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Effects of endurance training at high altitude on diaphragm muscle properties

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Abstract. The biochemical, histochemical, and structural changes induced by endurance training and long-term exposure to high altitude were studied in the diaphragm muscle of rats exposed to simulated altitude (HA: $n = 16$; $P_b = 62$ kPa, 463 Torr; 4000 m) and compared to animals maintained at sea-level (SL: $n = 16$). Half of the animals in each group were trained (T) by swimming for 12 weeks, the other half were kept sedentary (S). Except for a small decrease in type I fibres in the HA-S group (−7%, $P<0.05$), in favour of type IIab and type IIb fibres, neither high-altitude exposure nor endurance training had an overall effect on fibre type distribution. The mean fibre cross-sectional area was found to be unaffected by altitude and/or chronic exercise. Capillary density was shown to be increased by both high-altitude exposure ($P<0.02$) and training ($P<0.001$), whereas capillary growth, estimated by the capillary/fibre ratio, was unaffected in both cases. Following endurance training, a modest increase in citrate synthase was shown to occur to the same extent in the HA-T and SL-T groups (+15% and +16% respectively, NS). Hexokinase increased following training ($P<0.05$) and high-altitude exposure ($P<0.001$). In normoxic and hypoxic animals, endurance training enhanced the ratio of the heart-specific lactate dehydrogenase isozyme LDH1 to total LDH activity (+59%, $P<0.01$; +92%, $P<0.05$ respectively). It may be hypothesized that the increased glucose phosphorylation capacity observed in diaphragm muscle contributes to the reduction of glycogen utilization during exercise.

Key words: Rat diaphragm – Endurance training – High-altitude exposure – Fibre-type composition – Capillary bed – Enzyme activities – Hexokinase

Introduction

In mammals, the diaphragm is the main inspiratory muscle, and like the heart, it is chronically active. Locomotor skeletal muscles subjected to increased use undergo adaptative cellular responses due to an increase in respiratory capacity (for review see [11]). The nature and the importance of these adaptations depend on the type, intensity, and daily duration of the exercises performed [8].

Like other skeletal muscles, the diaphragm is able to adapt to increased use, and these cellular adaptations are of a histochemical and biochemical nature [15]. It is now well known that, in the rat, the diaphragm increases its contractile activity during exercise since both tidal volume and ventilatory frequency are increased [17]. Previous studies have shown that chronic exercise failed to provoke changes in fibre type distribution [12]. However, Lieberman et al. [16] have shown that the diaphragm of trained guinea-pigs has 20% more red fibres (stained for succinic dehydrogenase activity) than are found in control animals. Few published reports have demonstrated an increase in the oxidative capacity of the diaphragm following endurance training [12, 23]. Nevertheless, conflicting results have been reported by Metzger and Fitts [22]. They have shown that, unlike the response of limb skeletal muscle, high-intensity training has only a minor effect on the biochemical properties of both the ventral costal and crural diaphragm regions of the rat.

It appears that chronic hypoxia leads to an increase in the frequency of contractions in respiratory muscles. Olson and Dempsey [24] observed an 80%–90% increase in the pulmonary minute ventilation (determined at rest) of rats acclimatized to high altitude (4300 m). On the other hand, it has been shown that, in diaphragm muscle, high-altitude exposure of long duration reduces the mean fibre cross-sectional area and shortens the oxygen diffusion distances [30].

Some previous data have shown that, in human subjects, exercise performed at high altitude elicits a significant increase in ventilatory response [7, 20]. These results provide indirect evidence of the increase in workload on respiratory muscles during exercise performed in hypoxic environments. On the other hand, it was clearly shown that, whereas prolonged normoxic exercise failed to induce significant glycogen utilization in the right costal di-

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aphragm region in the rat, prolonged hypoxic exercise ($FIO_2 = 0.12$) substantially reduced glycogen concentration and elevated lactate accumulation [9]. These data give indirect evidence of increased diaphragmatic work during prolonged exercise in acute hypoxia.

However, there are no previously published reports concerning the nature and extent of adaptative changes observed in the diaphragm after training exercise performed at high altitude. Thus, the purpose of this study was to explore the biochemical, histochemical and tissue capillarity changes that occur in the rat diaphragm after endurance training, and/or long-term exposure to a simulated hypobaric altitude (4000 m).

Materials and methods

Animal care. A group of 32 male albino rats, Wistar strain, weighing approximately 175–180 g were randomly assigned to one of two groups: high-altitude (HA) and sea-level residents (SL). All animals were housed 4 per cage with a dark/light cycle of 12 h/12 h, and maintained on a standard diet and water ad libitum.

Altitude was simulated in a hypobaric chamber. During the first 2 days, the pressure was maintained at 85 kPa (635 Torr; 1500 m). It was gradually reduced until it came down to 62 kPa (463 Torr; 4000 m) after 12 days. In each series (SL or HA), the animals were assigned to a sedentary (S) or a trained group (T). Thus the 32 rats were divided into four groups, each with 8 animals: sea-level sedentary (SL-S), sea-level trained (SL-T), high-altitude sedentary (HA-S) and high-altitude trained (HA-T).

Exercise-trained animals were subjected to a swimming programme. Complete details of the training regimen have been published elsewhere [3, 4]. Exercise-trained animals were maintained in a small hypobaric chamber and moved to a larger chamber for their swimming sessions. Swimming was performed in cylinder tanks in which the water was regulated at 36 °C. On the first day, the rats swam for 20 min. The swimming time was progressively increased to 1 h/day, repeated 5 days/week, for 12 consecutive weeks. Each exercise session was carried out at sea-level for the SL-T group, and in a large hypobaric chamber for the HA-T group.

Muscle preparation. The animals were randomly divided up 24 h after the last exercise session and sacrificed in four groups. They were anaesthetized with sodium pentobarbitone, administered intraperitoneally (50 mg kg^{-1} body mass). Their diaphragms were excised immediately after exsanguination. In order to select the most responsive diaphragm region, and in accordance with Powers et al. [25], two segments were excised from the costal region. The right dorsal costal portion was quickly frozen in liquid nitrogen for biochemical assays. The right ventral costal portion was cleaned of excess blood, adipose and connective tissue, mounted in an embedding medium (TEK O.C.T. compound), and frozen in isopentane cooled to freezing point by liquid nitrogen for histochemical analysis. All samples were stored at −80 °C until biochemical and histochemical procedures were conducted.

Histochemical methods. Serial transverse sections (18–20 μm) were cut in a cryostat at −20 °C. After being incubated in alkaline and acid buffers, fibre types were stained for myofibrillar ATPase and classified according to the method of Brooke and Kaiser into groups I, IIa, or IIb [5]. In addition, intermediate fibres, types IIc and IIab, were taken into account. After preincubation at pH 4.32, type IIc fibres showed staining characteristics intermediate between type I and type II fibres [5]. After preincubation at pH 4.55, type IIab fibres showed staining characteristics intermediate between type IIa and type IIb fibres [13]. The general fibre composition of the muscle for each fibre type was determined by using a number of fields equally distributed over the biopsy, with a minimum of 1000 fibres/sample.

Fibre areas. The fibre cross-sectional areas (A_{fcs}) of each main type of fibre were determined using a Leitz texture analysis system (TAS Leitz, Heerbrugg, Switzerland). Sections were examined under a Leitz Orthoplan microscope connected to a Plumbicon camera (Bosh type TKY9, Wild Leitz, Rueil, France) with an analog-to-digital conversion system. Each fibre area was determined using a spotlight. Geometric operations were performed on the digitized image using an LSI microprocessor. On an average, 60 cross-sectional areas of type I fibres and 80–100 of type IIa and IIb fibres were measured in each muscle sample. For each sample, the mean A_{fcs} was determined as the sum of the product of the A_{fcs} of each fibre type and its percentage observed in the biopsy.

Capillary staining. Staining for myofibrillar ATPase, after preincubation at pH 4, was used to visualize the capillaries, according to the method indicated by Sillau and Banchero [29]. Capillaries were identified on a screen coupled with a light microscope (Reichert type Visopan, by Leica, Heerbrugg, Switzerland). Several areas were randomly selected on each sample, and they represented a total area, A. The capillary bed was appraised according to the following parameter: capillary density was calculated as the number of capillaries in the total area A divided by the area of A; the capillary-to-fibre ratio (C/F) was determined as capillary density to fibre density, where fibre density represents the mean number of fibres per square millimeter [21].

Biochemical methods. All samples were placed in tubes and lyophilized. Blood, fat and connective tissue were removed from muscles in an air-conditioned room maintained at 20 °C, with 25% relative humidity. The samples were weighed in the room and homogenized at a dilution level of 1/400. Homogenization was carried out at local temperature (between 0 and −4 °C) in a 0.3 M phosphate buffer with a pH of 7.7, and containing 0.05% bovine serum albumin. Enzyme activities were determined on this homogenate by using NAD/NADH enzymatic fluorometric tests at 25 °C with a Gilson spectra/glofluorometer, according to the Lowry and Passoneau method [18]. They were then expressed on the basis of the amount of substrate utilized (μmol) min^{-1} g muscle $protein^{-1}$. Muscle protein was determined according to the method described by Lowry et al. [19]: bovine serum albumin served as the standard, and a Beckman spectrophotometer was used. The activities of several enzymes were determined: 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) was used to estimate the β-oxidation of the fatty acids; citrate synthase (EC 4.1.3.7) was used to measure citric acid cycle activity; hexokinase (EC 2.7.1.1) was used to test glucose phosphorylation capacity, and lactate dehydrogenase (EC 1.1.1.27) was used to determine lactate production. Lactate dehydrogenase isozyme 1, associated with the oxidation of lactate to pyruvate, was tested using homogenates thermo-inactivated at 65 °C and 2-oxobutyrate as substrate, according to the method described by Karlson et al. [14].

Statistics. A multifactor analysis of variance was used to examine the global effects of long-term exposure to high altitude and endurance training on the following response variables: main and intermediate fibre types distributions, mean fibre cross-sectional area, muscle capillarity and cellular enzyme activities. In addition, interaction of high-altitude and endurance training was examined on each response variable. Comparisons between groups were tested using a Student's t-test in order to examine the specific effect of both endurance training on SL or HA animals, and high-altitude exposure on sedentary or trained rats. A level of $P<0.05$ was selected to indicate statistical significance. Values are expressed as means ± the standard error of the mean (SEM).

Results

Body mass

We reported elsewhere that exposure to high altitude reduced the growth rate of animals [3, 4]. During the experimental period, body mass gain was estimated to be

217% in SL-S animals, and 137% in HA-S rats. During the same period SL-T animals gained less body mass than HA-T rats: 127%, versus 168%. Moreover, training-induced reduction in growth-rate was more obvious in SL than in HA rats: by the end of the experimental period, the mean body mass of SL-T animals was 13% less than that of SL-S rats ($P<0.01$), whereas it was 4% less in HA-T than in HA-S rats (NS).

Fibre type distribution

Neither high-altitude exposure nor endurance training affected the percentage of fibre types I, IIa, or IIb (Table 1). However, the percentage of the intermediate fibre type IIab was increased by long-term exposure to high altitude ($P<0.025$) to a similar extent in both sedentary and trained animals. On the other hand, in animals that remained sedentary, type I fibres decreased with altitude (−7%, $P<0.05$). This decrease corresponded to an increased proportion of type IIab ($P<0.05$) and to a slight enhancement in the percentage of type IIb fibres (NS).

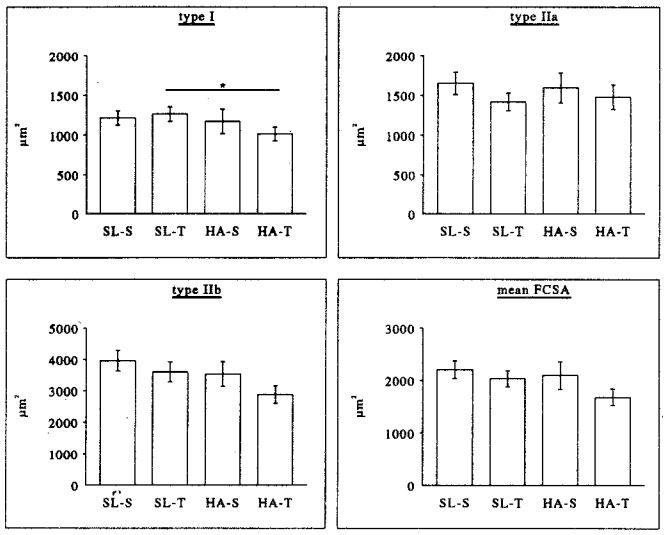

Fig. 1. Changes in fibre cross-sectional area (*FCSA*) observed in diaphragm muscle after chronic hypoxia and/or endurance training. Values are means ± SE; *$P<0.05$

Fibre areas

The fibre cross-sectional area (A_{fcs}) of each main fibre type was as a whole unaffected by high altitude and/or chronic exercise (Fig. 1). Only a significant decrease in type 1 A_{fcs} was observed in animals trained at high altitude in comparison with those trained at sea-level (−19.7%, $P<0.05$). A high degree of heterogeneity was shown to occur among the A_{fcs} values of the three main muscle fibre types and among the animals. This fact was confirmed by a high level of dispersion among the means. Consequently, the mean A_{fcs} was unaffected by both high-altitude exposure and exercise training (Fig. 1). On the other hand, it was shown that the changes in mean A_{fcs} were not statistically dependent on body mass. These date showed that, in the diaphragm, there was no linear model allowing the mean A_{fcs} (i.e. the dependent variable) to be related to body mass (i.e. the independent variable): $r = 0.25$, $P>0.17$.

Diaphragm capillarity

Capillary density increased with both high-altitude exposure (overall effect significant at $P<0.02$) and endurance training (overall effect significant at $P<0.001$; Fig. 2). The increase with endurance training was more significant in animals acclimatized to high altitude (+25%, $P<0.01$) than in animals living at sea level (+22%, $P<0.05$). It was observed that capillary density (CD) decreased hyperbolically with mean A_{fcs}: $1/CD = 2.464 \times 10^{-3} + (9.060 \times 10^{-7} \times \text{mean } A_{fcs})$, $r = 0.72$, $P<0.001$. This relationship was ascertained for all animals maintained sedentary or exercise-trained at high altitude or in sea-level environments. Neither chronic exercise nor long-term exposure to high altitude produced significant changes in C/F (Fig. 2).

Biochemical results

The data resulting from the determinations of enzyme activities showed that citrate synthase was increased following the training programme (overall effect significant at $P<0.05$; Table 2). The results showed an increase in citrate synthase activity, which was observed to a similar extent in sea-level-trained animals (+16%) and in high-

Table 1. Fibre type distribution in the right dorsal costal sections of the diaphragm in sea-level (SL) or high-altitude (HA) animals, trained (T) or maintained sedentary (S)[a]

Animal group	Fibre type				
	I (%)	IIc (%)	IIa (%)	IIab (%)	IIb (%)
SL-S ($n = 8$)	37.58 ± 0.49	0.47 ± 0.01	26.89 ± 1.47	2.27 ± 0.41	32.77 ± 1.36
SL-T ($n = 8$)	38.18 ± 1.19	0.16 ± 0.04 *1	26.47 ± 1.54	2.26 ± 0.35	32.92 ± 0.93
HA-S ($n = 8$)	34.95 ± 1.09 *2	0.16 ± 0.03 *3	26.71 ± 1.52	3.33 ± 0.34 *2	34.84 ± 1.82
HA-T ($n = 7$)	38.97 ± 1.78	0.35 ± 0.11	25.50 ± 1.89	3.36 ± 0.53	31.81 ± 1.63
Overall effect of training	NS	NS	NS	NS	NS
Overall effect of altitude	NS	NS	NS	$P<0.025$	NS
Interaction altitude/training	NS	$P<0.05$	NS	NS	NS

[a] Data are means ± SE

*1 $P<0.01$, compared with sedentary groups; *2 $P<0.05$, *3 $P<0.01$, compared with sea-level groups

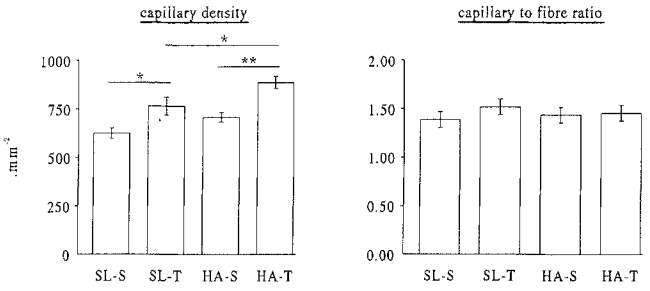

Fig. 2. Effects of high altitude exposure and chronic exercise on diaphragm capillarity in rats. Values are means±SE; $*P<0.05$ $**P<0.01$

altitude-trained animals (+15%), but there was no statistical significance in either case. The activity of the key enzyme involved in β-oxidation of fatty acids (3-hydroxyacyl-CoA dehydrogenase) was unaffected by the training programme or by chronic hypoxia. Hexokinase activity (i.e. the enzyme representative of glucose phosphorylation) was increased as a consequence of exercise training ($P<0.05$) and long-term exposure to high altitude (overall effect significant at $P<0.001$; Table 2). At both sea level and high altitude, the training programme resulted in a decrease in lactate dehydrogenase activity ($P<0.001$); this decrease was the same for animals trained at sea-level (−21%, $P<0.01$) and high altitude (−28%, $P<0.01$). Chronic exercise increased the ratio of the heart-specific isozyme (LDH 1) to total lactate dehydrogenase activity by +92% in sea-level animals ($P<0.05$) and by +59% in high-altitude-trained animals ($P<0.01$). The total lactate dehydrogenase activity and the distribution of the LDH 1 seemed to be unaffected by chronic hypoxia.

Discussion

The results of this study showed that exercise training performed at high altitude had only moderate structural and morphological effects on the diaphragm muscle. Likewise, slight but non-significant variations in the oxidative capacity of the muscle following training were observed to be present to the same degree in animals trained at high altitude and sea-level.

The significant increase in capillary density following chronic exercise at sea-level was the result of two factors, first the non-significant decrease in mean A_{fcs} (−8%), second the non-significant increase in capillary growth (C/F ratio +9%). Despite the slight decrease in mean A_{fcs}, there was an apparent increase in the percentage of the area occupied by type I fibres (i.e. the percentage of type I fibres×mean A_{fcs} of type I fibres/mean A_{fcs}: 21% in the SL-S group, versus 24% in the SL-T group ($P<0.05$). This trend towards an increase in the area occupied by type I fibres appears to be related to the slight increase observed in citrate synthase activity, a key enzyme of the tricarboxylic acid cycle (+16%). These adaptative changes were observed under conditions of endurance training using a mild exercise that corresponds to 50%−65% of the maximum aerobic capacity of a rat [28]. The results obtained under normoxic conditions agree with previous work showing that training programmes of high intensity have only minor effects on histochemical and metabolic properties of the diaphragm [12, 22, 23].

Short-term exposure to hypoxia is known to induce ventilatory acclimatization, which differs greatly among species (for review see [6]). In acclimatized rats, the rise observed in minute ventilation is relatively large and is attributable to an increase in both breathing frequency and tidal volume [24]. Snyder et al. [30] reported that chronic hypoxia does not modify the percentage of oxidative fibres. Under our conditions, the slight decrease in the percentage of type I fibres observed in sedentary animals subjected to long-term exposure to high altitude (−7%, $P<0.05$) did not conflict with these results since no change was observed when the percentages of type I and IIa fibres were pooled. The percentage in the area occupied by type I fibres remained constant: 21% in the SL-S group versus 20% in the HA-S group. Thus, it appears that the increased percentage of fast-twitch fibres observed in rats acclimatized to high altitude was minimized by the non-significant changes in the A_{fcs} of the slow-twitch fibres. On the other hand, we did not find statistical changes in C/F, and these results confirm that chronic hypoxia (up to 62 kPa, 463 Torr, barometric pressure) does not stimulate the development of new capillaries [30]. As with training at sea-level, enhanced capillary density following prolonged exposure to high altitude did

Table 2. Effects of training (T) and high altitude (HA) on metabolic adaptations of diaphragm muscle

Animal group	Enzyme activity (IU/g)[a]				LDH1/total
	CS	HAD	HK	LDH	
SL-S (n = 8)	72.41±3.72	52.86±2.26	7.77±0.47	2129±147	0.036±0.006
SL-T (n = 8)	84.23±4.97	48.73±3.03	9.04±0.37 *1	1689±40 *2	0.069±0.013 *1
HA-S (n = 8)	79.41±3.96	50.07±2.88	9.87±0.79 *3	2379±129	0.042±0.007
HA-T (n = 7)	91.24±5.24	51.38±1.91	11.05±0.41 *4	1699±113 *2	0.067±0.005 *2
Overall effect of training	$P<0.05$	NS	$P<0.05$	$P<0.001$	$P<0.005$
Overall effect of altitude	NS	NS	$P<0.001$	NS	NS
Interaction altitude/training	NS	NS	NS	NS	NS

[a] Values are means±SE for citrate synthase (CS), hydroxyacyl-CoA dehydrogenase (HAD), hexokinase (HK), and lactate dehydrogenase (LDH) LDH1/total, isozyme1-to-total LDH activity ratio

*1 $P<0.05$, *2 $P<0.01$ compared with sedentary groups; *3 $P<0.05$, *4 $P<0.01$ compared with sea-level groups

not result in real increased muscle capillarity but is dependent on both a slight mean A_{fcs} reduction (−5%) and a non-significant increase in C/F (+3.2%).

The main results of this study emphasized the slight changes observed in the diaphragm after exercise training performed at high altitude. It has been shown that short-term acclimatization to moderate altitude (3100 m) induced a significant increase in exercise ventilation at moderate (+31% to +33%) and heavy work loads (+43%) [7]. It seemed likely that under our experimental conditions, the expected ventilatory responses to swimming would be 30% greater at high altitude than at sea-level. No previous studies concerning the adaptations of the diaphragm muscle to chronic exercise performed at high altitude have been found to date. Although no significant linear relationship between mean A_{fcs} and body mass was observed, mean A_{fcs} values undoubtedly tended to decrease with both endurance training and high-altitude exposure. The decrease of only 8% in body mass as a result of training performed at high altitude, appears only partly to explain the increase in fibre density. The reasons for this difference remain unclear but a proliferation in diaphragm muscle fibres may be questioned. The increase in capillary density in HA-T rats, in comparison with both SL-T and HA-S rats, was primarily related to a non-statistically significant decrease in mean A_{fcs} (−17% and −20% respectively), whereas C/F remained unaffected (−4% and +1% respectively). The variations observed in capillary density in HA-T rats in comparison with HA-S animals (+25%) appear to be of the same magnitude as those recorded in mean A_{fcs} (−20%). All these data strongly suggest that both training exercise and/or exposure to high altitude are insufficient stimuli for enhancing capillary growth in the diaphragm. Also, the biochemical changes reported here under conditions of training at high altitude are consistent with previous data observed in skeletal muscle trained at sea-level. This was verified for citrate synthase activity (overall increase at $P<0.05$) and for the ratio of the heart-secific isoform to total lactate dehydrogenase activity (overall increase at $P<0.005$) [10, 11]. It has been previously hypothesized that in rat diaphragm muscle, a high level of glucose 6-phosphate contributes to preserving the glycogen stores under conditions of severe hyperventilation and acute hypoxia ($FIO_2 = 0.12$) [9]. Our data show that chronic exercise enhances hexokinase activity in rats living both at sea level, and at high altitude. Under these two conditions of tachypneic hyperventilation, increased hexokinase activity contributes to the increase in glucose 6-phosphate concentration. These data are in accordance with the results of Fregosi and Dempsey [9], who suggested that glucose phosphorylation capacity plays a key role in the preservation of diaphragm glycogen stores.

There is ample evidence that the metabolic cost of free swimming is lower than that of running [28]. Since the magnitude of the biochemical adaptations reported in skeletal muscle is strongly related to both intensity and daily duration of the exercise [8], the use of free swimming as a training exercise can be evoked partly to explain the moderate adaptative changes observed in diaphragm muscle. Moreover, the relationship existing between exercise intensity and the magnitude of the adaptative changes appears to be consistent with the fact that metabolic adaptations observed in diaphragm muscle after endurance training are smaller than those recorded after training with inspiratory-flow-resistive loads [1, 15]. We have described elsewhere the adaptative changes observed in hindlimb muscles under the same experimental conditions [3, 4]. The results showed that training performed at high altitude enhanced the percentage of type IIa fibres in the extensor digitorum longus muscles (+20%, $P<0.05$). In this fast-twitch muscle, the oxidative enzyme activities (citrate synthase and hydroxyacyl-CoA dehydrogenase) were increased to a greater extent when training was performed at high altitude (+24% and +36% respectively, $P<0.01$) than at sea-level (+12% and +8% respectively, NS). The present data, obtained under the same experimental conditions using swimming exercise, ascertain the functional and metabolic alterations induced by repeated mild exercise at high altitude on hindlimb skeletal muscles.

Metzger and Fitts [22] have shown that although the diaphragm muscle has a heterogeneous fibre population, intermediate to that of fast- and slow-twitch muscles, its oxidative capacity is higher than that of the soleus muscle, a slow-twitch muscle comprised primarily of type I fibres [2]. These findings are consistent with the fact that the histochemical method for fibre identification is based on the pH lability of the ATPase activity, and that this method does not characterize the metabolic profiles of the fibres. Many studies have emphasized a wide variation in the enzyme content of fibres of the same type [26]. The oxidative capacity of the diaphragm muscle contrasts with its intermediate fibre type composition. Therefore, it seems, in accordance with Moore and Gollnick [23], that the elevated respiratory capacity of the diaphragm cannot be attributed to a high percentage of slow-twitch oxidative fibres, but is rather the result of a high level of mitochondrial enzymes in all three main fibre types; it is likely that this is induced by the chronic activity of this muscle. On the other hand, the diaphragm's glycolytic capacity appears to be intermediate to that of slow- and fast-twitch muscles [23, 27]. All these data suggest, according to Metzger and Fitts [22], that the lower responsiveness of the diaphragm muscle to endurance training at high altitude could be partly related to its high level of oxidative capacity.

In summary, the results reported here show a lack of major changes in histochemical properties in the diaphragm muscle in response to endurance training performed at sea-level or at high-altitude environments. The capillary density increased with high-altitude exposure and training, without significant changes in C/F ratio. Training and altitude exposure increased the glucose phosphorylation capacity, and it may be hypothesized that this adaptative change contributes to glycogen sparing. In contrast to the effects in hindlimb muscles, the local barometric pressure had no drastic effect on oxidative capacity induced by the training programme on the diaphragm. It appears clearly that the diaphragm muscle adapts minimally to endurance training performed at high altitude.

References

1. Akabas SR, Bazzy AR, DiMauro S, Haddad GG (1989) Metabolic and functional adaptation of the diaphragm to training with resistive loads. J Appl Physiol 66:529–535
2. Ariano MA, Armstrong RB, Edgerton VR (1972) Hindlimb muscle fiber populations of five mammals. Histochem Cytochem 21:51–55
3. Bigard AX, Brunet A, Guezennec CY, Monod H (1991) Skeletal muscle changes after endurance training at high altitude. J Appl Physiol 71:2114–2121
4. Bigard AX, Brunet A, Guezennec CY, Monod H (1991) Effects of chronic hypoxia and endurance training on muscle capillarity in rats. Pflügers Arch 419:225–229
5. Brooke MH, Kaiser KK (1970) Muscle fiber types: how many and what kind? Arch Neurol 23:369–379
6. Dempsey JA, Forster HV (1982) Mediation of ventilatory adaptations. Physiol Rev 62:262–346
7. Dempsey JA, Forster HV, Birnbaum ML, Reddan WG, Thoden J, Grover RF, Rankin J (1972) Control of exercise hyperpnea under varying durations of exposure to moderate hypoxia. Respir Physiol 16:213–231
8. Dudley GA, Abraham WM, Terjung RL (1982) Influence of exercise intensity and duration on biochemical adaptations in skeletal muscle. J Appl Physiol 53:844–850
9. Fregosi RF, Dempsey JA (1986) Effects of exercise in normoxia and acute hypoxia on respiratory muscle metabolites. J Appl Physiol 60:1274–1283
10. Gollnick PD, Struck PJ Bogyo T (1967) Lactic dehydrogenase activities of rat heart and skeletal muscle after exercise and training. J Appl Physiol 22:623–627
11. Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831–838
12. Ianuzzo CD, Noble EG, Hamilton N, Dabrowski B (1982) Effects of streptozotocin diabetes, insulin treatment, and training on the diaphragm. J Appl Physiol 52:1471–1475
13. Ingjer F (1979) Effects of endurance training on muscle fibre ATPase activity, capillary supply and mitochondrial content in mean. J Physiol (Lond) 294:419–432
14. Karlsson J, Fritz K, Sjodin B, Gollnick PD, Saltin B (1974) Distribution of LDH isozymes in human skeletal muscle. Scand J Clin Lab Invest 33:307–312
15. Keens TG, Chen V, Patel P, O'Brien P, Levison H, Ianuzzo CD (1978) Cellular adaptations of the ventilatory muscles to a chronic increased respiratory load. J Appl Physiol 44:905–908
16. Lieberman DA, Maxwell LC Faulkner JA (1972) Adaptation of guinea pid diaphragm to aging and endurance training. Am J Physiol 222:556–560
17. Lind F, Hesser CM (1984) Breathing patterns and lung volume during exercise. Acta Physiol Scand 120:123–129
18. Lowry OH, Passonneau JV (1972) A flexible system of enzymatic analysis, 1st edn. Academic Press, New York
19. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
20. Malhotra MS, Sen-Gupta J (1976) Work capacity at altitude. Med Sport (Basel) 9:165–177
21. Maxwell LC, Withe TP, Faulkner JA (1980) Oxidative capacity, blood flow, and capillarity of skeletal muscle. J Appl Physiol 49:627–633
22. Metzger JM, Fitts RH (1986) Contractile and biochemical properties of diaphragm: effects of exercise training and fatigue. J Appl Physiol 60:1752–1758
23. Moore RL, Gollnick PD (1982) Response of ventilatory muscles of the rat to endurance training. Pflügers Arch 392:268–271
24. Olson RE, Dempsey JA (1978) Rats as a model for humanlike ventilatory adaptation to chronic hypoxia. J Appl Physiol 44:763–769
25. Powers SK, Lawer J, Criswell D, Dodd S, Grinton S, Bagby G, Silverman H (1990) Endurance-training-induced cellular adaptations in respiratory muscles. J Appl Physiol 68:2114–2118
26. Saltin B, Gollnick PD (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: Handbook of physiology, section 10. Skeletal muscle. Williams and Wilkins, Baltimore, pp 555–631
27. Sembrowich WL, Knudson MB, Gollnick PD (1977) Muscle metabolism and cardiac function of the myopathic hamster following training. J Appl Physiol 43:936–941
28. Shepherd RE, Gollnick PD (1976) Oxygen uptake of rats at different work intensities. Pflügers Arch 362:219–222
29. Sillau AH, Banchero N (1977) Effects of hypoxia on capillarity density and fibre composition in rat skeletal muscle. Pflügers Arch 370:227–232
30. Snyder GK, Wilcox EE, Burnham EW (1985) Effects of hypoxia on muscle capillarity in rats. Respir Physiol 62:135–140