (Fig. 2*A*). Resting RCV was not significantly increased between SL and HA (2.52 ± 0.43 vs. 2.57 ± 0.60 liters) but was higher at

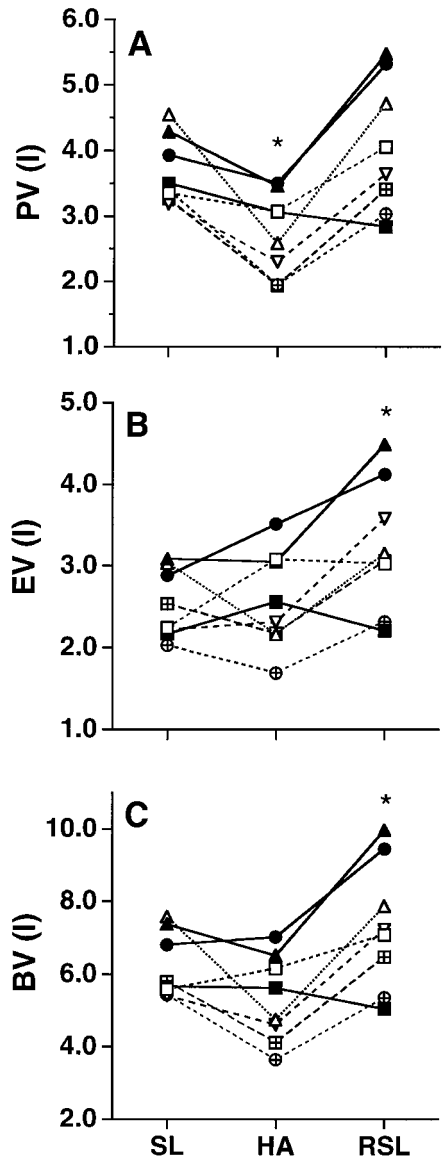

Fig. 2. Individual variations ($n = 8$) in plasma volume (PV; *A*), erythrocyte volume (EV; *B*), and blood volume (BV; *C*) between SL, HA, and RSL. Intravascular volumes were measured at rest in a sitting position, after a 30-min rest period in this position. *$P < 0.05$ HA or RSL vs. SL.

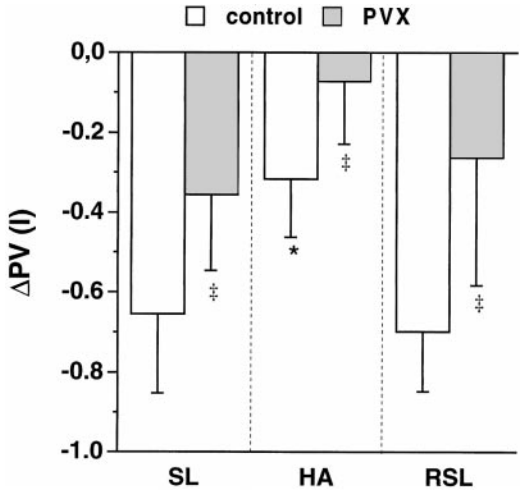

Fig. 3. Decrease in plasma volume (ΔPV) induced by maximal exercise without (control) or with PVX, at SL, HA, and RSL. Values are means ± SD ($n = 8$). *$P < 0.05$ HA vs. SL; ‡$P < 0.05$ PVX vs. control.

RSL (3.24 ± 0.80 liters; Fig. 2*B*). BV was not significantly decreased ($P = 0.06$) at HA compared with SL (5.30 ± 1.21 vs. 6.20 ± 0.90 liters) but was higher at RSL (7.31 ± 1.76 liters; Fig. 2*C*).

ΔPV induced by maximal exercise was similar before and after hypobaric exposure but reduced at HA (Fig. 3). For a given absolute workload that corresponded to the maximal workload at HA, ΔPV was the same in the three altitude conditions, with values of −310 ± 18, −317 ± 15, and −337 ± 22 ml at SL, HA, and RSL, respectively.

During maximal exercise at HA, heart rate was 22% lower than at SL, whereas pulmonary ventilation did not change (Table 1). $\dot{V}O_{2max}$, expressed in liters per minute, was lower at HA than at SL, and $\dot{V}O_{2max}$ recovery remained incomplete (−14%) 1–3 days after the 31-day decompression (Table 1). $\dot{V}O_{2max}$, expressed per kilogram of body mass, decreased 58% between SL and HA and remained 11% lower ($P = 0.1$) than the original SL value after hypobaric exposure (Fig. 4). The discrepancy between both percentages for $\dot{V}O_{2max}$ recovery (14 vs. 11%) was related to the 3.9% decrease in body mass between SL and RSL studies.

Hct and [Hb] were increased by altitude exposure at rest and at $\dot{V}O_{2max}$, whereas plasma protein concentration was raised at rest only. Plasma [Alb] did not change at HA (Table 2).

Hypobaric hypoxia altered neither mean plasma [Ren] nor [Aldo] at rest or at $\dot{V}O_{2max}$ (Fig. 5). Conversely, the exercise-induced increase in plasma [ANF] was blunted at HA (Fig. 5). After altitude exposure, resting [Ren] and [Aldo] tended to be higher than at SL, and both [Ren] and [Aldo] responses to exercise were exaggerated.

Finally, the decrease in $\dot{V}O_{2max}$ at HA was related to the concomitant decline in PV_{rest} ($\%\dot{V}O_{2max} = 0.497 \times \%PV_{rest} - 48.267$; $r = 0.72$, $P < 0.05$) but not in PV at maximal exercise (Fig. 6*A*). $\dot{V}O_{2max}$ decrease at HA was also related to the concomitant decline in resting BV (BV_{rest}; $\%\dot{V}O_{2max} = 0.257 \times \%BV_{rest} - 54.625$; $r = 0.73$; $P < 0.05$) and in BV at maximal exercise (Fig. 6*B*). Furthermore, $\dot{V}O_{2max}$ recovery at RSL was related to the concomitant expansion *1*) of PV_{rest} ($\%\dot{V}O_{2max} = 0.614 \times PV_{rest} - 16.113$; $r = 0.74$, $P < 0.05$) and of PV at maximal exercise (Fig. 6*C*), and *2*) of BV_{rest} ($\%\dot{V}O_{2max} = 0.632 \times \%BV_{rest} - 20.628$; $r = 0.74$; $P < 0.05$) and of BV at maximal exercise (Fig. 6*D*).

Effects of PVX. At each altitude, PVX significantly attenuated exercise-induced ΔPV (Fig. 3). PVX did not influence any of the cardiopulmonary variables except $\dot{V}O_{2max}$ at HA, which increased 9% ($P < 0.05$; Table 1). When expressed per kilogram of body mass, $\dot{V}O_{2max}$ was also enhanced 9% by PVX at HA (Fig. 4). Conversely, PVX had no effect on $\dot{V}O_{2max}$ at SL or RSL. PVX did not alter heart rate at maximal exercise or at 50% of $\dot{V}O_{2max}$ (Table 1).

Table 1. *Cardiopulmonary data during maximal exercise, before, during, and after HA exposure, and without and with plasma volume expansion*

	Sea Level		6,000 m		Return to Sea Level	
	Control	PVX	Control	PVX	Control	PVX
HR, beats/min						
R	68 ± 11	68 ± 7	96 ± 8*	98 ± 9†	74 ± 8	73 ± 12
$E_{50\%}$	133 ± 9*	135 ± 11*	130 ± 10*	129 ± 10*	137 ± 8*	138 ± 9*
E	191 ± 5*	192 ± 8*	150 ± 15*†	149 ± 16*†	191 ± 10*	187 ± 6*
$\dot{V}E$, l/min						
R	11 ± 2	12 ± 4	21 ± 5†	23 ± 4†	13 ± 2	14 ± 3
E	149 ± 24*	160 ± 12*	145 ± 19*	149 ± 13*	157 ± 15*	157 ± 18*
$\dot{V}O_2$, l/min						
R	390 ± 52	371 ± 48	354 ± 60	380 ± 62	360 ± 78	357 ± 76
E	4,210 ± 577*	4,201 ± 514*	1,684 ± 248*†	1,839 ± 258*†‡	3,612 ± 538*†	3,623 ± 374*†
Sa_{O_2}, %						
R	98 ± 0.5	98 ± 0.0	85 ± 2.3†	85 ± 2.2†	98 ± 0.5	99 ± 0.8
E	97 ± 1.0	97 ± 0.5	72 ± 3.0*†	71 ± 3.8*†	97 ± 1.2	97 ± 1.1

Values are means ± SD. Heart rate (HR), ventilation ($\dot{V}E$), O_2 uptake ($\dot{V}O_2$), and arterial oxygen saturation (Sa_{O_2}) were measured at rest (R), at 50% of maximal O_2 uptake ($E_{50\%}$) for HR, and at the cessation of maximal exercise (E) at sea level (SL), at the simulated high altitude of 6,000 m (HA), and on return to sea level (RSL), without (control) and with plasma volume expansion (PVX) during exercise. *$P < 0.05$ exercise vs. rest; †$P < 0.05$ HA or RSL vs. SL; ‡$P < 0.05$ PVX vs. control.

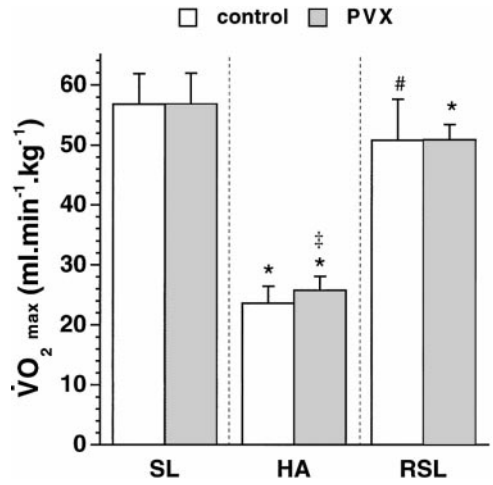

Fig. 4. Maximal oxygen uptake ($\dot{V}O_{2max}$) in control and PVX groups at SL, HA, and RSL. Values are means ± SD ($n = 8$). *$P < 0.05$ HA or RSL vs. SL; #$P = 0.1$ RSL vs. SL; ‡$P < 0.05$ PVX vs. control.

[Ren] response to exercise was reduced, and [Aldo] response tended to be reduced by PVX at RSL only; however, [ANF] response to exercise was increased with PVX at SL and HA (Fig. 5). Finally, we found that the more PV_{rest} decreased with altitude exposure, the more $\dot{V}O_{2max}$ was improved by PVX at HA ($\%\dot{V}O_{2max} = -1.157 \times \%PV_{rest} - 13.263$; $r = 0.89$, $P < 0.005$). The relationship between BV_{rest} and $\dot{V}O_{2max}$ was also significant ($\%\dot{V}O_{2max} = -0.613 \times \%BV_{rest} + 1.323$; $r = 0.93$; $P < 0.001$). The increase in $\dot{V}O_{2max}$ with PVX at HA was also related to the altitude-induced decrement in PV or BV during maximal exercise (Fig. 7).

DISCUSSION

The main finding of the present study was that acute PVX during incremental exercise to exhaustion slightly improved $\dot{V}O_{2max}$ at HA in acclimatized subjects. Conversely, plasma expander infusion at SL, before and after a 31-day gradual decompression up to 253 mmHg, did not alter $\dot{V}O_{2max}$. This study also provided insight on the recovery period after extreme altitude exposure. Our results indicate that PV and BV expansion occur during RSL because PV and BV were 0.7 and 1.1 liters higher, respectively, after RSL compared with at SL. At RSL, $\dot{V}O_{2max}$ did not return completely to SL values. [Ren] and [Aldo] responses to exercise were exaggerated at RSL, the only time they were affected by PVX.

HA exposure. In the present study, exposure to the simulated altitude of 6,000 m (after an 18-day gradual decompression starting at 4,350 m) provoked a 58% decrease in $\dot{V}O_{2max}$, which was higher than decreases previously reported for similar altitudes by West et al. (30) and during Operation Everest II by Cymerman et al. (3) (50 and 47%, respectively). However, the magnitude of the differences observed between the present and previous studies (58 vs. 47–50%) may be due to the large range in individual responses, which could be equal to or greater than the differences between these means.

Despite the presence of a strong hypoxic stimulus at HA and the fall in inspired gas density, which reduces the work of breathing, ventilation at maximal exercise was not found to be increased between SL and HA. However, this lack of increase in maximal ventilation is consistent with the findings of Operation Everest II at an almost identical altitude (3).

The beneficial effect of PVX on $\dot{V}O_{2max}$ demonstrated during acclimatization to severe hypoxia, but not at SL, raises the question of the involvement of PV shifts in the limitation of maximal O_2 transport at HA. At SL, after acute PVX, $\dot{V}O_{2max}$ was found to be either enhanced (2) or unchanged (11, 12, 14). In the present basal condition, infusion during exercise did not improve maximal O_2 transport, suggesting that, if resting PV is within the normal range, the magnitude of ΔPV

Table 2. *Plasma protein, albumin, and lactate concentrations during maximal exercise, before, during, and after HA exposure, with and without PVX*

	Sea Level		6,000 m		Return to Sea Level	
	Control	PVX	Control	PVX	Control	PVX
Hct, %						
R	46.1 ± 3.0	45.3 ± 2.6	54.0 ± 3.0†	53.2 ± 3.0†	48.8 ± 4.1†	48.8 ± 3.2†
E	50.9 ± 3.0*	47.9 ± 2.9*‡	56.6 ± 2.3*†	54.0 ± 2.9†‡	53.5 ± 3.8*†	50.4 ± 2.9†‡
[Hb], g/dl						
R	14.9 ± 1.5	14.8 ± 0.6	17.1 ± 1.3†	16.8 ± 0.9†	15.5 ± 1.5	15.3 ± 1.1
E	16.8 ± 1.5*	15.3 ± 0.4*‡	17.9 ± 1.0*†	17.1 ± 1.1†‡	17.0 ± 1.5*	15.7 ± 1.0‡
[Protein], g/l						
R	73.8 ± 3.9	74.6 ± 2.9	78.0 ± 4.3†	76.4 ± 2.9	70.5 ± 3.5†	69.9 ± 4.6†
E	85.3 ± 6.8*	78.6 ± 4.8*‡	84.3 ± 2.3*	78.4 ± 2.1‡	80.1 ± 4.8*	73.8 ± 4.1*†‡
[Alb], g/l						
R	47.1 ± 3.0	46.3 ± 2.7	47.6 ± 3.6	47.5 ± 2.4	44.2 ± 3.9†	44.1 ± 2.1
E	53.8 ± 4.2*	49.4 ± 3.6*‡	51.4 ± 3.7*	47.2 ± 2.7‡	49.6 ± 4.1*†	47.1 ± 4.3*
[Lac^-], mmol/l						
R	1.7 ± 0.6	1.8 ± 0.3	2.0 ± 0.4	2.1 ± 0.4	1.8 ± 0.5	1.8 ± 0.7
E	15.0 ± 2.6*	15.1 ± 3.5*	6.0 ± 1.0*†	6.9 ± 2.2*†	12.6 ± 2.2*†	12.3 ± 2.6*†

Values are means ± SD. Venous hematocrit (Hct), hemoglobin concentration ([Hb]), and venous plasma concentrations of proteins ([protein]), albumin ([Alb]), and lactate ([Lac^-]) were measured at rest and at the cessation of maximal exercise at SL, HA, and RSL, with and without PVX. *$P < 0.05$ exercise vs. rest; †$P < 0.05$ HA or RSL vs. SL; ‡$P < 0.05$ PVX vs. control.

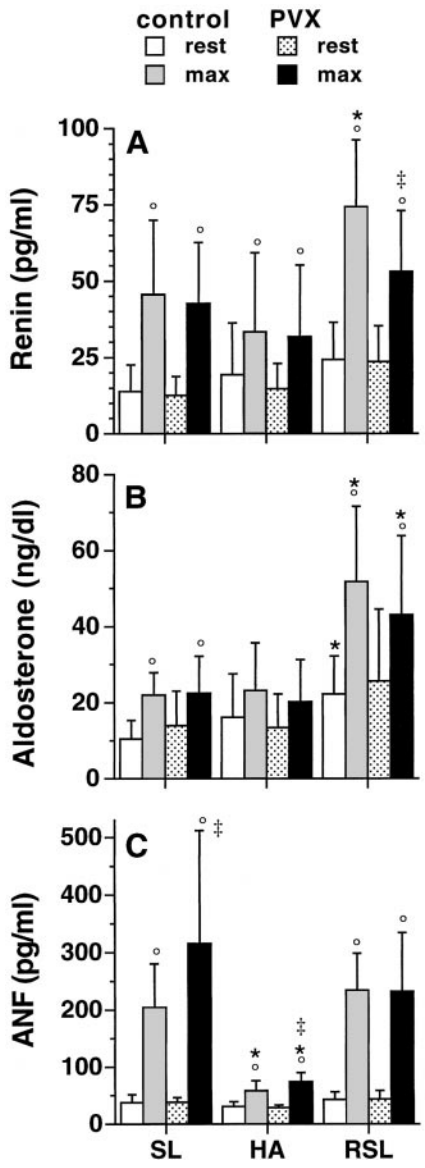

Fig. 5. Plasma concentrations of renin (*A*), aldosterone (*B*), and atrial natriuretic factor (ANF; *C*) at rest and at maximal exercise in control and PVX groups at SL, HA, and RSL. Values are means ± SD ($n = 8$). °$P < 0.05$ exercise vs. rest; *$P < 0.05$ HA or RSL vs. SL; ‡$P < 0.05$ PVX vs. control.

associated with exercise would not contribute to limit $\dot{V}O_{2max}$. Prolonged exposure to HA was associated with a 26% decrease in PV_{rest}, which was in agreement with previous data obtained at similar altitudes (16). This plasma loss was unlikely to be due to dehydration and/or increased diuresis because water intake and urine output were well preserved over the first 16 days in hypobaria (Westerterp, unpublished observation). Conversely, the significant decrease in total circulating protein between SL and HA (from 273 to 213 g) may be a primary factor in PV loss, as suggested previously (26).

The insignificant increase in RCV observed in this study, from 35 ml/kg at SL to 37 ml/kg at HA, may appear surprising. Classically, acclimatization is associated with a rise in red cell mass. However, recent work using an erythrocyte-labeling method indicated that RCV was unchanged after 13 days at 4,300 m (26). Moreover, Grover et al. (5) demonstrated that RCV (determined by rebreathing carbon monoxide) was not increased during the first 2 wk at 4,300 m, i.e., most individuals showed little or no increase or decrease in RCV during this period. Indeed, a recent review has emphasized the considerable individual variability in the RCV response to altitude (5). These data therefore support the idea that red cell mass expansion may take more than 2 wk of altitude exposure, despite high levels of erythropoietin.

Finally, exercise-induced ΔPV was similar at SL and RSL but decreased at HA (Fig. 3). This lower leak of intravascular fluid at high altitude was primarily related to the reduced maximal exercise intensity in this condition, whereas the shorter exercise time was of less importance (8).

At HA, although the fall in $\dot{V}O_{2max}$ was too great to be reversed to SL values by the infusion, the 219 ml (mean value) of plasma expander improved $\dot{V}O_{2max}$ by 9%. This observation supports the hypothesis that the depressed circulating volume during maximal exercise in prolonged hypoxia may participate in the limitation of $\dot{V}O_{2max}$. Previous studies completed at lower altitudes demonstrated that $\dot{V}O_{2max}$ was not ameliorated after isovolemic hemodilution (25) or erythrocyte infusion (31). However, we are not aware of other reports investigating acute PVX during maximal exercise in similar altitude conditions.

It is of interest to discuss the respective roles of acclimatization- and exercise-induced ΔPV on $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ decrement at HA was significantly related to the concomitant decrease in PV_{rest} or BV_{rest} and to the decrease in BV at maximal exercise (Fig. 6*B*). Second, the more PV_{rest} or BV_{rest} and PV or BV at maximal exercise (Fig. 7, *A* and *B*) were depressed at high altitude the greater the effect of PVX on O_2 transport. On the other hand, exercise at HA provoked an additional decrease in PV of ~11% (317 ml), which was reduced to ~3% (72 ml) with PVX. However, the individual improvement in $\dot{V}O_{2max}$ with PVX was poorly related to the PVX-induced reduction in plasma loss during exercise (results not shown). Thus, if PV loss is a limiting factor of O_2 transport at high altitude, the mechanism could be primarily linked to the large leak of plasma (~950 ml) associated with prolonged exposure to hypoxia, whereas supplementary ΔPV occurring during exercise would be of less importance.

Because we did not measure any parameters of central or peripheral circulation, we were not able to determine which mechanism PVX used to enhance $\dot{V}O_{2max}$ at HA. One could hypothesize that, facing a depressed circulating volume, even a relatively small amount of PV expansion improved venous return, thus improving cardiac output and blood flow to active muscles. Despite a concomitant reduction in blood O_2-carrying capacity, the net result would be an amelioration in muscle O_2 delivery. Alternatively, if muscle oxygenation was impaired at HA by high blood viscosity,

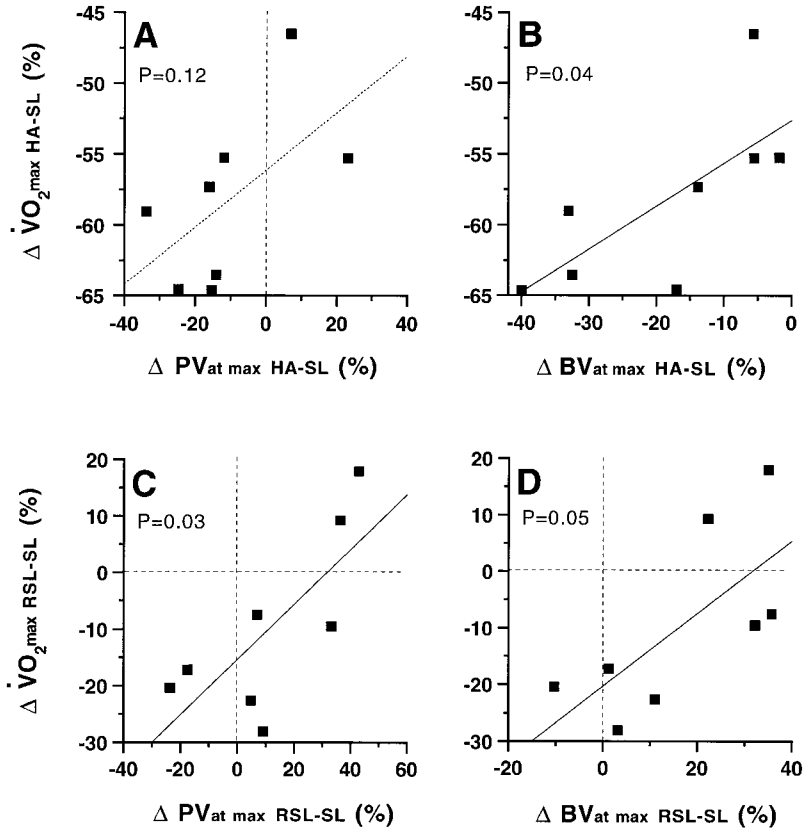

Fig. 6. Relationships between ΔPV (*A*) and blood volume decrease (ΔBV; *B*) at maximal exercise (PV_{atmax} and BV_{atmax}) and maximal oxygen uptake ($\Delta\dot{V}O_{2max}$) induced by HA exposure and relationships between ΔPV_{atmax} (*C*), ΔBV_{atmax} (*D*), and $\Delta\dot{V}O_{2max}$ at RSL compared with SL ($n = 8$). Regression equations: $y = 0.202x - 56.097$, $r = 0.58$, $P = 0.12$ (*A*); $y = 0.303x - 52.598$, $r = 0.72$, $P < 0.05$ (*B*); $y = 0.486x - 15.345$, $r = 0.74$, $P < 0.05$ (*C*); and $y = 0.643x - 20.250$, $r = 0.70$, $P = 0.05$ (*D*).

rather than by volume depletion, PVX could be beneficial by blunting the viscosity increase. However, this idea is not supported by the data of Sarnquist et al. (25), which indicated that $\dot{V}O_{2max}$ at 5,400 m was not improved after isovolemic hemodilution. In that study, the remarkable finding was that $\dot{V}O_{2max}$ did not change and was not impaired, despite the lower O_2-carrying capacity associated with hemodilution (25).

Finally, the fact that maximal heart rate was not reduced with PVX, both at SL and HA, is consistent with other data obtained at SL that indicate no change in maximal heart rate with an acute PVX of 450–600 ml (2, 14). Conversely, maximal heart rate at SL was reduced after a 700-ml PVX because of an increase in stroke volume (11). The lack of a significant effect of PVX on maximal heart rate in the present study was, therefore, most likely due to the limited volume of fluid infused.

Recovery after HA exposure. Experiments performed at RSL provided additional insights on our hypothesis. Even if $\dot{V}O_{2max}$ recovery was not complete at RSL, $\dot{V}O_{2max}$ (expressed in $ml \cdot min^{-1} \cdot kg^{-1}$) was only 11% lower than the initial value. In comparison, during Operation Everest II, $\dot{V}O_{2max}$ after extreme altitude exposure (within 2 days after the end of decompression) was more reduced, being lower than the SL value by 20% (3). It is of interest to relate the restoration of aerobic performance to PV recovery. In eight subjects, six experienced "spontaneous" PVX between SL and RSL. This observation is not in agreement with other

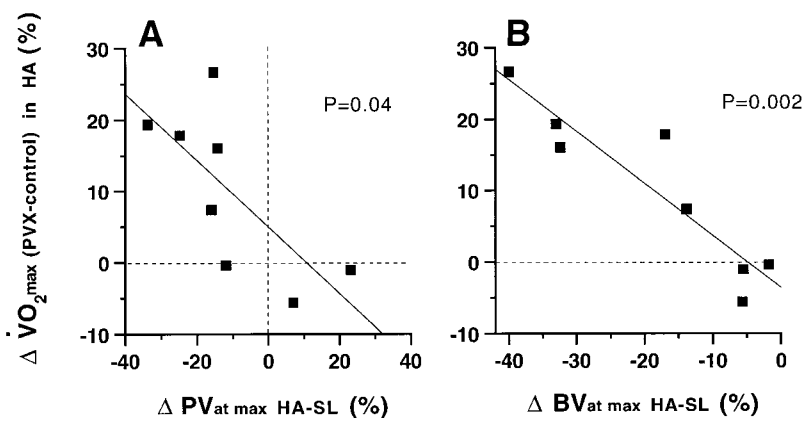

Fig. 7. Relationship between decrease in ΔPV_{atmax} (*A*) and ΔBV_{atmax} (*B*) associated with HA exposure and improvement in $\dot{V}O_{2max}$ with PVX at HA ($n = 8$). Regression equations: $y = -0.465x + 5.012$, $r = 0.72$ (*A*); $y = -0.729x - 3.606$, $r = 0.92$ (*B*).

data obtained at altitudes ≤4,300 m, indicating a subnormal PV during early recovery from altitude exposure (4, 26). Nevertheless, this phenomenon of PVX at RSL seems to play a role in $\dot{V}O_{2max}$ recovery (Fig. 6*C*). Finally, the fact that infusion had no effect on $\dot{V}O_{2max}$ at SL is not surprising, because PV_{rest} was normal or even supranormal.

Hormonal response. The lack of a significant decrease in [Ren] and [Aldo] at HA, as classically observed in hypoxia (15, 18, 32), could be partly explained by the great variability of our sample. Some subjects showed a suppression of exercise-induced rises in [Ren] and [Aldo] at HA, and others showed no change at all. However, the 15% decrease in BV experienced by our subjects at HA may have stimulated these sodium- and water-retaining hormones. Thus the antagonist effects of hypoxemia and hypovolemia may explain why [Ren] and [Aldo] did not change significantly at HA. The fact that the [ANF] response to exercise was reduced at HA, as demonstrated in previous studies (1, 18, 28), is probably explained by the large drop in heart rate and absolute work load. However, the most surprising hormonal responses occurred after recompression. At that time, [Ren] and [Aldo] responses to exercise were exacerbated, and infusion partially inhibited these responses, whereas [ANF] was not further increased. Globally, these hormonal changes indicated a trend toward an antidiuretic effect (increased renin-aldosterone activity) associated with RSL, which probably accounts for the concomitant PVX. This phenomenon could be related to a transient imbalance between two antagonist effects associated with reoxygenation, i.e., *1*) an immediate withdrawal of hypoxia-induced inhibition and *2*) a delayed suppression of hypovolemia-induced stimulation on the renin-aldosterone system.

In summary, this study demonstrated that acute PVX expansion, despite a concomitant reduction in [Hb], slightly ameliorated $\dot{V}O_{2max}$ at HA but not before or after hypoxic exposure. Thus alterations in PV associated with acclimatization may be involved in the impairment of O_2 transport at HA. Furthermore, the PVX observed at RSL, which was probably mediated by a rebound in renin and aldosterone secretion, could also be implicated in $\dot{V}O_{2max}$ recovery. From a practical point of view, the deleterious effect of a depressed circulating volume on aerobic performance at altitude stresses the importance of adequate hydration in the high mountain environment.

We thank the administrative and technical crews of COMEX S.A. for assistance provided to the investigators and subjects during the study; Kim Bodin, Emmanuel Cauchy, Guillaume Despiau, Jean-François Finance, Mathieu Gayet, Guillaume Sabin, Philippe Serpollet, and Alexandre Héritier for patience and courage during this exceptional experience; and Vincent Marchand as well.

This study was made possible by grants from the Région Provence Alpes Côte d'Azur and Ministère Jeunesse et Sports.

This study was part of a larger study (Operation Everest III) investigating several physiological, physiopathological, and psychological mechanisms during exposure to extreme and prolonged hypobaric hypoxia.

REFERENCES

1. **Bouissou P, Richalet J-P, Galen FX, Lartigue M, Larmignat P, Devaux F, Dubray C, and Kéromès A.** Effect of β-adrenoceptor blockade on renin-aldosterone and α-ANF during exercise at altitude. *J Appl Physiol* 67: 141–146, 1989.
2. **Coyle EF, Hopper MK, and Coggan AR.** Maximal oxygen uptake relative to plasma volume expansion. *Int J Sports Med* 11: 116–119, 1990.
3. **Cymerman A, Reeves JT, Sutton JR, Rock PB, Groves BM, Malconian MK, Young PM, Wagner PD, and Houston CS.** Operation Everest II: maximal oxygen uptake at extreme altitude. *J Appl Physiol* 66: 2446–2453, 1989.
4. **Greenleaf JE, Bernauer EM, Adams WC, and Juhos L.** Fluid-electrolyte shifts and $\dot{V}O_{2max}$ in man at simulated altitude (2,287 m). *J Appl Physiol* 44: 652–658, 1978.
5. **Grover RF, Selland MA, McCullough RG, Dahms TE, Wolfel EE, Butterfield GE, Reeves JT, and Greenleaf JE.** β-Adrenergic blockade does not prevent polycythemia or decrease in plasma volume in men at 4,300 m altitude. *Eur J Appl Physiol* 77: 264–270, 1998.
6. **Hannon JP, Chinn KS, and Shields JL.** Effects of acute high altitude exposure on body fluids. *Fed Proc* 28: 1178–1184, 1969.
7. **Hansen JM, Olsen NV, Feldt-Rasmussen B, Kanstrup IL, Déchaux M, Dubray C, and Richalet J-P.** Albuminuria and overall capillary permeability of albumin in acute altitude hypoxia. *J Appl Physiol* 76: 1922–1927, 1994.
8. **Harrison MH.** Effects on thermal stress and exercise on blood volume in humans. *Physiol Rev* 65: 149–209, 1985.
9. **Hultgren H.** *High Altitude Medicine*. Stanford, CA: Hultgren, 1997, p.101–108.
10. **Huszczuk A, Whipp BJ, and Wasserman K.** A respiratory gas exchange simulator for routine calibration in metabolic studies. *Eur Respir J* 3: 465–468, 1990.
11. **Kanstrup IL and Ekblom B.** Acute hypervolemia, cardiac performance, and aerobic power during exercise. *J Appl Physiol* 52: 1186–1191, 1982.
12. **Kanstrup IL and Ekblom B.** Blood volume and hemoglobin concentration as determinants of maximal aerobic power. *Med Sci Sports Exerc* 16: 256–262, 1984.
13. **Lundvall J, Mellander S, Westling H, and White T.** Fluid transfer between blood and tissues during exercise. *Acta Physiol Scand* 85: 258–269, 1972.
14. **Mier CM, Domenick MA, Turner NS, and Wilmore JH.** Changes in stroke volume and maximal aerobic capacity with increased blood volume in men and women. *J Appl Physiol* 80: 1180–1186, 1996.
15. **Olsen NV.** Ventilation, hypocapnia, and hypoxia: effects on renal function. In: *Women at Altitude*, edited by Houston CS and Coates G. Burlington, VT: Queen City Printers, 1997, p. 284–299.
16. **Pugh LGCE.** Blood volume and hemoglobin concentration at altitudes above 18,000 ft (5,500 m). *J Physiol (London)* 170: 344–354, 1964.
17. **Richalet J-P.** The heart and adrenergic system in hypoxia. In: *Hypoxia: The Adaptations*, edited by Sutton JR, Coates G, and Remmers JE. Philadelphia, PA: Decker, 1990, p. 231–240.
18. **Richalet J-P, Déchaux M, Bienvenu A, Souberbielle J-C, Antezana A-M, and Cauchy E.** Erythropoiesis and renal function at the altitude of 6,542 m. *Jap J Mountain Med* 15: 135–150, 1995.
19. **Richalet J-P, Rathat C, Kéromès A, Herry J-P, Larmignat P, Garnier M, and Pilardeau P.** Plasma volume, body weight, and acute mountain sickness. *Lancet* 1: 525, 1983.
20. **Richalet J-P, Robach P, Jarrot S, Schneider J-C, Mason NP, Cauchy E, Herry J-P, Gardette B, and Gortan C.** Operation Everest III (COMEX '97): effects of prolonged and progressive hypoxia on humans during a simulated ascent to 8,848 m in a hypobaric chamber. In: *Hypoxia: Into The Next Millennium*, edited by Roach RC, Wagner PD, and Hackett PH. New York: Kluwer Academic/Plenum, 1999, p. 297–317.
21. **Robach P, Biou D, Herry J-P, Deberne D, Letournel M, Vaysse J, and Richalet J-P.** Recovery processes after repeated

supramaximal exercise at the altitude of 4,350 m. *J Appl Physiol* 82: 1897–1904, 1997.
22. **Rose MS, Houston CS, Fulco CS, Coates G, Sutton JR, and Cymerman A.** Operation Everest II: nutrition and body composition. *J Appl Physiol* 65: 2545–2551, 1988.
23. **Saltin B.** Limitations to performance at altitude. In: *Hypoxia: The Tolerable Limits*, edited by Sutton JR, Houston CS, and Coates J. Indianapolis, IN: Benchmark, 1988, p. 9–34.
24. **Saltin B.** Exercise and the environment: focus on altitude. *Res Q Exerc Sport 67*: S1–S10, 1996.
25. **Sarnquist FH, Schoene RB, Hackett PH, and Townes BD.** Hemodilution of polycythemic mountaineers: effects on exercise and mental function. *Aviat Space Environ Med* 57: 313–317, 1986.
26. **Sawka MN, Young AJ, Rock PB, Lyons TP, Boushel R, Freund BJ, Muza SR, Cymerman A, Dennis RC, Pandolf KB, and Valeri CR.** Altitude acclimatization and blood volume: effects of exogenous erythrocyte volume expansion. *J Appl Physiol* 81: 636–642, 1996.
27. **Sutton JR, Reeves JT, Wagner PD, Groves BM, Cymerman A, Malconian MK, Rock PB, Young PM, Walter SD, and Houston CS.** Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *J Appl Physiol* 64: 1309–1321, 1988.
28. **Vuolteenaho O, Koistinen P, Martikkala V, Takala T, and Leppöluoto J.** Effect of physical exercise in hypobaric conditions on atrial natriuretic peptide secretion. *Am J Physiol Regulatory Integrative Comp Physiol* 263: R647–R652, 1992.
29. **Wagner PD.** Determinants of maximal oxygen uptake and utilization. *Ann Rev Physiol* 58: 21–50, 1996.
30. **West JB, Boyer SJ, Graber DJ, Hackett PH, Maret KH, Milledge JS, Peters RM Jr, Pizzo CJ, Samaja M, Sarnquist FH, Schoene RB, and Winslow RM.** Maximal exercise at extreme altitudes on Mount Everest. *J Appl Physiol* 55: 688–698, 1983.
31. **Young AJ, Sawka MN, Muza SR, Boushel R, Lyons T, Rock PB, Freund BJ, Waters R, Cymerman A, Pandolf KB, and Valeri CR.** Effects of erythrocyte infusion on $\dot{V}O_{2max}$ at high altitude. *J Appl Physiol* 81: 252–259, 1996.
32. **Zaccaria M, Rocco S, Noventa D, Varnier M, and Opocher G.** Sodium regulating hormones at high altitude: basal and postexercise levels. *J Clin Endocrinol Metab* 83: 570–574, 1998.