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Paradoxical effects of endurance training and chronic hypoxia on myofibrillar ATPase activity

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1UMR 866 Institut National de la Recherche Agronomique, University of Montpellier I, Faculty of Sport Sciences, Montpellier, France; 2Department of Anatomy and Physiology, University of Padova, Padova, Italy; 3Brunel University, School of Sport and Education, West-London, UK; 4EA 2426 Laboratory of Cardiovascular Adaptations to Exercise, Avignon, France; 5UMR 5236 Centre National de la Recherche Scientifique, University of Montpellier I, France; and 6Department of Physics and Applied Mathematics, Faculty of Chemistry, University of Bucharest, Bucharest, Romania

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Roels B, Reggiani C, Rebuffel C, Lionne C, Iorga B, Obert P, Tanguy S, Gibault A, Jouglé A, Travers F, Millet GP, Candau R. Paradoxical effects of endurance training and chronic hypoxia on myofibrillar ATPase activity. Am J Physiol Regul Integr Comp Physiol 294: R1911–R1918, 2008. First published April 16, 2008; doi:10.1152/ajpregu.00210.2006.—This study aimed to determine the changes in soleus myofibrillar ATPase (m-ATPase) activity and myosin heavy chain (MHC) isoform expression after endurance training and/or chronic hypoxic exposure. Dark Agouti rats were randomly divided into four groups: control, normoxic sedentary (N; n = 14), normoxic endurance trained (NT; n = 14), hypoxic hypoxia (H; n = 10), and hypoxic endurance trained (HT; n = 14). Rats lived and trained in normoxia at 760 mmHg (N and NT) or hypobaric hypoxia at 550 mmHg (H and HT). m-ATPase activity was measured by rapid flow quench technique; myosin subunits were analyzed with mono- and two-dimensional gel electrophoresis. Endurance training significantly increased m-ATPase activity (P < 0.01), although an increase in MHC-I content occurred (P < 0.01). In spite of slow-to-fast transitions in MHC isoform distribution in chronic hypoxia (P < 0.05) no increase in m-ATPase was observed. The rate constants of m-ATPase were 0.0350 ± 0.0023 s⁻¹ and 0.047 ± 0.0050 s⁻¹ for H and HT. Thus, dissociation between variations in m-ATPase and changes in MHC isoform expression was observed.

Changes in active myofibrillar ATPase activity were analyzed in myosin light chain isoform (MLC) as the main determinant of ATPase activity. Thus, hypoxia seems to affect the structural and biochemical properties of skeletal muscles by inducing a transformation from type I to type II fibers. Opposite to chronic hypoxia, endurance training is likely to induce a decrease in fast fibers and an increase in slow-fiber proportion. For instance, Green et al. (18) demonstrated that a prolonged (15 wk) endurance training induced a fiber transformation from fast-type IIa to slow type I on the basis of enzyme histochemistry and MLC distribution. Endurance training (10 wk) also induced changes in the distribution patterns of fast alkali MLC isoforms detectable within MLC (triplet of isomyosins with distinct MLC complement, FM1b, FM2b, and FM3b) of the fastest isoform MHC-IIb (3). Surprisingly, endurance training seems to induce an increase in the actomyosin ATPase activity and maximal shortening velocity in the slow-twitch soleus muscle and a decrease in the vastus lateralis (34). These observations of changes in ATPase activity and myosin isoform shifts appear to be paradoxical, as ATP hydrolyzed by myosin represents the main component of ATP consumption during contraction. These changes in myosin isoform expression probably regulate ATP consumption. Actually, there is strong evidence that myosin isoforms are the main determinant of ATPase activity, at least at low temperature in vitro. In this study, we analyzed myosin isoform composition and myofibrillar ATPase activity in soleus muscles of rats exposed to normoxia or hypoxia with or without endurance training to assess the variations of soleus myofibrillar ATPase (m-ATPase) activity that accompany the changes in MHC isoform expression under training and/or chronic hypoxia conditions.

METHODS

Animal model. Sixteen-week-old, sea level native dark Agouti male rats (260 ± 23 g), obtained from Harlan Laboratories (Gannat, Puy de Dôme, France), were randomly assigned to one of the following four groups: hypobaric hypoxia with endurance training sessions (HT rats, n = 14), hypobaric hypoxia without training (H rats, n = 10), normoxia with endurance training sessions (NT rats, n = 14), or normoxia without training (N rats, n = 14).

Hypoxic exposure protocol. Hypobaric hypoxia was obtained in steel chambers [fitted with a clear plastic glass door to illuminate and observe the animals] using a specific vacuum pump (Becker Mot63, Rambouillet, France) as previously described by Melin et al.
were quickly removed. In each animal, one soleus was immediately anesthetized with intraperitoneal ketamine and xylazine (28). All rats were maintained for 5 wk in their own environment, at a barometric pressure of 760 mmHg (Pr0, ~159 mmHg, altitude ~80 m) for N and NT, or 550 mmHg (Pr0, ~105 mmHg, altitude ~2,800 m) for H and HT. The level and duration of the hypoxic stimulus were suggested to be sufficient for inducing adaptations, while still well tolerated by the rats and without any negative effects of the hypoxic stimulus, such as muscle mass loss, sickness, or fatigue, compared with controls (10). H and HT animals were fed ad libitum with free access to tap water. Because of the hypoxic effect on food intake and consequently animal growth, a pair-fed regimen was applied to the two other groups, with free access to tap water. To assure there was no decrease in body weight due to the hypoxic exposure, the rats’ body weight was measured before and after the protocol. Room temperature was maintained at 21°C using air-conditioning. Not more than four animals were kept at the same time in each cage. A 12:12-h light-dark cycle was applied. All procedures were performed in agreement with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publications No. 85-23, revised 1996) and with the approval of the French Ministry of Agriculture.

**Training program.** Twenty-five training sessions were conducted in NT and HT rats during the 5 wk of environmental exposure; that is, five sessions a week followed by two consecutive days of rest at the end of the week. Training consisted in wheel-driven running (EA 2426, Avignon, France) at 80% of maximal aerobic velocity (MAV); that is, the maximal speed reached at the end of the incremental test described below. To define accurately the training intensities, MAV was evaluated before the study period in normoxia or hypoxia. MAVs were also measured during the third week to adjust the training intensities. MAVs were evaluated using a driven wheel during a continuous incremental exercise test to exhaustion. Under normoxia, the driven wheel was set at a speed of 10 m/min for 2 to 3 min, after which the speed was increased by 4 m/min every 90 s until 85–90% of the expected MAV was reached. Then the speed was increased by 0.5–1 m/min every 60 s until MAV was reached. Hypoxic MAV was evaluated using the same protocol, but with a starting speed of 7 m/min. In both cases, we considered that MAV was reached when the rat was not able to follow the speed imposed by the driven wheel anymore and turned into the wheel more than three times. MAV reproducibility was evaluated during a preliminary study in five dark Agouti rats submitted to three MAV tests, each test being performed with a 2-day interval. Individual variation coefficients were lower than 4.6%, suggesting a good reliability of the measurements. Training lasted 5 wk and was conducted at the same relative intensity for both groups (i.e., 80% of normoxic MAV for NT and 80% of hypoxic MAV for HT). Each session lasted about 20 min in the first week and was increased up to 60 min in the last week. Rats were trained at the same P0,2 experienced for the rest of the day. The running skills of the N and H group were maintained by performing two sessions a week of 10 min at 40% of MAV. This training program was chosen, as it has been used in our laboratory for several years and is effective to induce changes in MAV, muscular, and cardioadrenal parameters (16, 28, 31).

**Myofibril preparation.** At the end of the 5-wk training protocol, all of the rats were anesthetized with intraperitoneal ketamine and xylazine injection (50 mg/kg body mass), and killed. Both soleus muscles were quickly removed. In each animal, one soleus was immediately frozen in liquid nitrogen (N2) for electrophoretic analysis, and one soleus was immersed in ice-cooled Ringer buffer (50 mM Tris-HCl, pH 7.0, 100 mM NaCl, 2 mM KCl, 2 mM MgCl2, 1 mM EGTA, 2 mM DTT, 0.2 mM PMSF, 10 μM leupeptin, 5 μM pepstatin, and 0.5 mM NaN3) at slack length. From these latter soleus muscles, myofibrils were prepared as previously described (11). The total myosin head concentration in the myofibrillar suspension was measured by absorption at 280 nm of a 1/20 dilution of the suspension in 2% SDS (23). Sarcomere length of the myofibrils was measured as previously described (11). Ten to twenty myofibrils were analyzed, and their sarcomere lengths were averaged.

**Myofibrillar ATPase activity.** ATPase measurements were carried out in a home-built, thermostatically controlled, rapid flow quench apparatus (4). Experiments were performed at 4°C to slow down the unloaded shortening velocity (V0), and thus, to increase the duration of the unloaded shortening phase (t0). The chosen temperature of 4°C, although slowing the shortening velocity down, still allows the myofibrils to behave and contract normally and offers the possibility to measure the rate constant of the cleavage step in the cross bridge cycle, as validated by Stehle et al. (36). In the rapid flow quench apparatus, myofibrils were mixed with [γ-32P]ATP (2.5 μM myosin heads + 25 μM [γ-32P]ATP in reaction mixture at 4°C in Ca2+-activated condition). Reaction mixtures were quenched in acid (22% TCA and 1 mM KH2PO4) at different times, and the [32P]Pi produced was assayed. Reaction mixtures with incubation times from 200 ms to tens of seconds were obtained with this apparatus. For longer incubation times, myofibrils and [γ-32P]ATP were mixed in a thermostated beaker, where one sample of the reaction mixture was taken and quenched every 10–15 s. The amounts of total Pi (i.e., free Pi plus myosin head bound Pi) were determined in the quenched reaction mixtures by the filter paper method of Reimann and Umfleet (32). Relaxing and activating buffers were used to obtain relaxed and Ca2+-activated ATPase rates, respectively. Buffers consisted of 50 mM Tris-acetate, pH 7.4, 100 mM KC, 5 mM MgCl, and either 0.1 mM CaCl2, 2 mM Mg-acetate for the activating buffer (+Ca2+) or 2 mM EGTA, and 5 mM Mg-acetate for the relaxing buffer (without Ca2+). Experiments were carried out in paired conditions to control external factors. In the same day, the steady-state rates of myosin ATPase activity were measured in training vs. control group in hypoxia vs. control group with precisely the same procedure of myofibril preparation, the same buffers, and temperature. Three independent sets of experiments on myofibrillar ATPase parameters, with different myofibrils samples, were carried out to ensure intertrial consistency.

**Modeling of the ATPase activity kinetics.** The first phase of steady-state ATP hydrolysis corresponds to the ATPase activity of Ca2+-activated myofibrils during unloaded shortening. The second phase that is slower was shown to represent the ATPase activity of overcontracting myofibrils (26). Overcontraction starts when the thinfilaments of the two halves of the sarcomeres start to overlap with each other. The whole kinetics of ATP hydrolysis was modeled with a three-component model, one exponential (transient Pi burst) and two linear (steady states): [Total Pi]/[myosin head] = [A(1 – e−kobs·t)] + kobs·t + U· [A + kobs·tobs + kobs (t – t0)], where [Total Pi]/[myosin head] is the amount of total phosphate determined per myosin head (mol of ATP hydrolyzed/mol of myosin head), A and kobs are the amplitude (mol/mol) and the rate constant (s−1) of the Pi burst respectively, kobs is the rate constant (s−1) of the steady state during unloading shortening, kobs is the rate constant (s−1) of the steady state during overcontraction, t0 is the time break (s) corresponding to the unloading shortening phase duration, U = 0, when t < t0 and U = 1, when t > t0. The third component starts at the offset of the first fast linear phase. The model parameters were determined using an iterative process that minimizes the sum of the mean squares between the total phosphate measured and that predicted by the model.

**Titration of active myosin heads.** In Pi burst experiments, the titration, that is, the number of fully active ATPase sites per total sites, was estimated from the amplitude (A) of the transient Pi burst. Experiments were performed in relaxed conditions because, first, A is similar in relaxed and in Ca2+-activated conditions, the equilibrium constant of the cleavage step that controls the Pi burst amplitude occurring in detached state whatever the condition. Second, A is...
determined more accurately in relaxed than in activated conditions, because the steady state is slower. Third, with myofibrils, A is similar to the amplitude of titration experiments (cold ATP chase), because the equilibrium constant of the cleavage step is large (23, 27).

Stopped-flow experiments. This type of experiment allows the measurements of the kinetics of ATP binding induces myosin head detachment from the thin filament and of the evaluation of the ATP cleavage step.

The experiment was carried out in a Hi-Tech Scientific stopped-flow apparatus (model SF-61 DX2, Hi-Tech Limited, Salisbury, UK). The excitation wavelength was 295 nm, and emission was >320 nm using a cut-off filter (WG320, Hi-Tech Limited, Salisbury, UK) to measure the tryptophan fluorescence. For each experimental condition, a series of 12 shots were carried out and averaged. The fluorescence of the myofibrillar solution without ATP, was measured just before the commencement of the experiment and was set as background. This latter was subtracted to the signal of the reaction mixture with ATP. For further details, see Stehle et al. (36).

Myosin isoform determination. Muscles to be used for electrophoresis were quickly frozen in liquid N2 after dissection, pulverized with ATP. For further details, see Oakley et al. (29). The excitation wavelength was 295 nm, and emission was measured using a cut-off filter (WG320, Hi-Tech Limited, Salisbury, UK) to measure the tryptophan fluorescence. For each experimental condition, a series of 12 shots were carried out and averaged. The fluorescence of the myofibrillar solution without ATP, was measured just before the commencement of the experiment and was set as background. This latter was subtracted to the signal of the reaction mixture with ATP. For further details, see Stehle et al. (36).

RESULTS

Training adaptations and body weight. MAV increased (P < 0.05) from 37.6 ± 3.1 and 37.1 ± 4.7 m/min for NT and HT, respectively at the beginning of the protocol under normoxic conditions to 43.7 ± 2.6 and 44.0 ± 3.7 m/min for NT and HT, respectively, after 5 wk of training. Initially, there was no significant difference in body mass between groups. The rats’ body weight increased significantly (P < 0.05) after the 5-wk protocol, but without any differences between groups: N: 243 ± 8.0 g vs. 258 ± 23 g; NT: 237 ± 21 g vs. 248 ± 25 g; H: 244 ± 21 g vs. 258 ± 21 g; and HT: 244 ± 11 g vs. 249 ± 14 g before and after the protocol, respectively.

Sarcomere length. The sarcomere length of soleus myofibrils (2.4 μm) was in accordance with the reported values in the literature. It was homogeneous among myofibrils of each group. The myofibrils were rectilinear and not aggregated.

Ca2+-activated ATPase activity. Myofibril ATPase activity was followed over 200 s and, in the presence of Ca2++, the three distinct phases mentioned above could be identified (Fig. 1): 1) a fast monoeXponential phase on the milliseconds time scale, corresponding to a transient Pi burst phase of amplitude A (kinetics, kobs, not obtained on the timescale used), 2) a fast linear phase with a slope kF, corresponding to the myofibrill ATPase activity during unloaded shortening, 3) a slow linear phase with a slope kS, corresponding to the myofibrill activity during overcontraction. Because this last phase is not physiological, no further interest was given to kS. The transition between kF and kS occurs at time t0, which is inversely related to the unloaded shortening speed. The three characteristic phases were described with the triphasic model detailed in the methods section. The value of the kinetic parameters kF and t0, essential for the comparison of ATPase activities, are given in Table 1. Three independent experiments using different myofibrillar preparations were performed, and the coefficient of variability for the three sets of the experiment was 7.8% ± 1.6%. This coefficient took into account experimental errors on myofibrils and ATP solution preparations and temperature fluctuations. Within each set of experiment, this kind of experimental error is likely to be lower, due to better control of external factors (same buffers, same day).
chronic hypoxic training group (n-baric hypoxia) (Table 1). Post hoc tests indicate a difference in the environment of exposure (normobaric normoxia or hypoxia) for the first series of separated experiments. The ATPase activities were constant among the three series of independent repetitions, and variance analysis showed a significant main effect of training as the slope, \( k_F \), was significantly higher (main effect \( P < 0.01 \)) in the training groups compared with the sedentary groups whatever the training status. Hypoxia produced an increase in the number of fully competent myosin heads (Table 2). As far as the effect of hypoxia, the values of Pi burst amplitude were similar in the H and control groups (0.49 and 0.44 mol/mol, respectively) suggesting that moderate hypoxia had no effect on the number of fully competent myosin heads.

**Cleavage step rate.** The rate of the ATP cleavage step was evaluated from tryptophan fluorescence stopped-flow experiments with relaxed myofibrils, as previously described. The slow-phase rate constant had similar values in N (8.5 ± 1.2 s\(^{-1}\)) and in NT groups (9.7 ± 2.7 s\(^{-1}\)), suggesting that training did not affect the cleavage step kinetics. The cleavage step rates were evaluated only in the normoxic groups because the hypoxic effect on m-ATPase did not reach significance level.

**MHC and MLC isoform identification and quantification.** Table 2 shows the distribution of slow and fast MHC and MLC isoforms of soleus, determined by one-dimensional gel electrophoresis. Examples of MHC and MLC separation performed with precisely the same protocol can be seen in Fig. 1 in Bozzo et al. (9). In hypoxic conditions, the proportion of the fast isoforms was increased (\( P < 0.01 \) and \( P < 0.05 \) for MHC-IIIX and MHC-IIIB, respectively), and the proportion of slow isoforms was decreased (\( P < 0.01 \)) compared with the normoxic control and training groups. Thus, a slow-to-fast transition was induced by hypoxia. Training led to an increase in MHC-I (\( P < 0.01 \)), indicating an opposite effect to hypoxia. Training in hypoxic conditions caused a reduction of the slow-to-fast transition induced by hypoxia alone, as indicated by the decreased proportion of MHC-2A. Training effect was less pronounced than the hypoxic one.

**MLC isoform expression** showed similar changes to those observed in MHC isoforms. Hypoxia produced an increase in fast isoforms (\( P < 0.001 \) for both MLC-1f+3 and MLC-2f) and a decrease in slow isoforms (\( P < 0.001 \) for both MLC-1s and MLC-2s). These changes were partially reversed by training in hypoxic conditions. Again, the training effect was less pronounced than hypoxic effect, and its effect was detectable only for MLC-1. MLC-1s increase and MLC-1f+3 decrease (\( P < 0.01 \)).

Two-dimensional gel electrophoresis resolved the regulatory MLC isoforms in several spots, corresponding to variants with

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**Fig. 1.** Training effect on the soleus myofibrillar ATPase activity at 4°C in normoxia (A) and hypoxia (B) for the first series of separated experiments. The reaction mixture (2.5 μM myosin heads + 25 μM [γ-\(^{32}\)P]ATP) was quenched in acid at the times indicated (between 200 ms and 250 s), and the \(^{32}\)Pi was determined. \( k_F \) represents the slope of the fast linear phase; \( t_B \) represents the duration of the shortening phase, \( t_B \), was shorter (main effect \( P < 0.05 \)) in training groups compared with sedentary groups. Because shortening velocity is a function of \( t_B \), this decrease suggests an increase in the unloading shortening velocity with training. As far as hypoxia, no significant main effect was detected, and only a trend (\( P = 0.07 \)) for a decrease in \( k_F \) was observed in hypoxic groups, whatever the training status.

### Table 1. Average soleus myofibrillar ATPase activity (\( k_F \)) and duration of the shortening phase (\( t_B \)) in activated condition at 4°C in the four experimental groups

<table>
<thead>
<tr>
<th></th>
<th>N (( n = 14 ))</th>
<th>NT (( n = 14 ))</th>
<th>H (( n = 10 ))</th>
<th>HT (( n = 14 ))</th>
<th>Training Effect</th>
<th>Hypoxic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_F ), s</td>
<td>0.035 (0.0023)</td>
<td>0.047 (0.0050)</td>
<td>0.033 (0.0021)</td>
<td>0.036 (0.0032)</td>
<td>+24%†</td>
<td>–12%</td>
</tr>
<tr>
<td>( t_B ), s</td>
<td>77 (7)</td>
<td>62 (12)</td>
<td>78 (7)</td>
<td>64 (3)</td>
<td>–19%*</td>
<td>+3%</td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). N, normoxic group; NT, normoxic training group; H, chronic hypoxic group; HT, chronic hypoxic training group; \( k_F \), the slope of the fast linear phase; \( t_B \), duration of the shortening; †\( P < 0.05 \); *\( P < 0.01 \); \( n \) = number of muscles.
distinct isoelectric points [an example is given in Fig. 2 in Bozzo et al. (9)]. In the soleus, slow MLC-2 was the predominant regulatory light chain and appeared separated into two spots (s and s1), with different isoelectric points. A third smaller spot was also present and corresponded to a variant MLC-2f, whereas the acidic variant MLC-f1 was undetectable. The average proportions of the MLC-2 variants are shown in Table 3. No significant changes in the distribution between less acidic nonphosphorylated variants (s and f) and more acidic phosphorylated variants (s1 and f1, which was undetectable) were present in the soleus of the four experimental groups.

**DISCUSSION**

The aim of the present study was to determine whether endurance training and/or hypoxia caused different changes in myofibrillar ATPase activity in relation with the known changes in MHC isoform composition in rat soleus. Myofibrils were prepared from the soleus muscle of rats trained in normoxic or hypoxic conditions for 5 wk. The determination of myofibrillar ATPase activity and MHC isoform composition of soleus myofibrils showed that 1) endurance training increased significantly the myofibrillar ATPase activity, although a fast-to-slow shift in MHC isoform distribution took place; 2) in spite of a slow-to-fast transition in MHC isoform distribution observed, no concomitant increase was evident in hypoxic groups and in contrast chronic hypoxia tended \( (P = 0.07) \) to decrease the myofibrillar ATPase activity. It is generally accepted that MHC isoforms are the major determinants of myofibrillar ATPase activity. The issue has been investigated on many preparations from isolated myosin to single muscle fibers. The contribution of the present study points out a possible modulation of the relation between MHC isoforms and ATPase activity. Actually, in the present experimental conditions, ATPase activity and MHC isoforms appeared dissociated, and other molecular control mechanisms were evoked.

Potential limits. One could argue that the observed changes in ATPase activity were temperature dependent. However, we examined previously the three characteristic phases of myofibrill ATPase kinetics in unloading myofibrils under a large range of temperature (4 to 35°C), and the Arrhenius plots of all the kinetics constants, including the duration of shortening phase were linear, indicating that the energy of activation was constant for all temperatures studied; that is, no break in the Arrhenius plot has been observed (11). Therefore, one may argue that the same limiting steps are controlling the ATPase activity over this large range of temperature. At low temperatures, myofibrils displayed the same behavior than at high temperatures, and all the contractile characteristics (including the shortening phase) were conserved, the only difference being the rate of the reaction. Whatever the temperatures, ~3 ATP are consumed during the shortening phase. The chosen temperature of 4°C although slowing down the shortening velocity, still allows the myofibrils to behave and contract normally. Interestingly, myofibrils prevented from shortening with 1-ethyl-3-[3-dimethylaminopropyl]carbodimide hydrochloride) cross-linker (8% of the myosin head cross-linked) displayed a different kinetics than native myofibrils. Only one linear phase corresponding to an isometric action occurred, whereas in native myofibrils, two linear phases with different slopes were evi-

Table 2. Distribution of MHC and MLC isoforms of soleus determined by one-dimensional gel electrophoresis

<table>
<thead>
<tr>
<th></th>
<th>N (n = 14)</th>
<th>H (n = 10)</th>
<th>NT (n = 11)</th>
<th>HT (n = 13)</th>
<th>Training Effect</th>
<th>Hypoxia Effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-I</td>
<td>70.8 (8.8)</td>
<td>56.2 (4.4)</td>
<td>73.3 (8.7)</td>
<td>66.1 (10.2)</td>
<td>*</td>
<td>‡</td>
<td></td>
</tr>
<tr>
<td>MHC-IIA</td>
<td>25.8 (5.2)</td>
<td>34.5 (12.1)</td>
<td>25.4 (7.7)</td>
<td>24.2 (9.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC-IIIX</td>
<td>1.0 (1.9)</td>
<td>5.9 (7.4)</td>
<td>0.4 (1.2)</td>
<td>2.0 (2.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MHC-IIB</td>
<td>2.3 (6.8)</td>
<td>4.2 (7.4)</td>
<td>0.5 (1.6)</td>
<td>6.6 (5.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLC-1 s</td>
<td>81.1 (7.4)</td>
<td>70.4 (4.2)</td>
<td>81.8 (3.2)</td>
<td>78.1 (3.2)</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>MLC-1 f+3</td>
<td>18.8 (7.4)</td>
<td>29.4 (4.2)</td>
<td>18.2 (3.2)</td>
<td>21.9 (3.2)</td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>MLC-2 s</td>
<td>77.3 (6.2)</td>
<td>54.4 (4.2)</td>
<td>72.1 (3.3)</td>
<td>64.3 (2.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLC-2 f</td>
<td>22.7 (6.2)</td>
<td>45.6 (4.2)</td>
<td>27.9 (3.3)</td>
<td>35.7 (2.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). Number (n) of muscles examined is in parentheses. MHC I, slow-type myosin heavy chain; MHC IIA, intermediate-type myosin heavy chain; MHC IIIX, fast intermediate-type myosin heavy chain; MHC IIB, fast-type myosin heavy chain; MLC 1 s, slow myosin light chain type 1; MLC 1 f, regulatory fast myosin light chain type 1; MLC 3, fast myosin light-chain type 3; MLC 2 s, regulatory slow myosin light chain type 2; MLC 2 f, fast myosin light chain type 2. *P < 0.05; †P < 0.01; ‡P < 0.001.
Table 3. Distribution of slow and fast MLC-2 isoforms of soleus determined by two-dimensional gel electrophoresis

<table>
<thead>
<tr>
<th></th>
<th>N (n = 5)</th>
<th>NT (n = 4)</th>
<th>H (n = 4)</th>
<th>HT (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.9 (2.4)</td>
<td>71.9 (2.0)</td>
<td>73.1 (2.0)</td>
<td>72.0 (2.0)</td>
<td></td>
</tr>
<tr>
<td>26.1 (2.3)</td>
<td>28.1 (2.0)</td>
<td>26.9 (1.9)</td>
<td>28.0 (1.9)</td>
<td></td>
</tr>
<tr>
<td>100.0 (0.0)</td>
<td>100.0 (0.0)</td>
<td>100.0 (0.0)</td>
<td>100.0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means (SD). Number (n) of samples analyzed is indicated in parentheses.
and significant depressive effects of hypoxia on \( \text{Na}^+ - \text{K}^- \)-ATPase activity were reported \((1, 18, 19)\), suggesting an occurrence of an adaptive mechanism also present in ATPase pump activity.

Molecular mechanisms of ATPase activity. Most studies on the relationship between MHC isoforms and kinetic parameters as \( V_{\text{max}} \) or ATPase activity have compared muscles or single fibers with distinct MHC isoform composition. Few studies have analyzed whether kinetic parameters and MHC isoforms change concomitantly in the same muscle as an adaptation to new functional demands, such as endurance training or hypoxia. The present results show a dissociation, which implies that other factors, beside MHC isoforms, may modulate kinetic parameters. The first possible explanation lies in an increase in the number of myosin heads fully competent from an enzymatic point of view. Thus, the endurance training effect on myosin ATPase activity might be caused by a simple increase in the number of active heads. The assessment of titration of the active myosin heads based on Pi burst amplitude, however, suggests that training did not modify the number of fully competent myosin heads. In addition, the experimental approach adopted in this study allowed us to analyze, at least in part, the individual steps of the ATPase cycle. Because the steady-state rate is mainly controlled by the slowest step rates (rate-limiting steps), the increase of the ATPase activity caused by endurance training may be attributed to an increase in the rate of such rate-limiting steps. The steady state depends on the isomerization step that controls Pi release, \( k_h \), in the kinetics scheme of Bagshaw and Trentham \((2)\) and the cleavage step equilibrium constant \( \left( K_3 \right) \) by the following equation: 

\[
k_{\text{cat}} = k_4 \frac{K_3}{1 + K_3},
\]

where \( k_{\text{cat}} = k_{p} / [\text{active site}] \), \( k_4 \) is the rate constant of the isomerization step preceding Pi release, \( K_3 \) is the cleavage step equilibrium constant, \( K_3 = k_{+3} / k_{-3} \) with \( k_{+3} \) and \( k_{-3} \) the forward and backward cleavage step kinetic rate (e.g., 23). In soleus as in psoas myofibrils during unloaded shortening, the kinetics of production of total and free Pi indicate that the rate-limiting step is clearly an isomerization step preceding, and thus controlling, the Pi release. This conclusion is mainly based on the occurrence of a large total Pi burst and the lack of free Pi burst. Because the ATP cleavage occurs in an actin-detached configuration of myosin, its kinetics can be indifferently studied in activated or relaxed conditions. In this study, ATP cleavage was evaluated by means of the slow phase of tryptophan fluorescence signal upon mixing relaxed myofibrils with ATP in a stopped-flow apparatus, as proposed by Stehle et al. \((36)\). No training effect could be detected on the ATP cleavage kinetics. This suggests that the training effect on the steady-state ATPase is due to an increase in the slowest step, the rate of the isomerization step preceding Pi release. Several studies \((18, 33, 38)\) have proposed that regulatory myosin light chains (MLC-2) may play a role in the adaptations following training. These studies showed that the proportion of fast regulatory MLC (MLC-2f) is increased by endurance training in the slow muscles, and this would induce an increase in ATPase activity. After a 10-wk endurance progressive running program, the MLC-3f decreased by 61% \((P < 0.01)\) \((38)\). These authors suggested that this 10-wk progressive running program decreased the contractile velocity of type IIB fibers and that the observed training-induced decrease of the muscle contractile velocity might be due to changes in fast alkali MLC complements within a certain fiber type but also due to shifts in MHC-based fiber populations. In addition, Green et al. \((18)\) demonstrated that a prolonged \((15 \text{wk})\) endurance training induced a fiber transformation from fast type IIA to slow type I on the basis of enzyme histochemistry and MLC distribution. We hypothesize that the changes in ATPase activity and shortening duration might be attributable to an altered expression of fast MLC-2 in the slow type I fibers. This hypothesis is supported by the fact that light chains are known to be involved in the power stroke and that removal of light chains depresses force and velocity. However, in the present study, no significant change in MLC-2 isoforms between the sedentary \((N \text{ and } H)\) and the trained groups \((NT \text{ and } HT)\) were detected. The lack of changes in MHC II and MLC-2 isoform distribution under training effect might be explained, as suggested by Salmons \((33)\), assuming that a minimal duration and intensity of exercise is required to change in myosin subunits. Regulatory MLC can be phosphorylated and when MLC-2 is phosphorylated, force developed at submaximal calcium concentration is increased, that is, myofibrils become more sensitive to calcium activation. In the present study the level of MLC-2 phosphorylation was determined with two-dimensional gel electrophoresis, and no significant change in the MLC-2 phosphorylation was detected between sedentary and trained groups. Thus, MLC-2 phosphorylation cannot explain the increase in ATPase activity. Attempts to explain the lack of relationship between myofibrillar ATPase activity changes and the changes in the distribution of MLC isoforms and of their level of phosphorylation were not successful. In the same way as for the increase of myofibrillar ATPase due to endurance training, it is more likely that the enzymatic activity is influenced by other myofibrillar proteins different from myosin or by posttranslational modification of myosin, different from MLC-2 phosphorylation. The identification of such a myofibrillar protein or of the relevant posttranslational modifications of myosin is beyond the scope of the present study.

Conclusion. Although it is generally accepted that myofibrillar ATPase activity is controlled by MHC isoforms, the present study showed that under specific circumstances, dissociations might occur. In this study, the analysis of MHC isoform transition and myofibrillar ATPase changes after endurance training gave a first example that the quantitative variations in the myofibrillar ATP hydrolysis rate are a result of complex mechanisms depending undoubtedly on the myosin isoforms but also under the control of other less known factors. The increased myosin ATPase activity in response to training was not linked to any effect on the hydrolysis step rate, suggesting that the slowest step of the mechano-chemical cross-bridge cycle, that is, the isomerization step preceding Pi release, is involved in this adaptation. A second example was found in chronic hypoxia in which in spite of the shift of MHC isoforms from slow to fast type, no significant change in ATPase activity was evidenced. Changes in expression of MLC isoform and in the level of MLC phosphorylation could not explain the dissociation between myofibrillar ATPase activity and MHC isoforms. Therefore, changes in other sarcometric proteins or posttranslational modifications of myosin are likely to be involved.

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REFERENCES


