Maximal rate of blood lactate accumulation during exercise at altitude in humans

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Grassi, Bruno, Guido Ferretti, Bengt Kayser, Mauro Marzorati, Angelo Colombini, Claudio Marconi, and Paolo Cerretelli. Maximal rate of blood lactate accumulation during exercise at altitude in humans. *J. Appl. Physiol.* 79(1): 331–339, 1995.—The lower peak lactate accumulation in blood ($[La_b]_p$) at altitude may be associated with a reduced maximal glycolytic flux. Based on certain assumptions, the latter can be indirectly evaluated in vivo, during short supramaximal exercises, by measuring the maximal rate of lactate accumulation in blood ($\Delta[\dot{La}_b]_{max}$). $\Delta[\dot{La}_b]_{max}$ was determined on six white subjects at sea level (SL1), after ~1 wk (Alt1) and 4 wk (Alt2) of a 35-day sojourn at 5,050 m, and 1 wk after return to sea level (SL2). The subjects performed exercises of increasing duration (5, 15, 25, 35, 45 s or until exhaustion) on a bicycle ergometer at loads = 200% of the individual $\dot{W}_{max}$. The latter was previously determined in each condition as the greatest work rate that could be sustained for 2–4 min during an incremental exercise. Net $[La_b]$ accumulation ($\Delta[La_b]$) was measured after each exercise bout. $\Delta[La_b]$ resulted to be linearly related to exercise duration. The slopes of the individual $\Delta[La_b]$ vs. exercise duration lines were taken as $\Delta[\dot{La}_b]_{max}$. Exhaustion times were ~30–45 s in all conditions. $[La_b]_p$ (in mM) during recovery after the exhaustive load was higher at SL1 (10.22 ± 1.09; $\bar{x}$ ± SD) than at Alt1 (5.08 ± 0.82), Alt2 (8.13 ± 2.67), and SL2 (8.18 ± 1.43). $\Delta[\dot{La}_b]_{max}$ was lower at Alt1 (0.09 ± 0.02) and at Alt2 (0.17 ± 0.05) than at SL1 (0.25 ± 0.05) and SL2 (0.23 ± 0.06). Both $[La_b]_p$ and $\Delta[\dot{La}_b]_{max}$ increased during acclimatization. It is concluded that the lower $[La_b]_p$ at altitude was associated with a reduced $\Delta[\dot{La}_b]_{max}$. In the presence of a presumably normal maximal potential activity of glycolytic enzymes, as described by several authors, the reduced $\Delta[\dot{La}_b]_{max}$ is indicative of an upstream inhibition of glycolysis at altitude.

chronic hypoxia; altitude acclimatization; lactate paradox; lactic capacity; glycolysis

PEAK LACTATE ACCUMULATION in blood ($[La_b]_p$) during recovery following exhaustive maximal or supramaximal exercise is known to be reduced by chronic hypoxia, either from altitude exposure (6, 11, 18, 25, 36) or from a prolonged sojourn in a decompression chamber (19). Several hypotheses have been put forward to explain this phenomenon (7, 35, 37), among which one is based on a restriction of the maximal flux of substrates along the glycolytic pathway. This hypothesis, however, appears to be challenged by the finding that, according to most studies on this topic, the maximal potential activity of key glycolytic enzymes is unchanged in chronic hypoxia compared with normoxia (20, 23, 39). Only Green et al. (21) described, after high-altitude acclimatization, a slight reduction (−14%) of the maximal potential activity of phosphofructokinase, the key regulating enzyme of glycolysis. The maximal potential activity of glycolytic enzymes, however, does not provide information concerning the in vivo maximal energy flux along the glycolytic pathway. The latter could in fact be affected also by changes in the velocity constants of some rate-limiting enzymes, and/or by some form of upstream inhibition, possibly attributable to β-adrenergic modulation of glycogenolysis (1, 4, 40) and/or to a reduced neuromuscular activation (2, 19, 26).

According to Margaria et al. (27), during supramaximal exercise leading to exhaustion in <1 min, *1*) net $[La_b]$ accumulation ($\Delta[La_b]$) is linearly related to exercise duration (t); and *2*) above a given workload, the rate of $\Delta[La_b]$ accumulation ($\Delta[\dot{La}_b]$) attains a maximum ($\Delta[\dot{La}_b]_{max}$). $\Delta[\dot{La}_b]_{max}$ can be considered to a certain extent (see DISCUSSION) an indirect index of the maximal glycolytic flux, particularly considering the shortness of the exercise protocol, which should render the influence of lactate removal on $\Delta[\dot{La}_b]_{max}$ negligible (5). $\Delta[\dot{La}_b]_{max}$ could therefore represent a valuable index for comparing, in vivo and noninvasively, the maximal glycolytic flux at sea level and at altitude in humans. According to the hypothesis that $[La_b]_p$ in chronic hypoxia is reduced because of a restricted maximal flux of substrates along the glycolytic pathway, $\Delta[\dot{La}_b]_{max}$ should be lower at altitude than at sea level. We therefore measured $\Delta[\dot{La}_b]_{max}$ and $[La_b]_p$ on a group of lowlanders before, during, and after 5 wk of exposure to an altitude of 5,050 m.

SUBJECTS, METHODS, AND EXPERIMENTAL PROCEDURES

Subjects

Six healthy white males (age 31.7 ± 4.0 yr; mean ± SD) participated in the study after providing an informed consent. The percentage of body fat was determined by plicometry (10). Leg muscles volume (LMV) was estimated by a volumetric reconstruction based on heights and circumferences (24).

Methods and Experimental Procedures

The experiments were performed in four conditions: at sea level (Milan, Italy, 122 m) before departure (SL1), after ~1 wk (Alt1) and 4 wk (Alt2) of a 35-day sojourn at 5,050 m (Ev-K2-CNR Pyramid Laboratory, set near Lobuche, Khumbu, Nepal), and again at sea level 1 wk after return from altitude (SL2). The altitude laboratory was equipped with a stabilized electrical supply powered by a water turbine. Temperature inside the laboratory during the experiments ranged between 17 and 22°C. Exercises were carried out on a mechanically braked bicycle ergometer (Monark Ergomedic 818E). The

TABLE 1. *Work loads, exhaustion times and ($[La_b]_p$) in various experimental conditions*

Subject	SL1	Alt1	Alt2	Alt2 $NaHCO_3$	Alt2bis	SL2
			Work load, W			
1	600	360	420	420	510	660
2	480	300	360	360		480
3	480	300	360			440
4	420	240	300		360	425
5	360	240	300			420
6	420	300	360	360	450	500
Mean	460	290	350	380	440	488
±SD	±82	±45*†	±45*†	±35	±75§	±90
			Exhaustion times, s			
1	35	45	45	45	35	35
2	30	45	45	45		35
3	45	45	45			35
4	45	45	45		35	30
5	45	45	45			35
6	40	45	60	45	45	35
Mean	40	45	48	45	38	34
±SD	±6†	±0†	±6†	±0	±6§	±2
			$[La_b]_p$, mM			
1	9.84	5.83	11.56	8.09	7.11	9.95
2	10.38	6.00	10.47	10.12		9.66
3	11.38	5.28	8.98			7.46
4	10.23	3.80	5.32		4.34	6.26
5	11.15	4.59	7.34			8.41
6	8.33	4.98	5.11	6.33	6.03	7.34
Mean	10.22	5.08	8.13	8.18	5.83	8.18
±SD	±1.09	±0.82*†	±2.67*‡	±1.90	±1.40	±1.43*

$[La_b]_p$, peak lactate accumulation in blood. SL1, sea level, before departure; Alt1, after 1 wk at altitude; Alt2, after 4 wk at altitude; $Alt2_{bis}$, 100% maximal mechanical power output at Alt2 + 100% maximal mechanical power output at SL1. * Significantly different from SL1. † Significantly different from SL2. ‡ Significantly different from Alt1. § Significantly different from Alt2.

electrocardiogram was continuously monitored (Cardioline ETA 150) throughout the tests.

The experimental protocols in each session were as follows.

Protocol 1. On *day 1*, the maximal mechanical power output ($\dot{W}_{max}$) was determined. $\dot{W}_{max}$ was defined as the greatest work rate that could be sustained for 2–4 min in the course of an incremental exercise (30 W added every 4 min) up to voluntary exhaustion. The latter was defined as the inability to maintain the imposed pedaling frequency.

Protocol 2. On *day 2*, the subjects performed exercise bouts of increasing duration (5, 15, 25, 35, 45 s or until exhaustion) at 200% of the individual $\dot{W}_{max}$ determined the day before. The exercise bouts were separated by sufficient time to allow a complete recovery of the investigated variables. In particular, no exercise bout was performed until resting blood lactate concentration ($[La_b]$) was below 1.5 mM. Pedaling frequencies were kept constant during each exercise bout and varied among subjects between 70 and 90 rpm. Before work, at the end of each exercise bout, and at various times (1, 3, 5, 7 min) during inactive recovery, 20 μl of arterialized capillary blood were taken from an earlobe for the determination of $[La_b]$ by an electroenzymatic method (Kontron lactate analyzer 640). Arterialization was achieved by prior application of a hyperemia-inducing ointment (Trafuril, Ciba-Geigy). One or more puncture(s) by a lancet of the earlobe allowed rather easily blood collections from the same or a nearby site after each exercise bout. $\Delta[\dot{L}a_b]$ during each exercise bout (highest $[La_b]$ value during recovery minus resting $[La_b]$) was calculated. During the recovery following the exhaustive load, blood lactate washout kinetics was determined from blood samples taken at various times (1, 3, 5, 7, 9, 13, 19, 25, 35, 45, and 60 min) until $[La_b]$ approached resting values. The highest $[La_b]$ during recovery after the exhaustive load was taken as $[La_b]_p$. On three subjects only, and limited to Alt2, blood lactate washout kinetics was also followed during active recovery, i.e., with the subjects exercising at a workload corresponding to ~30% of $\dot{W}_{max}$ determined at Alt2.

Protocol 3. At Alt2, in addition to *protocols 1* and *2*, on *day 3* the same measurements described in *protocol 2* were repeated on three of the subjects after the oral administration, ~1 h before the first exercise bout, of $NaHCO_3$ at a dose of 0.3 g/kg body wt, diluted in 400 ml of water (Alt2 $NaHCO_3$). No subjective adverse effects from the ingestion of $NaHCO_3$ were noticed.

Protocol 4. At Alt2, in addition to *protocols 1* and *2*, on *day 4* the same measurements described in *protocol 2* were repeated on three of the subjects exercising at a workload corresponding to 100% of the $\dot{W}_{max}$ determined at Alt2 + 100% of the $\dot{W}_{max}$ determined at SL1 ($Alt2_{bis}$).

Calculations

Individual $\Delta[La_b]$ values obtained for each exercise bout were plotted against t, and regression lines were drawn. The slope ($\Delta[\dot{L}a_b]_{max}$) and the intercept with the abscissa of the individual $\Delta[La_b]$ vs. t functions were considered for data analysis. Average $\Delta[La_b]$ values vs. t functions were also calculated by using the data obtained on all subjects in each experimental session.

Statistics

The values were given as means ± SD. The values obtained in the various experimental sessions were compared by one-way analysis of variance (ANOVA). A Tukey's test was utilized to discriminate between significantly different pairs.

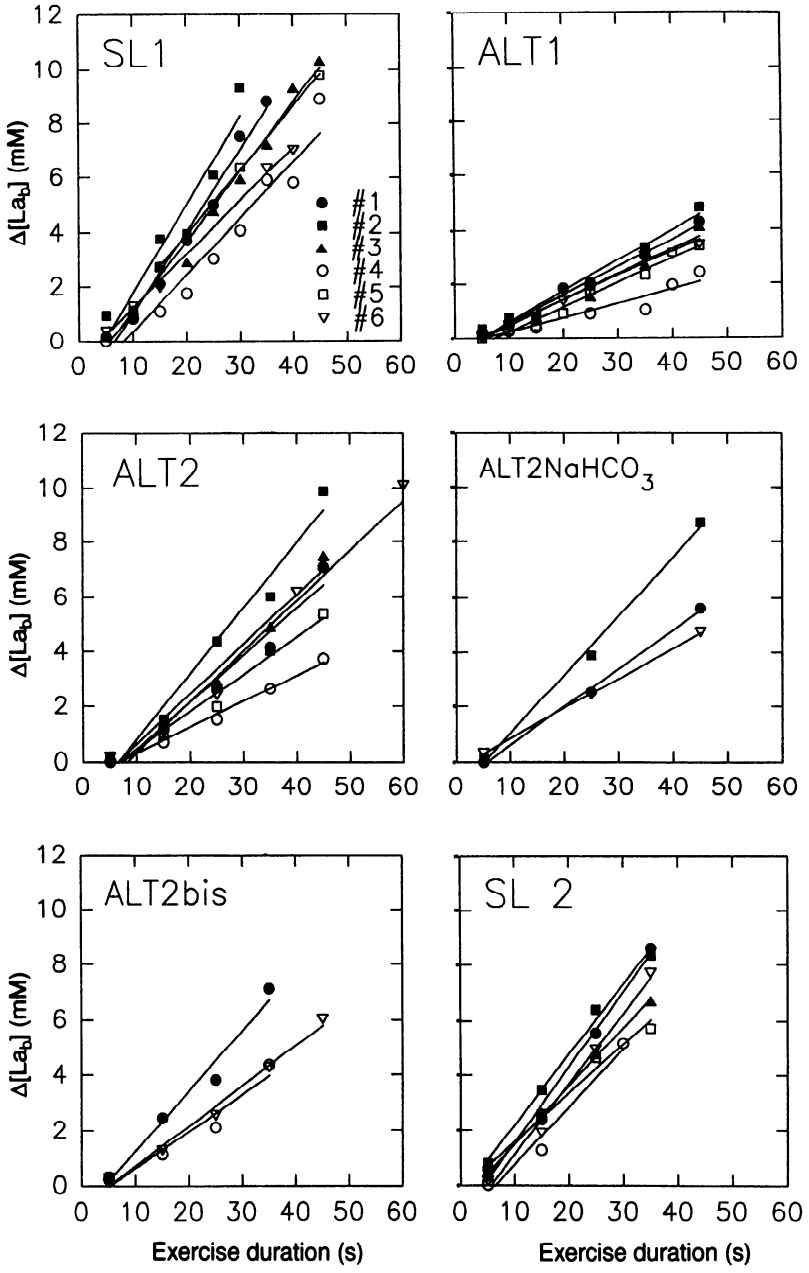

FIG. 1. Net lactate accumulation in blood ($\Delta[La_b]$) as a function of exercise duration in various experimental conditions. SL1, before departure, sea level; Alt1, after 1 wk at altitude (5,050 m); Alt 2, after 4 wk at altitude; $Alt2_{bis}$, 100% maximal mechanical power output at Alt2 + 100% maximal mechanical power output at SL1; SL2, after return, sea level. Individual linear regression lines are also shown. See text for further details.

For comparison between results obtained at Alt2 and Alt2 $NaHCO_3$ and at Alt2 and $Alt2_{bis}$, a Student's t-test (two-tailed; unpaired analysis) was utilized. The level of significance was taken at $P < 0.05$. These statistical analyses were performed by using a commercially available software package (Statgraphics 5.0, STSC). The power of the tests and the minumum detectable differences were calculated according to standard methods (41).

RESULTS

The body weight (in kg) of the subjects was 75.9 ± 12.4 at SL1, 72.7 ± 12.1 at Alt1, 70.4 ± 12.9 at Alt2, and 72.2 ± 12.7 at SL2. The percentage of body fat was 18.4 ± 7.4 at SL1, 17.4 ± 7.0 at Alt1, 17.6 ± 6.7 at Alt2, and 17.8 ± 6.5 at SL2. LMV (in liters) was 6.88 ± 0.90 at SL1, 6.63 ± 0.93 at Alt1, 6.04 ± 0.99 at Alt2, and 6.43 ± 1.04 at SL2.

The workloads imposed on the subjects in the various experimental conditions, the exhaustion times, and $[La_b]_p$ are shown in Table 1. $\dot{W}_{max}$ at Alt1 and Alt2 were ~35 and 25% lower, respectively, than at SL1, whereas at SL2 $\dot{W}_{max}$ was not significantly different than at SL1. Exhaustion times ranged in all sessions between ~30 and 45 s. $[La_b]$ values at rest were not significantly different in the various conditions and ranged between 1 and 1.5 mM. $[La_b]_p$ values at Alt1, Alt2, and SL2 were ~50, 20, and 20% lower, respectively, than at SL1. No significant differences in $[La_b]_p$ were observed between Alt2 and $Alt2_{bis}$ nor between Alt2 and Alt2 $NaHCO_3$ for the three investigated subjects.

Figure 1 illustrates that $\Delta[La_b]$ was linearly related to t in all subjects and in all experimental sessions; very high correlation coefficients ($0.97 < r < 1.00$) were obtained. Individual values of $\Delta[\dot{L}a_b]_{max}$ are shown in

TABLE 2. *Slopes of the individual $\Delta[La_b]$ vs. time linear regression lines ($\Delta[\dot{La}_b]_{max}$) in various experimental conditions*

Subject	SL1	Alt1	Alt2	Alt2 $NaHCO_3$	Alt2bis	SL2
1	0.30	0.10	0.19	0.14	0.22	0.30
2	0.32	0.11	0.24	0.21		0.26
3	0.26	0.09	0.18			0.21
4	0.21	0.06	0.09		0.10	0.15
5	0.24	0.09	0.13			0.26
6	0.20	0.09	0.15	0.11	0.14	0.18
Mean	0.25	0.09	0.17	0.15	0.15	0.23
±SD	+0.05	+0.02*†	±0.05*†‡	±0.05§	±0.06	±0.06

$\Delta[La_b]$, not blood lactate accumulation; $\Delta[\dot{La}_b]_{max}$, maximal rate of lactate accumulation in blood. * Significantly different from SL1. † Significantly different from SL2. ‡ Significantly different from Alt1. § Significantly different from Alt2.

Table 2; they were significantly lower at Alt1 and Alt2 than at SL1 and significantly higher at Alt2 than at Alt1, whereas no significant differences were observed between the values obtained at SL1 and at SL2. The values obtained at Alt2 $NaHCO_3$ and at $Alt2_{bis}$ were not significantly higher than those at Alt2. The risk of incurring in a type II error (i.e., not rejecting the null hypothesis when it is in fact false) was determined by calculating the power of the tests (41). The latter was 0.9 (minimum detectable difference = 0.05) for the ANOVA test performed on the data at SL1, Alt1, Alt2, and SL2, indicating a risk of type II error acceptably low (41). On the other hand, the smaller number of subjects tested at Alt2 $NaHCO_3$ and at $Alt2_{bis}$ does not allow to exclude, with acceptable confidence, that the absence of differences could be attributable to the small sample size. A direct comparison of $\Delta[\dot{La}_b]_{max}$ obtained in the various conditions is made in Fig. 2, in which the average $\Delta[La_b]$ values vs. t functions are shown. Individual values of the intercept with the abscissa of the $\Delta[La_b]$ vs. t functions are shown in Table 3. The intercepts ranged between 4 and 7 s.

Blood lactate washout curves during inactive recovery following the exhaustive exercises are shown in Fig. 3. In all experimental conditions, $[La_b]$ increased during the first 4–6 min of recovery and, after reaching a peak ($[La_b]_p$), it diminished progressively according to a monoexponential function of time, with half times of ~11–13 min. No significant differences were observed, in the various conditions, in the delay for $[La_b]_p$ during recovery, as well as in the half times of the ensuing $[La_b]$ decrease. In Fig. 4, the mean data of Fig. 3 are expressed as a ratio of $[La_b]_p$ obtained in each condition (i.e., as $[La_b]/[La_b]_p$), thereby normalizing for the different absolute values. From Fig. 4 it can be noticed that the $[La_b]/[La_b]_p$ washout kinetics were very similar in all experimental conditions. At Alt2, the delays for reaching the peak of $[La_b]/[La_b]_p$ were not significantly different during inactive and active recovery, whereas the ensuing $[La_b]/[La_b]_p$ decrease was significantly faster during active recovery (Fig. 5).

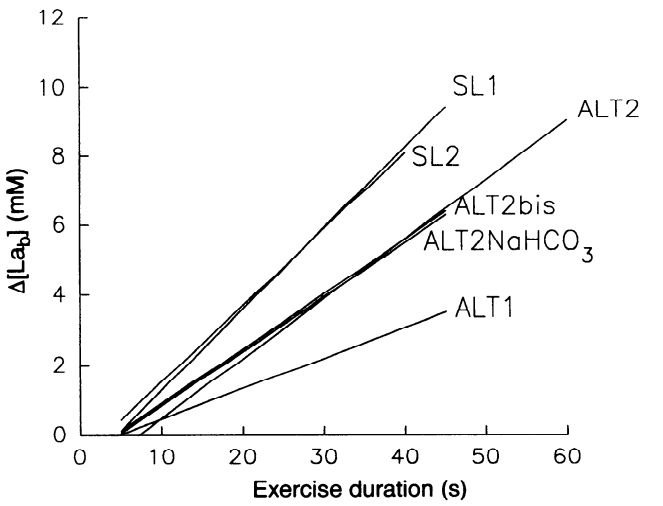

FIG. 2. $\Delta[La_b]$ as a function of exercise duration in various experimental conditions. Average linear regression lines, calculated by using data obtained from all subjects in each experimental session, are shown. See text for details.

DISCUSSION

Maximal Glycolytic Flux at Altitude

The significantly lower $\Delta[\dot{La}_b]_{max}$ obtained at Alt1 and Alt2 compared with SL1 and SL2 is compatible with the hypothesis of a reduced maximal glycolytic flux at altitude, even in the presence of a normal maximal potential activity of key glycolytic enzymes, as previously described (20, 23, 39). The decreased maximal glycolytic flux could be, at least in part, responsible for the lower $[La_b]_p$ observed by several authors (6, 11, 18, 19, 25, 36) as well as in the present study in chronic hypoxia compared with normoxia. Both $\Delta[\dot{La}_b]_{max}$ and $[La_b]_p$ increased in the course of acclimatization.

On the basis of the present results, no direct inferences can be made as to the cause(s) of the reduction of maximal glycolytic flux at altitude. Some hypotheses, however, are briefly discussed here.

Hypothesis 1: reduced blood buffering capacity. Cerretelli et al. (6) hypothesized that the reduced maximal glycolytic flux could be associated with a reduced buffer capacity, consequential to the renal compensation of the respiratory alkalosis secondary to the hypoxic hyperventilation. In the present study, the measurements of $\Delta[\dot{La}_b]_{max}$ were repeated at Alt2, on three subjects, after the administration of a $NaHCO_3$ load. The latter was previously shown to induce a normalization of blood bicarbonate buffers, i.e., a condition of compensated respiratory alkalosis (25). The exercise bouts were performed ~1 h after the $NaHCO_3$ load, so that any effects of the latter on urinary volumes and, as a consequence, on the volumes of intra- and extracellular fluids should have been negligible when the exercises were performed. $\Delta[\dot{La}_b]_{max}$ [as well as $[La_b]_p$, confirming recent results by Kayser et al. (25)], did not increase after $NaHCO_3$ loading. Thus the above hypothesis (6) was not supported by the present experiments, even though, as mentioned above, the small number of subjects tested at Alt2 $NaHCO_3$ does not allow to exclude with acceptable confidence the risk of a type II statistical error.

Hypothesis 2: reduced muscle mass. Muscle mass re-

TABLE 3. *Intercepts (in s) with the abscissa of the individual $\Delta[La_b]$ vs. time linear regression lines in various experimental conditions*

Subject	SL1	Alt1	Alt2	Alt2 $NaHCO_3$	Alt2bis	SL2
1	7	4	8	6	4	5
2	5	4	7	5		1
3	6	5	6			3
4	8	6	6		5	6
5	3	4	7			6
6	4	4	6	2	5	1
Means	6	5	7	4	5	4
±SD	±2	±1	±1*†	±2	±1	±2

* Significantly different from SL2. † Significantly different from Alt1.

duction measured in the present study was similar to that previously observed on subjects exposed to chronic hypoxia (12, 30). Such decrease might per se be responsible for the reduced $\Delta[\dot{L}a_b]_{max}$ at altitude. However, for the present study, its influence is expected to be negligible, considering that *1*) the percentage decreases in $\Delta[\dot{L}a_b]_{max}$ at altitude (−64% at Alt1 and −32% at Alt2), compared with SL1, were much greater than these of LMV (−4% at Alt1 and −12% at Alt2); and *2*) the most marked decrease in $\Delta[\dot{L}a_b]_{max}$ occurred at Alt1, when the LMV decrease was very small.

Hypothesis 3: reduced muscle glycogen stores. Muscles biopsies were not performed in the present study, so that one cannot exclude that some glycogen depletion occurred on altitude exposure or during the exhaustive exercises conducted during 2–3 consecutive days. Previous determinations of muscle glycogen stores in chronic hypoxia, however, showed only minor reductions (21, 38) or no reduction at all (20) compared with normoxia. Short (albeit very intense) exercise bouts are known to induce, both in normoxia and in acute hypoxia, only minor changes in muscle glycogen levels (17, 22 29). In a recent review, Fitts (13) concluded that glycogen depletion can only be causative in fatigue during prolonged endurance exercise requiring between 65 and at most 90% of a subject's maximal O_2 consumption. During an incremental exercise up to exhaustion, lasting ~15–30 min, like that carried out on *day 1*, glycogen depletion has been shown to be small, both in normoxia and in chronic hypoxia (20). In the present study, at least 24 h of rest were allowed between the exhaustive exercises, which should lead to a complete recovery of glycogen stores. Therefore, it seems rather unlikely that glycogen depletion could represent a major factor responsible for the observed decrease of $\Delta[\dot{L}a_b]_{max}$ at altitude.

A reduction of the maximal glycolytic flux, likely not attributable to one of the mechanisms described in *hypotheses 1–3*, in the presence of a normal maximal potential activity of the key glycolytic enzymes, makes it tempting to hypothesize that the inhibition of glycolysis at altitude takes place "upstream." Upstream may indicate the following.

Hypothesis 4: β-adrenergic control of glycogenolysis, as proposed by various authors (1, 4, 40), even though

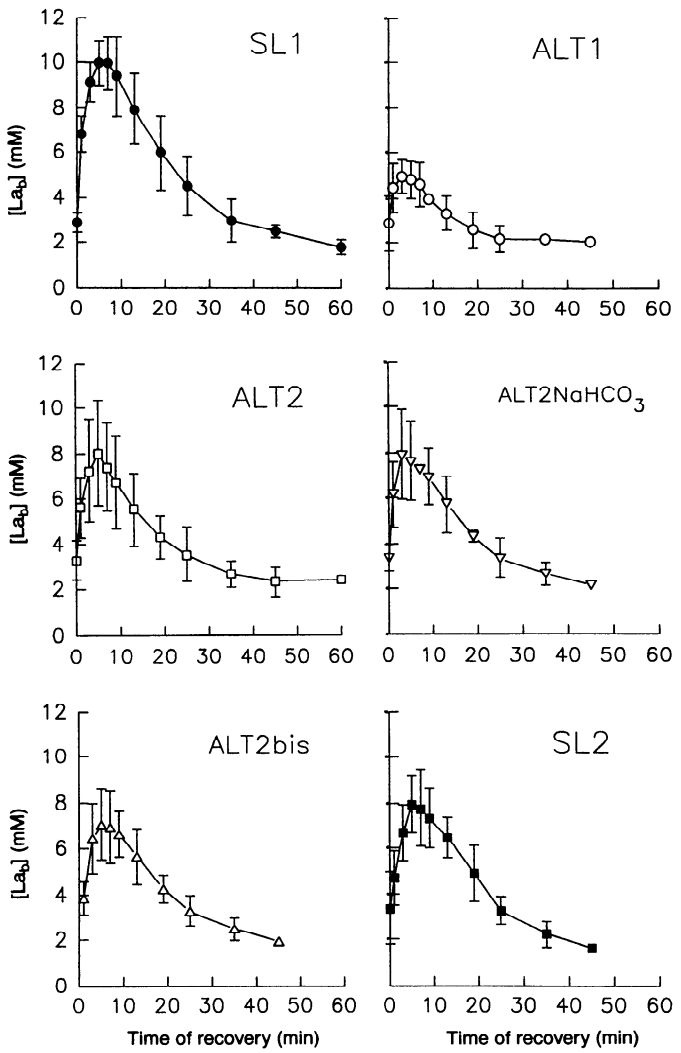

FIG. 3. Lactate concentration in blood ($[La_b]$) washout curves during inactive recovery following exhaustive exercises in various experimental conditions. Data are expressed as means ± SD of individual values. See text for details.

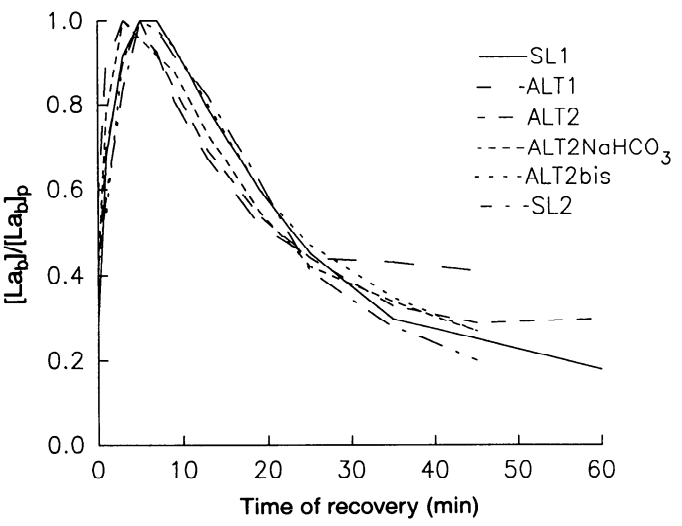

FIG. 4. $[La_b]$ washout curves during inactive recovery following exhaustive exercises in various experimental conditions. Data (expressed as means ± SD of individual values) are presented as a ratio of peak $[La_b]$ obtained in each experimental condition (i.e., as $[La_b]/[La_b]_p$). See text for details.

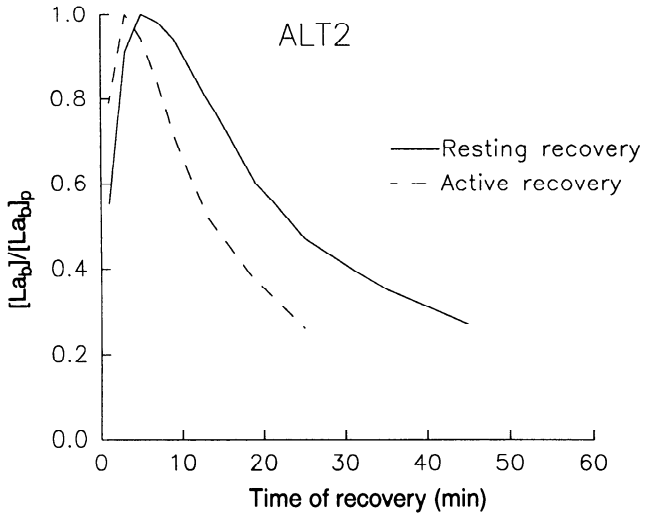

FIG. 5. $[La_b]$ washout curves during inactive and active recovery following exhaustive exercises at Alt2. Data (expressed as means ± SD of individual values) are presented as $[La_b]/[La_b]_p$. See text for details.

the same group recently published data according to which the sympathoadrenal system does not entirely account for the lactate changes observed during exercise at high altitude (28).

Hypothesis 5: reduced neuromuscular activation (2, 19, 26). It has recently been shown that at exhaustion from maximal cycling at altitude the quadriceps rather surprisingly do not show electromyographic signs of fatigue (26), suggesting a central inhibition of their maximal neuromuscular activation. According to Green et al. (19) such response could be beneficial, being aimed at protecting the body against the potential damage of metabolic acidosis in a condition characterized by marked hypoxia and loss of buffering capacity. The described phenomenon could also be aimed at preserving some of the available O_2 for the needs of vital organs, such as the respiratory muscles, which during intense exercises at altitude must sustain particularly elevated loads. Indeed, in these conditions, signs of diaphragmatic fatigue have recently been detected (8).

In any case, whatever the mechanism responsible for the upstream inhibition of glycolysis, according to the present results it appears to be partially offset during acclimatization, and it was no more effective 1 wk after return of the subjects to sea level.

The intercepts of the $\Delta[La_b]$ vs. t functions with the abscissa, indicating the delay after exercise onset before the occurrence of any lactate accumulation in blood, ranged in the various experimental sessions between 4 and 7 s. These results are in agreement with those obtained in normoxic conditions by previous authors, who determined [La] in muscle biopsies taken immediately after supramaximal (1.2 $\dot{W}_{max}$) bicycle ergometer exercises (33). According to these authors, no lactate production occurred for $t < 6$ s.

Critique of Methods, Validity of Assumptions

The conclusions of the present study are based on the assumption that during short supramaximal exercises the maximal rate of lactate production by the muscles can be evaluated, at least indirectly, from the maximal rate of lactate appearance in blood (i.e., $\Delta[\dot{L}a_b]_{max}$).

The initial assumption was that the measured $\Delta[\dot{L}a_b]$ values were actually maximal (i.e., $\Delta[\dot{L}a_b]_{max}$). Margaria et al. (27) measured $\Delta[\dot{L}a_b]_{max}$ during exercises performed on a treadmill, whereas for logistic reasons we utilized a bicycle ergometer. We assumed that exercises at 200% of $\dot{W}_{max}$, leading to exhaustion in ~30–45 s, would maximally activate glycolysis. To test this hypothesis, one should perform, as Margaria et al. did, also exercises at higher workloads (250 or 300% of $\dot{W}_{max}$) to check whether $\Delta[\dot{L}a_b]$ further increases or not, in the latter case being considered maximal. Such extremely high workloads could not be carried out in a controlled manner by some of the present subjects. In fact, only three subjects were able to perform at SL1 workloads corresponding to 250% of $\dot{W}_{max}$. In these subjects, no significant increases in $\Delta[\dot{L}a_b]$ (0.25 ± 0.04) were found compared with those obtained at 200% $\dot{W}_{max}$ (0.24 ± 0.03). To induce exhaustion within 30–45 s, the subjects performed exercises at the same relative workload (200% $\dot{W}_{max}$) both at sea level and at altitude. Thus the load imposed on the subjects at Alt1 and Alt2 was lower, in absolute terms, than at SL1. To stress glycolysis to the same extent as at SL1, both in relative and in absolute terms, and to check whether an increase in absolute workload would affect $\Delta[\dot{L}a_b]$ at altitude, the latter was determined on three subjects, at Alt2, at a workload corresponding to 100% of the $\dot{W}_{max}$ determined at Alt2 + 100% of the $\dot{W}_{max}$ determined at SL1, i.e., $Alt2_{bis}$. $\Delta[\dot{L}a_b]$ was not greater at $Alt2_{bis}$ than at Alt2 (although the lower number of subjects did not allow to exclude with acceptable confidence the risk of type II error) and was significantly lower at $Alt2_{bis}$ than at SL1. Considering that *1*) both at SL1 and at Alt2 (on the three tested subjects) an increase in workload did not cause a significant increase in $\Delta[\dot{L}a_b]$; *2*) in all conditions $[La_b]_p$ values were similar or even higher than the $[La_b]_p$ observed after different experimental protocols (6, 18); and *3*) in all conditions the subjects reached exhaustion in 30–45 s; then the assumption that the observed $\Delta[\dot{L}a_b]$ values were indeed the greatest that the subjects could attain (i.e., $\Delta[\dot{L}a_b]_{max}$) seems legitimate, particularly for SL1, Alt2, and SL2. Some caution is necessary for the results obtained at Alt1.

The second assumption was that $\Delta[\dot{L}a_b]_{max}$ allows an indirect evaluation of the maximal glycolytic flux. Blood lactate accumulation, as it is well known, is the result of a complex interaction of factors such as lactate production by muscles, lactate transport and diffusion from muscles to the extracellular fluids and blood, and lactate uptake by muscles, liver, and other organs (3, 9). At the whole body level, however, $\Delta[La_b]$ has been quantitatively related to the overall energy output from anaerobic sources (9). According to Brooks et al. (4), changes in arterial [La] at altitude mainly reflect changes in lactate production, not changes in lactate disposal. Lactate uptake by muscles and other organs is concentration dependent (4) and, therefore, since higher $[La_b]$ values were present at sea level, any differ-

ence in blood lactate disposal between sea level and altitude would mean that the differences of the glycolytic flux between the two conditions are even more pronounced than those detected by $\Delta[\dot{L}a_b]_{max}$. Moreover, and perhaps most importantly, the short-term nature of the exercise protocol adopted for the present study should render the influence of lactate removal on $[La_b]_p$ and $\Delta[\dot{L}a_b]_{max}$ negligible (5). If a limitation exists in the maximal rate of lactate transport and diffusion from muscle to blood (32), then such limitation would be more marked at sea level, i.e., in a situation in which muscle lactate concentration after exhaustive exercise is higher than at altitude (19). If this holds true, the differences of $\Delta[\dot{L}a]_{max}$ between sea level and altitude would be even more pronounced in muscle than those observed in blood. The rate of lactate efflux from the cell is reduced by high $[H^+]$ and by low $[HCO_3^-]$ (brackets indicate concn) outside the cell (34). Our subjects at altitude were in a situation of partially compensated respiratory alkalosis, e.g., with pH and $[HCO_3^-]$ in arterialized blood of 7.47 ± 0.02 and 16.8 ± 2.7 mM at Alt1, and of 7.45 ± 0.03 and 15.7 ± 1.8 mM at Alt2 (18). The lower than normal $[H^+]$ and $[HCO_3^-]$ outside the cell would therefore influence in opposite directions the rate of lactate efflux from muscle. Although these influences cannot be easily quantitated, the net result should be a situation not significantly different from that at sea level, also considering that at Alt2 $NaHCO_3$ loading did not have any significant effect on $\Delta[\dot{L}a_b]_{max}$ or on the blood lactate washout kinetics. In any case, significant differences, between sea level and altitude, in the rate of lactate efflux from muscles to blood and/or in the rates of lactate utilization as a fuel or as a substrate for gluconeogenesis, would have likely resulted in differences in the blood lactate washout curves during recovery. By contrast, in the present study, these curves during inactive recovery were very similar in all experimental sessions, as discussed below. The similarity of the situation, with regard to blood lactate disposal, between sea level and altitude is further supported by the observation that at Alt2 blood lactate disappearance kinetics was faster during active recovery than during inactive recovery, in agreement with similar observations at sea level (16). This indicates that the reserve ability to clear blood lactate by exercising muscles was substantially unchanged at altitude compared with sea level, although in this respect some caution is necessary, since these experiments were conducted only on three of the subjects and only at Alt2. Considering all this, the assumption that $\Delta[\dot{L}a]_{max}$, as determined in the present exercise protocol, allows an indirect evaluation of the maximal glycolytic flux appears legitimate.

$[La_b]_p$

$[La_b]_p$ at Alt1 was ~50% of that found at SL1. Such a decrease confirms previous observations at altitudes similar (6) or identical (18) to that of the present study, although by means of different exercise protocols. In the course of acclimatization $[La_b]_p$ increased, even without reaching preexpedition values. One week after return to sea level $[La_b]_p$ was still lower than that observed preexpedition. It is noteworthy that two of the subjects (*subjects 1* and *2*, see Table 2) presented at Alt2 $[La_b]_p$ values slightly higher than those obtained at SL1, even in the presence of a still reduced $\Delta[\dot{L}a_b]_{max}$. In these subjects, therefore, after 4 wk of altitude acclimatization the maximal lactic capacity, as evaluated by the present exercise protocol, was back to normal. This observation, although based only on two subjects (the best fit in the group, as desumed from their $\dot{W}_{max}$ [see Table 1]), in conjunction with the higher $[La_b]_p$ and $\Delta[\dot{L}a_b]_{max}$ observed at Alt2 compared with Alt1, seems of interest in the discussion about the mechanisms underlying the reduced maximal lactic capacity at altitude. Indeed, $[La_b]_p$ determined by adopting a different exercise protocol (i.e., incremental bicycle ergometer exercises lasting 15–30 min), in the same environmental conditions of the present study, was found to decrease during the acclimatization process (18). This indicates that the reduced $[La_b]_p$ at altitude is, at least in part, related to the specific exercise protocol: during short supramaximal exercises, the factor(s) responsible for this reduction are partially or completely offset by acclimatization, whereas this does not appear to be the case for exercises of longer duration.

Incidently, the $[La_b]_p$ values obtained at Alt2 on *subjects 1* and *2* (11.56 and 10.47 mM, respectively; see Table 1) appear quite extraordinary. Arterialized blood $[HCO_3^-]$ levels at Alt2 were, in these subjects at rest, equal to 17.3 and 14.3 mM, respectively (18). At the end of the exhaustive exercises, if we assume an arterial P_{CO_2} of ~15 Torr, their arterialized blood pH can be calculated to be, according to the Henderson-Hasselbalch equation, equal to 7.20 and 7.06, respectively.

Blood Lactate Washout Kinetics

A descriptive analysis of the blood lactate washout curves during the resting recovery of the exhaustive exercises was also performed. These curves were not influenced by altitude exposure and resulted, in all conditions, in close agreement with the model proposed by Oyono-Enguelle et al. (31). According to these authors, blood lactate washout kinetics is influenced by the origin of the blood samples (arterial, arterialized, or venous) (31), by the intensity (14), and by the duration (15) of the preceding exercise. All of these factors were standardized in the present study. Not standardized were the $[La_b]_p$ values, which were significantly lower at altitude compared with sea level. However, according to di Prampero (9), blood lactate washout kinetics after exhaustive exercises at sea level are similar for $[La_b]_p$ values in the range of 4–16 mM, which include those obtained in the present study. Possible inferences from the finding of very similar blood lactate washout curves in all conditions, as related to the validity of the previously mentioned assumptions, were discussed above. A more practical consideration is that at altitude the timing of blood sampling for the determination of $[La_b]_p$ during the recovery after exhaustive exercises can be the same as at sea level. The results of the present study partially

confirm previous observations by Cerretelli et al. (6), who observed at altitude, after a relatively longer delay, a blood lactate disappearance kinetics similar to that described at sea level.

Conclusions

The finding of a reduced $\Delta[\dot{L}a_b]_{max}$ at altitude seems compatible with the hypothesis of a reduced maximal glycolytic flux, which could be at least in part responsible for the lower $[La_b]_p$. Both $\Delta[\dot{L}a_b]_{max}$ and $[La_b]_p$ increased during the acclimatization process. Considering that in chronic hypoxia the maximal potential activity of glycolytic enzymes seems preserved, it can be hypothesized that an inhibition of glycolysis at altitude takes place upstream. Upstream may indicate, besides the rather unlikely possibility of some depletion of glycogen stores, the β-adrenergic modulation of glycogenolysis, and/or, most likely, the level of neuromuscular activation.

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REFERENCES

1. **Bender, P. R., B. M. Groves, R. E. McCullough, R. G. McCullough, L. Trad, A. J. Young, A. Cymerman, and J. T. Reeves.** Decreased exercise muscle lactate release after altitude acclimatization. *J. Appl. Physiol.* 67: 1456–1462, 1989.
2. **Bigland-Ritchie, B., and N. K. Vollestad.** Hypoxia and fatigue: how are they related? In: *Hypoxia. The Tolerable Limits*, edited by J. R. Sutton, C. S. Houston, and G. Coates. Indianapolis, IN: Benchmark, 1988, p. 315–326.
3. **Brooks, G. A.** Current concepts in lactate exchange. *Med. Sci. Sports Exercise* 23: 895–906, 1991.
4. **Brooks, G. A., G. E. Butterfield, R. R. Wolfe, B. M. Groves, R. S. Mazzeo, J. R. Sutton, E. E. Wolfel, and J. T. Reeves.** Decreased reliance on lactate during exercise after acclimatization to 4,300 m. *J. Appl. Physiol.* 71: 333–341, 1991.
5. **Brooks, G. A., E. E. Wolfel, B. M. Groves, P. R. Bender, G. E. Butterfield, A. Cymerman, R. S. Mazzeo, J. R. Sutton, R. R. Wolfe, and J. T. Reeves.** Muscle accounts for glucose disposal but not blood lactate appearance after acclimatization to 4,300 m. *J. Appl. Physiol.* 72: 2435–2445, 1992.
6. **Cerretelli, P., A. Veicsteinas, and C. Marconi.** Anaerobic metabolism at high altitude: the lactacid mechanism. In: *High Altitude Physiology and Medicine*, edited by W. Brendel and R. A. Zink. New York: Springer-Verlag, 1982, p. 94–102.
7. **Cerretelli, P., B. Grassi, and B. Kayser.** Anaerobic metabolism at altitude: recent developments. In: *Hipoxia: Investigaciones Básicas y Clínicas*, edited by F. León-Velarde and A. Arregui. Lima: IFEA-UPCH, 1993, p. 167–179.
8. **Cibella, F., G. Cuttita, B. Kayser, M. Narici, and F. Saibene.** Respiratory muscle fatigue at high altitude (Abstract). *Physiologist* 35: 228, 1992.
9. **Di Prampero, P. E.** Energetics of muscular exercise. *Rev. Physiol. Biochem. Pharmacol.* 89: 143–222, 1981.
10. **Durnin, J. V. G. A., and J. Womersley.** Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged 16 to 72 years. *Br. J. Nutr.* 32: 77–97, 1974.
11. **Edwards, H. T.** Lactic acid in rest, and work at high altitude. *Am. J. Physiol.* 116: 367–375, 1936.
12. **Ferretti, G., H. Hauser, and P. E. di Prampero.** Maximal muscular power before and after exposure to chronic hypoxia. *Int. J. Sports Med.* 11, *Suppl.* 1: S31–S34, 1990.
13. **Fitts, R. H.** Cellular mechanisms of muscle fatigue. *Physiol. Rev.* 74: 49–94, 1994.
14. **Freund, H., S. Oyono-Enguelle, A. Heitz, J. Marbach, C. Ott, P. Zoulomian, and E. Lampert.** Work rate-dependent lactate kinetics after exercise in humans. *J. Appl. Physiol.* 61: 932–939, 1986.
15. **Freund, H., S. Oyono-Enguelle, A. Heitz, J. Marbach, C. Ott, and M. Gartner.** Effect of exercise duration on lactate kinetics after short muscular exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 58: 534–542, 1989.
16. **Gisolfi, F., S. Robinson, and E. S. Turrell.** Effects of aerobic work performed during recovery from exhausting work. *J. Appl. Physiol.* 21: 1767–1722, 1967.
17. **Gollnick, P. D., R. B. Armstrong, W. L. Sembrovich, R. E. Sheperd, and B. Saltin.** Glycogen depletion pattern in human skeletal muscle fibers after heavy exercise. *J. Appl. Physiol.* 34: 615–618, 1973.
18. **Grassi, B., B. Kayser, M. Marzorati, A. Colombini, C. Marconi, and P. Cerretelli.** Peak blood lactate concentration and blood buffering capacity during high-altitude acclimatization and deacclimatization in humans (Abstract). *Physiologist* 35: 211, 1992.
19. **Green, H. J., J. R. Sutton, P. Young, A. Cymerman, and C. S. Houston.** Operation Everest II: muscle energetics during maximal exhaustive exercise. *J. Appl. Physiol.* 66: 142–150, 1989.
20. **Green, H. J., J. R. Sutton, A. Cymerman, P. M. Young, and C. S. Houston.** Operation Everest II: adaptations in human skeletal muscle. *J. Appl. Physiol.* 66: 2454–2461, 1989.
21. **Green, H. J., J. R. Sutton, E. E. Wolfel, J. T. Reeves, G. E. Butterfield, and G. A. Brooks.** Altitude acclimatization and energy metabolic adaptations in skeletal muscle during exercise. *J. Appl. Physiol.* 73: 2701–2708, 1992.
22. **Greenhaff, P. L., M. E. Nevill, K. Soderlund, K. Bodin, L. H. Boobis, C. Williams, and E. Hultman.** The metabolic responses of human type I and II muscle fibres during maximal treadmill sprinting. *J. Physiol. Lond.* 478: 149–155, 1994.
23. **Howald, H., D. Pette, J.-A. Simoneau, A. Uber, H. Hoppeler, and P. Cerretelli.** Effects of chronic hypoxia on muscle enzyme activities. *Int. J. Sports Med.* 11, *Suppl.* 1: S10–S14, 1990.
24. **Jones, P. R. M., and J. Pearson.** Anthropometric determination of leg fat and muscle plus bone volumes in young male and females adults. *J. Physiol. Lond.* 204: 63P–67P, 1969.
25. **Kayser, B., G. Ferretti, B. Grassi, T. Binzoni, and P. Cerretelli.** Maximal lactic capacity at altitude: effect of bicarbonate loading. *J. Appl. Physiol.* 75: 1070–1074, 1993.
26. **Kayser, B., M. Narici, T. Binzoni, B. Grassi, and P. Cerretelli.** Fatigue and exhaustion in chronic hypobaric hypoxia: influence of exercising muscle mass. *J. Appl. Physiol.* 76: 634–640, 1994.
27. **Margaria, R., P. Cerretelli, and F. Mangili.** Balance and kinetics of anaerobic energy release during strenuous exercise in man. *J. Appl. Physiol.* 19: 623–628, 1964.
28. **Mazzeo, R. S., G. A. Brooks, G. E. Butterfield, A. Cymerman, A. C. Roberts, M. Selland, E. E. Wolfel, and J. T. Reeves.** β-Adrenergic blockade does not prevent the lactate response to exercise after acclimatization to high altitude. *J. Appl. Physiol.* 76: 610–615, 1994.
29. **McLellan, T. M., M. F. Kavanagh, and I. Jacobs.** The effect of hypoxia on performance during 30 s or 45 s supramaximal exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 60: 155–161, 1990.
30. **MacDougall, J. D., H. J. Green, J. R. Sutton, G. Coates, A. Cymerman, P. Young, and C. S. Houston.** Operation Everest II: structural adaptations in skeletal muscle in response to extreme simulated altitude. *Acta Physiol. Scand.* 142: 421–427, 1991.
31. **Oyono-Enguelle, S., M. Gartner, J. Marbach, A. Heitz, C. Ott, and H. Freund.** Comparison of arterial and venous blood lactate kinetics after short exercise. *Int. J. Sports Med.* 10: 16–24, 1989.
32. **Roth, D. A., and G. A. Brooks.** Lactate transport is mediated

by a membrane-bound carrier in rat skeletal muscle sarcolemmal vescicles. *Arch. Biochem. Biophys.* 279: 377–385, 1990.

33. **Saltin, B., and B. Essén.** Muscle glycogen, lactate, ATP and CP in intermittent exercise. In: *Muscle Metabolism During Exercise*, edited by B. Pernow and B. Saltin. New York: Plenum, 1971, p. 419–424.
34. **Sutton, J. R., N. L. Jones, and C. J. Toews.** Effect of pH on muscle glycolysis during exercise. *Clin. Sci. Lond.* 61: 331–338, 1981.
35. **Sutton, J. R., and G. J. F. Heigenhauser.** Lactate at altitude. In: *Hypoxia: the Adaptations*, edited by J. R. Sutton, G. Coates, and J. E. Remmers. Toronto: Dekker, 1990, p. 94–97.
36. **West, J. B., S. J. Boyer, D. J. Graber, P. H. Hackett, K. H. Maret, J. S. Milledge, R. M. Peters, Jr., C. J. Pizzo, M. Samaja, F. H. Sarnquist, R. B. Schoene, and R. M. Winslow.** Maximal exercise at extreme altitudes on Mount Everest. *J. Appl. Physiol.* 55: 688–698, 1983.
37. **West, J. B.** Lactate during exercise at altitude. *Federation Proc.* 45: 2953–2957, 1986.
38. **Young, A. J., W. J. Evans, A. Cymerman, K. B. Pandolf, J. J. Knapik, and J. T. Maher.** Sparing effect of chronic high-altitude exposure on muscle glycogen utilization. *J. Appl. Physiol.* 52: 857–862, 1982.
39. **Young, A. J., W. J. Evans, E. C. Fisher, R. L. Sharp, D. L. Costill, and J. T. Maher.** Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence. *Eur. J. Appl. Physiol. Occup. Physiol.* 52: 463–466, 1984.
40. **Young, A. J., P. M. Young, R. E. McCullough, L. G. Moore, A. Cymerman, and J. T. Reeves.** Effect of beta-adrenergic blockade on plasma lactate concentration during exercise at high altitude. *Eur. J. Appl. Physiol. Occup. Physiol.* 63: 315–322, 1991.
41. **Zar, J. H.** *Biostatistical Analysis* (2nd ed.). Englewood Cliffs, NJ: Prentice Hall, 1984.