Eur J Appl Physiol (1984) 52: 463–466

European Journal of
Applied
Physiology
and Occupational Physiology

Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence

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Summary. The influence of short-term high-altitude (HA) residence on intramuscular pH and skeletal muscle enzyme activity of sea-level (SL) residents was investigated. Vastus lateralis muscle samples were obtained by biopsy from rested subjects ($n = 5$) at SL (50 m) and on the 18th day of HA residence (4,300 m) for determination of glycogen phosphorylase, hexokinase, malate dehydrogenase, and total lactate dehydrogenase activities. A second group of subjects ($n = 6$) performed cycle exercise of the same absolute intensity (mean ± SE = 195 ± 5 W) at SL and on the 15th day of residence at HA. Before and immediately after exercise, vastus lateralis muscle samples were obtained for the determination of intramuscular pH, and venous blood was obtained for determination of lactate concentration. The first group of subjects showed no significant changes in skeletal muscle enzyme activity after 18 days at HA. The second group of subjects were instructed to exercise for exactly 30 min, and all but one could complete the entire bout at SL. However, at HA, none could continue 30 min, and time to exhaustion (mean ± SE) was 11.9 ± 1.6 min. Resting intramuscular pH was not significantly different after HA residence as compared to SL. The fall in intramuscular pH was less with exercise on day 15 at HA than during SL exercise. Likewise, the increase in blood lactate concentration with exercise at HA was less than at SL. These data indicate that, after 15–18 days of HA residence, limitations in exercise performance are not due to inordinate intramuscular acidosis or to changes in the activity of glycolytic and oxidative enzymes.

Key words: Hypoxia – Intramuscular pH – Exercise – Muscle enzymes – Acclimation

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Introduction

Sea-level residents rapidly taken to high altitude experience a decrement in aerobic work capacity. This impairment is reflected by a reduction in both maximal oxygen uptake ($\dot{V}_{O_2\,max}$) and endurance for submaximal exercise. When high altitude exposure is continued for several days, adaptation to hypoxia results in an improvement in submaximal performance. Maher et al. (1974) reported that the endurance time (cycling at 75% $\dot{V}_{O_2\,max}$) of subjects residing for 12 days at 4,300 m was increased by 45% on day 12 as compared to day 2. Likewise, Horstman et al. (1980) observed a 59% increase in time to exhaustion (treadmill running at 85% $\dot{V}_{O_2\,max}$) between day 16 and day 1 of residence at 4,300 m. If the fundamental relationship between endurance capacity, $\dot{V}_{O_2\,max}$ and rate of metabolism of energy substrate is unchanged at high altitude, then the improvement in endurance time during the first few weeks of altitude residence must be a result of increased $\dot{V}_{O_2\,max}$ (Gleser and Vogel 1973) or changes in skeletal muscle metabolism which retard the rate at which muscle glycogen stores are depleted (Gleser and Vogel 1973; Hultman 1967).

It has been shown that $\dot{V}_{O_2\,max}$ does not change significantly during the first 15 days of residence at 4,300 m (Young et al. 1982). However, increased mobilization and utilization of free fatty acids during exercise with associated sparing of muscle glycogen stores have been found after 18 days at altitude (Young et al. 1982). Similar metabolic adaptations have been observed to result from chronic endurance training (Holloszy and Booth 1976). The purpose of the present investigation was to determine if short-term HA exposure would influence the activity of skeletal muscle enzymes similar to the effect of chronic exercise. It was hypothesized that, as a result of altitude exposure, skeletal muscle hexokinase and malate dehydrogenase activity would be increased,

and skeletal muscle glycogen phosphorylase and lactate dehydrogenase would be decreased. Furthermore, since the rate of glycolysis is affected by altering the pH of the reaction medium (Gevers and Dowdle 1963; Trivedi and Danforth 1966), it was also hypothesized that altitude exposure would result in a change in intramuscular pH.

Methods

Test subjects. Eleven male soldiers volunteered as subjects for this investigation after being fully informed of the nature and requirements of the study. All were sea-level (SL) residents, and none had lived at or visited high altitude (HA) for at least 10 months prior to participation in the experiments. The men participated in routine Army physical training; however, none were highly trained. $\dot{V}_{O_2\,max}$ (mean ± SE) was 46.0 ± 1.2 $ml \cdot min^{-1} \cdot kg^{-1}$ at SL. The subjects were asked to maintain physical activity at their usual level and not begin any new training program. Throughout the study, the subjects ate and drank ad libitum. The physical characteristics of the subjects (mean ± SE) were as follows: age, 21.8 ± 0.8 years; height, 176.0 ± 2.2 cm; and weight, 76.0 ± 1.4 kg.

Design. This investigation consisted of two series of experiments. Five subjects participated in the first series in which the influence of short-term altitude exposure on the activity of four skeletal muscle enzymes was studied. Samples of vastus lateralis muscle to be assayed for enzyme activity were obtained while these subjects were in a rested condition at SL (50 m) and again at HA (4,300 m) on day 18 of continuous residence. The remaining six subjects participated in the second series of experiments in which the change in intramuscular pH during exercise was measured at SL and on day 15 of continuous residence at 4,300 m. For these experiments, the subjects were instructed to cycle continuously for 30 min. Absolute exercise intensity (mean ± SE) was 195 ± 5 W at both SL and HA. Before, and immediately after exercise, samples of vastus lateralis muscle and venous blood were obtained to ascertain intramuscular pH and venous lactate concentration. Oxygen consumption was measured each minute of exercise.

Tissue samples. Muscle samples were obtained by biopsy (Bergstrom 1962). In the first series of experiments, specimens were quickly freed of connective tissue, divided into several pieces, and then frozen in liquid N_2. Samples obtained at SL were stored in liquid N_2 until enzyme analyses were performed. At HA, the samples were frozen in liquid N_2, packed in dry ice for shipment, and then returned to liquid N_2. Duplicate analyses were carried out for activity of glycogen phosphorylase (Costill et al. 1976), hexokinase (Mansour et al. 1966), malate dehydrogenase (Costill et al. 1979), and total lactate dehydrogenase (Bergmeyer 1974). In the second series of experiments, the entire muscle specimen was rapidly frozen by plunging the biopsy needle into liquid N_2 immediately upon withdrawing the needle from the muscle. The frozen samples were transferred to a vial and stored in liquid N_2 continuously (including shipment) until intramuscular pH was determined (Costill et al. 1982). The blood samples were obtained from a forearm vein. They were allowed to clot, and the serum was frozen for 24 h until lactate concentration was determined.

Exercise. All exercise was performed on electrically-braked cycle ergometers at a pedal frequency of 60 $rev \cdot min^{-1}$, paced by an electronic metronome. It was not possible to use the same ergometer at both SL and HA, so $\dot{V}_{O_2}$ was used to calibrate the exercise intensities on the two ergometers. Subjects breathed through a modified Otis-McKerrow valve connected to a # 3 pneumotach (Hewlett-Packard). Samples of expired air were analyzed for O_2 and CO_2 concentrations using gas analyzers (Applied Electrochemistry S-3A and Beckman LB-2, respectively). The computational procedures of Sue et al. (1980) were used to calculate $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (STPD) and $\dot{V}_E$ (BTPS). Prior to each experiment, gas analyzers were calibrated using gases of known composition.

Data analyses. The duplicate determinations of muscle enzyme activities, muscle pH, and blood lactate concentration were averaged. Differences between means of SL and HA enzyme activity were analyzed for statistical significance using Student's *t*-test. A three-way ANOVA and paired *t*-tests were used to study the influence of altitude, exercise, and individual subject variability on intramuscular pH and blood lactate. For all statistical compairsons, the level of significance was set at $P < 0.05$.

Results

Resting measures

Skeletal muscle enzyme activities. Table 1 shows the results of the enzyme analyses. There were no statistically significant changes in the activity of skeletal muscle glycogen phosphorylase, hexokinase, malate dehydrogenase, or total lactate dehydrogenase.

Exercise measures

Exercise and $\dot{V}_{O_2}$. At SL, $\dot{V}_{O_2}$ was (mean ± SE) 3.16 ± 0.26 $l \cdot min^{-1}$ (86 ± 2% $\dot{V}_{O_2\,max}$ at SL) and was not significantly different at HA where it was 3.05 ± 0.21 $l \cdot min^{-1}$ (97 ± 2% $\dot{V}_{O_2\,max}$ at HA). All but one of the subjects were able to complete the entire 30-min exercise bout at SL. The subjects were exhausted, and despite the strong verbal encouragement provided by the investigators, it is unlikely that they could have continued for much longer. At HA, strong verbal encouragement not withstanding, none of the

Table 1. Skeletal muscle enzyme activities

Enzyme	Activity ($\mu moles \cdot min^{-1} \cdot g^{-1}$ wet tissue)		
	Sea level	High altitude	*P*
Lactate dehydrogenase	186.60 ± 24.29	170.46 ± 19.87	NS
Malate dehydrogenase	55.39 ± 5.08	60.07 ± 4.90	NS
Glycogen phosphorylase	6.63 ± 0.76	6.07 ± 0.62	NS
Hexokinase	1.99 ± 0.11	2.37 ± 0.27	NS

Values are mean ± SE of samples collected at sea level and after 18 days of residence at 4,300 m. *n* = five male, sea level residents

subjects were able to exercise for the full 30 min, and time to exhaustion was (mean ± SE) 11.9 ± 1.6 min.

Intramuscular pH. Figure 1 shows the results of the intramuscular pH determinations, and Fig. 2 shows the results of the corresponding serum lactate determinations. For both intramuscular pH and serum lactate, the ANOVA revealed that, of the three factors (altitude, exercise, subjects), only the "exercise" factor had a significant main effect. There was, however, a significant interaction between the "exercise" and "altitude" factors on both intramuscular pH and serum lactate. To determine the meaning of these exercise-altitude interactions, the change (absolute difference) in pH and lactate from pre- to post-exercise was calculated for each subject, and a paired *t*-test used to compare the change at SL with the change at HA. Results of the test show that during exercise at HA there was a smaller fall in intramuscular pH than during exercise at SL. Similarly, there was a smaller rise in serum lactate with exercise at HA than at SL.

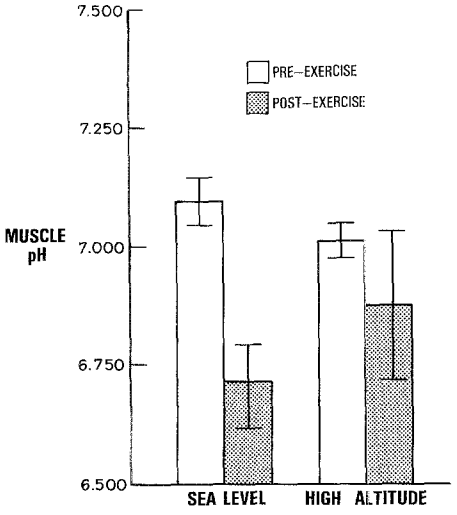

Fig. 1. Intramuscular pH (mean ± SE) of six sea level residents before and after exercise at sea level (50 m) and on the 15th day of residence at high altitude (4,300 m)

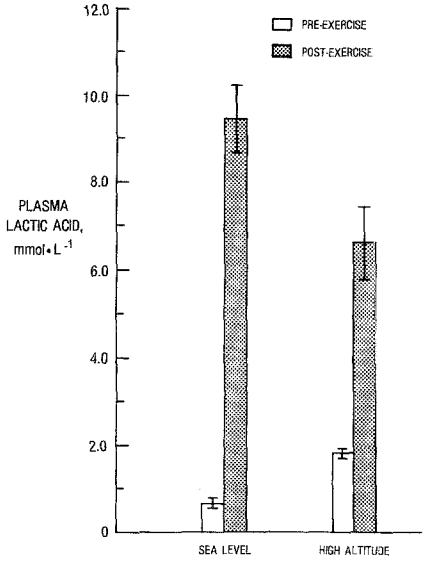

Fig. 2. Blood lactate concentration (mean ± SE) of six sea level residents before and after exercise at sea level (50 m) and on the 15th day of residence at high altitude (4,300 m)

Discussion

Sea-level residents chronically exposed to high altitude experience an adaptation in muscle metabolism during exercise (Young et al. 1982). This adaptation is similar to the metabolic adaptation associated with the increased endurance resulting from repeated bouts of endurance exercise at sea level, i.e., physical training has been shown to result in decreased glycogen utilization and increased mobilization of FFA during exercise (Holloszy and Booth 1976). Alterations in glycolytic and oxidative metabolic rates resulting either from induction or suppression of enzyme synthesis, or from changes in the factors which modulate the activity of enzymes (e.g., pH) are both known to be related to this response to physical training. Heretofore, the influence of chronic altitude exposure on enzyme activity and pH regulation in human skeletal muscle was not known. The muscle enzymes studied in the present investigation were not selected because they are the rate-limiting steps, but because they have been well studied in investigations of exercise-induced adaptations in muscle metabolism. It was thought that hexokinase and phosphorylase activities should be good indicators of glycolytic capacity, while lactate and malate dehydrogenase activities would reflect the oxidative capacity of the muscle.

An increase in skeletal muscle hexokinase activity, favoring increased use of glucose by the muscle and sparing of glycogen, has been shown to result from exercise (Holloszy and Booth 1976), but no change in hexokinase activity following altitude exposure was found in this investigation. Another enzymatic adaptation to chronic exercise is a decrease in the activity of both glycogen phosphorylase (Baldwin et al. 1973) and of lactate dehydrogenase (Baldwin et al. 1973; Holloszy and Booth 1976). The activity of neither of these enzymes was influenced by short-term residence at 4,300 m. However, both of these enzymes exist in two forms, and in the present study, only total activity was determined. Finally, activity of malate dehydrogenase, which is increased by physical training (Evans et al. 1979), was also

unchanged by altitude exposure. Thus, unlike the adaptation to chronic exercise, changes in the activity of skeletal muscle enzymes are not likely to account for changes in performance during short-term residence at altitude.

Changes in ambient pH affect the rate of glycogenolysis. Sutton et al. (1981) observed a reduction in the endurance time of men who were made acidotic before exercise, and concluded that acidosis had inhibited muscle glycolysis, thereby limiting glycogen utilization and reducing performance. In the present study, no significant difference was found between resting intramuscular pH at sea level and after 15 days of residence at 4,300 m. The drop in intramuscular pH with exercise was less at altitude compared to sea level. The increase in blood lactate with exercise was also less at altitude than at sea level. These observations indicate that formation of muscle lactate during exercise at altitude is inhibited via mechanisms other than those studied in this investigation. It seems reasonable to postulate that lactate production is reduced by an inhibition of glycolysis resulting from chronic HA exposure. As a result of chronic altitude exposure, others have shown that the number of mitochondria in tissue are increased (Reynafarje 1962). Cytoplasmic NADH concentration would be reduced since the mitochondrial shuttle and respiratory chain dehydrogenases have a higher affinity for NADH than the cytosol dehydrogenases (Lehninger 1970). A fall in cytosol NADH would favor direct oxidation of pyruvate to acetyl CoA instead of lactate.

Thus, in contrast to the effect of chronic exercise, the activity of the skeletal muscle enzymes hexokinase, total glycogen phosphorylase, malate dehydrogenase, and total lactate dehydrogenase was not changed by short-term altitude residence. Resting intramuscular pH was similarly unaffected by the high altitude exposure. Despite the fact that less blood lactate accumulated and that intramuscular pH was more alkaline with exercise at altitude than at sea level, exercise time was still shorter at high altitude.

Acknowledgements. The authors express their appreciation to Dr. Allen Cymerman for designing the respiratory exchange measuring system, and to Mr. Robert Feccia for his expert technical assistance.

Human subjects participated in this study after giving their free and informed voluntary consent. Investigators adhered to AR 7-25 and USAMRDC Regulation 70-25 on use of volunteers in research. The views, opinions and/or findings contained in this report are those of the authors and should not be construed an official Department of the Army position, policy or decision unless so designated by other official documentation.

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Accepted February 28, 1984