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Endurance training-induced changes in alkali light chain patterns in type IIB fibers of the rat

MASANOBU WADA,[1] SHUICHIRO INASHIMA,[1]
TAKASHI YAMADA,[2] AND SATOSHI MATSUNAGA[3]
[1]*Faculty of Integrated Arts and Sciences,* [2]*Faculty of Medicine, Hiroshima University, Higashihiroshima, 739-8521; and* [3]*Institute of Health Sciences and Physical Education, Osaka City University, Osaka, Japan 558-8585*

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Wada, Masanobu, Shuichiro Inashima, Takashi Yamada, and Satoshi Matsunaga. Endurance training-induced changes in alkali light chain patterns in type IIB fibers of the rat. *J Appl Physiol* 94: 923–929, 2003; 10.1152/japplphysiol.00549.2002.—The effects of endurance training on the expression of myosin were electrophoretically analyzed in the deep portion of vastus lateralis muscle from the rat. A 10-wk running program led to increases ($P < 0.01$) in myosin heavy chain (MHC) 2a and 2d with a decrease ($P < 0.01$) in MHC_{2b}. Training also evoked a rearrangement of the isomyosin pattern with decreases in fast isomyosin (FM) 1 ($P < 0.01$) and FM2 ($P < 0.05$) and a rise in intermediate isomyosin ($P < 0.01$). These changes were accompanied by a 61% decrease ($P < 0.01$) in myosin light chain (MLC) 3F (11.8 ± 2.7 vs. 4.6 ± 4.2%). Two-dimensional electrophoresis made it possible to separate the triplet of isomyosins (FMb) consisting of MHC_{2b}. Training elicited a 26% decrease ($P < 0.05$) in the FM1b fraction within FMb, i.e., FM1b/(FM1b + FM2b + FM3b) (24.2 ± 5.5 vs. 18.0 ± 4.3%). These changes resulted in a 10% decrease ($P < 0.05$) in the MLC_{3F} fraction, i.e., $MLC_{3F}/(MLC_{1F} + MLC_{3F})$, in FMb (44.9 ± 4.5 vs. 40.3 ± 3.2%). These results suggest that endurance training may exert the depressive effect on the contractile velocity of type IIB fibers and that a training-induced decrease in the contractile velocity of whole muscle may be caused by alterations in fast alkali MLC complements within a given fiber type as well as by transitions in MHC-based fiber populations.

myosin heavy chain; myosin light chain; isomyosin; isoform; electrophoresis

SKELETAL MUSCLE FIBERS EXHIBIT a remarkable adaptive ability that is exemplified by alterations in their phenotype in response to altered functional demands. This plasticity is based on the fact that multigene and alternative transcript splicing create multiple, thick- and thin-filament protein isoforms covering ranges of functional properties (for review, see Ref. 22). Rodent skeletal muscles are composed of slow type I and fast type II fibers; the latter can be subclassified into IIB, IID, and IIA fibers. These fiber types differ in their myosin heavy chain (MHC) composition. Thus type I, IIB, IID, and IIA fibers contain four distinct MHC isoforms, i.e., MHC_1, MHC_{2b}, MHC_{2d}, and MHC_{2a}, respectively (31).

Previous experiments clearly demonstrated the capacity of skeletal muscle to adapt to endurance training by qualitative and quantitative changes in fuel supply and catabolism, especially with regard to increased capacity of oxidative metabolic pathways (5). Endurance training also evokes transitions in MHC isoforms and MHC-based fiber types. As shown in studies on chronic low-frequency stimulation of rodent fast-twitch muscles, transitions induced by remarkably increased contractile activities appear to follow the order $MHC_{2b} \rightarrow MHC_{2d} \rightarrow MHC_{2a} \rightarrow MHC_1$ (for review, see Ref. 23). Although endurance training results in qualitatively similar transition processes as chronic stimulation, in most cases, transitions are limited to the fast-type subtypes and thus consist of a decrease in the faster MHC_{2b} isoform with an attendant increase in the slower MHC_{2a} isoform (1, 11, 21).

The four myosin light chains (MLC) are associated with the two myosin heads. The bound light chains consist of a pair of regulatory light chain, MLC_2, and a pair of alkali light chains, MLC_1 and/or MLC_3. Type II fibers comprise two distinct alkali light chains, MLC_{1F} and MLC_{3F}. MHC transitions within fast MHC isoforms resulting from increased contractile activity have been shown to be accompanied by an increase in MLC_{1F} at the expense of MLC_{3F} (3, 34). Our study on rat single fiber has revealed that, on average, type IIB fibers contain higher amounts of MLC_{3F} than type IID fibers, whereas the latter contain higher amounts of MLC_{3F} than type IIA fibers (36), indicating distinct affinities of MHC isoforms for fast alkali MLC complement. A study on in vivo synthesis rates of MLC has suggested that the activity-induced reduction in the MLC_{3F} content may be attributed, at least in part, to the decrement in MHC_{2b} displaying a high affinity for MLC_{3F} (17). In addition to differences in the fraction of MLC_{3F}, i.e., $MLC_{3F}/(MLC_{1F} + MLC_{3F})$, among the type II fiber subtypes, quantitative data from our study also showed that variations existed in the fraction within a

Address for reprint requests and other correspondence: M. Wada, Faculty of Integrated Arts and Sciences, Hiroshima Univ., 1-7-1 Higashihiroshima-shi, Hiroshima, Japan 739-8521 (E-mail: wada@hiroshima-u.ac.jp).

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given fiber type (36). Large scattering of the MLC_{3F} fraction indicates that each fiber type is composed of fibers identical with regard to their specific MHC complement but heterogeneous with regard to their fast alkali MLC composition. This raises the question of whether increased contractile activity, as occurs in sustained exercise, elicits an alteration in the distribution of fast alkali MLC, i.e., MLC_{1F} and MLC_{3F}, within a given fiber type as well as MHC transitions found in whole muscle.

These findings prompted us to investigate in more detail endurance training-induced changes in the distribution of MHC and MLC isoforms and isomyosins by means of several electrophoretic techniques. We have hypothesized that training would bring about an increase in MLC_{1F} at the expense of MLC_{3F} within isomyosins composed of MHC_{2b}. Our experiments conducted with rat skeletal muscle have suggested that training may exert the depressive effect on the contractile velocity of type IIB fibers.

METHODS

Animal care and experimental protocol. These experiments were approved by the University Committee for Use of Animals in Research at Hiroshima University. Five-week-old male Wistar rats weighing ~110 g were used in this study. All animals were housed in a temperature-controlled room (22°C) under conditions of an equal daily light and dark cycle and were fed chow and water ad libitum. The rats were randomly divided into a training (n = 9) and a control (n = 10) group. The training group participated in a 10-wk endurance program. Animals were conditioned 5 days/wk, utilizing an exercise program that involved both progressive intensity and duration. Treadmill grade remained constant at 10% decline throughout the training period. The rodents were initially run trained on a rodent treadmill at 25 m/min for 20 min/day. After 3 wk, the rats ran at 25 m/min for 70 min/day, and after 5 wk they ran at 30 m/min for 100 min/day. By the end of 7 wk, the rats were capable of running at 32.5 m/min for 2 h/day, and this level of intensity and duration was maintained for the remainder of the training program. From 36 to 48 h after the last exercise session, animals were killed after an intraperitoneal injection of pentobarbital sodium. The vastus lateralis muscles from both hindlimbs were quickly removed and cleaned of adipose and connective tissue. These muscles were separated into a superficial and a deep portion and stored at −80°C. Because the expression of myosin has previously been shown to be more sensitive to changes induced by endurance training in a deep portion of the vastus lateralis muscle (DVL) than in the other hindlimb muscles (15), DVL was selected for the study.

MLC electrophoresis. Small muscle pieces were homogenized in a glass homogenizer in 40 volumes of a solution containing 5 M urea, 2 M thiourea, 10 mM sodium pyrophosphate (PP), and 0.1% (vol/vol) 2-mercaptoethanol. Forty microliters of the resulting homogenate were directly applied to two-dimensional electrophoresis, according to O'Farrell (20). Electrophoresis in the first dimension was performed in a glass tube (130 × 2 mm internal diameter) by using 1.6% (pH 5–8) and 0.5% (pH 3–10) ampholines (Pharmacia) in 4.2% (mass/vol) polyacrylamide. Electrophoresis was run for 5 h at 500 V in a cold room (6°C). The second dimension consisted of a 15% polyacrylamide gel (36). Electrophoresis was first run for 1 h at a constant current of 30 mA and then for another 2 h at 60 mA.

Isomyosin electrophoresis. The muscles were pulverized under liquid nitrogen and extracted in 8 volumes of a solution consisting of (in mM) 100 sodium PP, 5 EGTA, 5 $MgCl_2$, 300 KCl, and 5 ATP, pH 8.6. The homogenate was centrifuged for 10 min at 10,000 *g*. The collected supernatant fraction was mixed with an equal volume of glycerol. The resulting myosin extracts were 20-fold diluted with a solution composed of 25 mM sodium PP, pH 8.6, 2 mM EGTA, 0.02% (mass/vol) bromophenol blue, and 25% (vol/vol) glycerol. Protein concentration was determined according to Bradford (9). PP-PAGE for isomyosin separation was performed according to the method of Wada et al. (32). The composition of 1.5-mm-thick slab gel was 4% (mass/vol) polyacrylamide, 0.11% (mass/vol) bisacrylamide, 0.25% (vol/vol) TEMED, 26.7 mM sodium PP, pH 8.6, 15 mM taurine, 8% (vol/vol) glycerol, 5 mM $MgCl_2$, and 0.06% (mass/vol) ammonium persulfate. After preelectrophoresis for 30 min, 3 μg of protein were loaded on the PP gel by using the vertical slab gel system Desaphor VA 150 (Desaga, Heidelberg, Germany). PP-PAGE was carried out at 0°C with a constant voltage of 120 V for 96 h.

MHC electrophoresis. Aliquots of crude myosin extracts utilized for isomyosin separation were 10-fold diluted with the following incubation medium: 62.5 mM Tris·HCl, pH 6.8, 2% (mass/vol) SDS, 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 0.02% (mass/vol) bromophenol blue. An amount of 0.3 μg of protein was applied to the previously described polyacrylamide (7%) gel electrophoresis in the presence of SDS (35). SDS-PAGE was run at 180 V for 48 h in a cold room.

Two-dimensional electrophoresis. Some of five bands separated by PP-PAGE were thought to contain more than a single isomyosin (see RESULTS) since it has been shown that, in rodent fast-twitch muscle, a total of nine fast isomyosins (FM) exist that result from combinations of three fast MHC isoforms with the alkali light chain MLC_{1F} and MLC_{3F} homodimers and MLC_{1F} and MLC_{3F} heterodimer (32). To evaluate more unequivocally the relative amounts of isomyosins, two-dimensional electrophoresis different from that of O'Farrell (20) was performed in this study, as previously described (35). An amount of 5 μg of protein was applied to the aforementioned PP-PAGE in the first dimension. The lane of the PP slab gel containing isomyosins was cut along the migrating direction of proteins after quick staining (<10 min) in 0.04% (mass/vol) Coomassie brilliant blue G-250 in 3.5% (vol/vol) perchloric acid. Separation in the second dimension was performed in SDS-PAGE used for MHC analysis.

Staining and densitometric evaluation. Gels for MLC isoform separation were stained with 0.25% (mass/vol) Coomassie brilliant blue R-250 in 45% (vol/vol) methanol and 10% (vol/vol) acetic acid, and were destained by diffusion in 45% methanol and 10% acetic acid. Other gels were silver-stained according to Oakley et al. (19). After the gels were stained, the percent distribution of various isoforms was estimated by densitometric evaluation. At least three electrophoretic analyses were performed on each sample.

Estimation of relative concentration of MLC_{3F} in FMs composed of MHC_{2b}. Two-dimensional electrophoresis used in this study provided the data on the relative distribution occupied by FM1b, FM2b, and FM3b in isomyosins (FMb), i.e., FM1b + FM2b + FM3b, composed of MHC_{2b} (see RESULTS). On the basis of the data, the MLC_{3F} fraction, i.e., $MLC_{3F}/(MLC_{3F} + MLC_{3F})$, present in FMb was calculated by the following equation

$$\%MLC_{3f} = \%FM2b \times 0.5 + \%FM1b$$

where $\%MLC_{3F}$ is the percent distribution of MLC_{3F} in fast alkali MLC, and %FM2b and %FM1b are the percent distributions of FM2b and FM1b in FMb, respectively.

Statistical analyses. Differences between control and trained conditions were tested for significance by using a two-tailed *t*-test. All comparisons were performed at the 95% confidence level. Data are presented as means ± SD.

RESULTS

MHC and MLC isoforms. In accordance with previous studies (2), four MHC isoforms were electrophoretically discerned in DVL and designated according to Termin et al. (31) as MHC_{2a}, MHC_{2d}, MHC_{2b}, and MHC_1 in order of increasing mobility (Fig. 1). The muscles of control rats displayed MHC_{2b} and MHC_{2d} as prominent isoforms together with relatively low amounts of MHC_{2a} and with trace of MHC_1. Training produced transitions in fast MHC isoforms with significant increases ($P < 0.01$) in MHC_{2a} and MHC_{2d} and attendant decreases ($P < 0.01$) in MHC_{2b} (Table 1). On the contrary, slow MHC isoform, MHC_1, was not affected by training.

The MLC complement of DVL was characterized by the coexistence of all five MLC isoforms found in fast and slow muscles of the rat (Fig. 2). According to the high amounts of fast MHC, fast MLC isoforms represent a major fraction in muscles under study. Although training tended to elevate the MLC_{1F} content, its increase from 25.9 to 29.1% was nonsignificant (Table 1). However, the MLC_{3F} content in trained DVL amounted to 4.6% and displayed a significant difference ($P < 0.01$) from that (11.8%) of control DVL. No differences in the amounts of slow MLC (MLC_{1S} and MLC_{2S}) and fast regulatory MLC (MLC_{2F}) were found between control and trained muscles.

Isomyosins. In myosin extracts from DVL consisting of both fast and slow MHC isoforms, five isomyosins were resolved and designated as FM1, FM2, FM3, intermediate isomyosin (IM), and slow isomyosin by using the terminology of d'Albis et al. (10) (Fig. 3). The muscle of control rat exhibited high amounts of FM3, FM2, and IM together with minor amounts of FM1 and slow isomyosin (Table 1). Training evoked a more than twofold increase in IM content ($P < 0.01$). In agreement with the above-described alterations in fast al-

Table 1. *Effects of endurance training on the percentage distribution of MHC and MLC isoforms and isomyosins in the deep portion of vastus lateralis muscle of the rat*

	Con	Tr
MHC, %		
MHC_1	5.8 ± 5.8	6.8 ± 6.3
MHC_{2a}	17.4 ± 5.7	32.4 ± 5.4†
MHC_{2d}	32.6 ± 4.0	40.9 ± 7.0†
MHC_{2b}	44.2 ± 9.4	19.9 ± 7.0†
MLC, %		
MLC_{1S}	6.1 ± 2.9	8.2 ± 4.0
MLC_{1F}	25.9 ± 2.4	29.1 ± 4.7
MLC_{2S}	7.9 ± 3.7	11.3 ± 4.2
MLC_{2F}	48.3 ± 5.3	46.8 ± 4.2
MLC_{3F}	11.8 ± 2.7	4.6 ± 4.2†
Isomyosin, %		
SM	4.4 ± 4.7	6.2 ± 6.0
IM	22.2 ± 7.7	49.3 ± 7.4†
FM3	31.2 ± 8.3	28.9 ± 10.5
FM2	32.6 ± 17.4	13.8 ± 9.5*
FM1	9.6 ± 3.2	1.8 ± 1.9†

Values are means ± SD. Con, control ($n = 10$); Tr, trained ($n = 9$); MHC, myosin heavy chain; MLC, myosin light chain; MHC_1, slow MHC; MHC_{2a}, MHC_{2d}, MHC_{2b}, fast MHC isoforms; MLC_{1S}, MLC_{2S}, slow MLC isoforms; MLC_{1F}, MLC_{2F}, MLC_{3F}, fast MLC isoforms; SM, slow isomyosin; IM, intermediate isomyosin; FM3, FM2, FM1, fast isomyosins. *$P < 0.05$; †$P < 0.01$, control vs. trained.

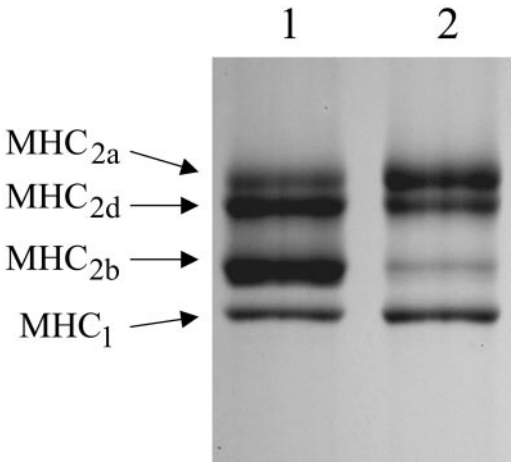

Fig. 1. Electrophoretic separation of myosin heavy chain (MHC) isoforms in the deep portion of vastus lateralis from control (*lane 1*) and trained (*lane 2*) rats. MHC_{2a}, MHC_{2d}, and MHC_{2b} were fast isoforms; MHC_1 was a slow isoform.

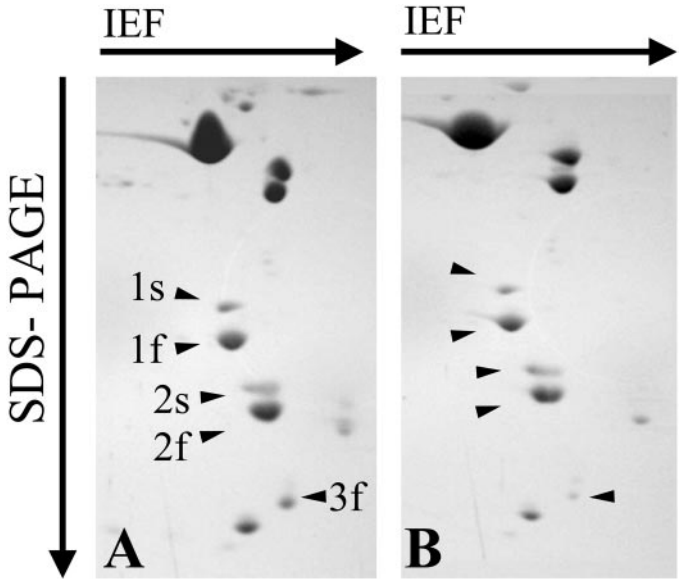

Fig. 2. Two-dimensional electrophoretic separation of myosin light chain isoforms in the deep portion of vastus lateralis from control (*A*) and trained (*B*) rats. Proteins were separated by isoelectric focusing (IEF) in the first dimension and then by SDS-PAGE in the second dimension. 1s, 2s, slow myosin light chains; 1f, 3f, fast alkali myosin light chains; 2f, fast regulatory myosin light chain.

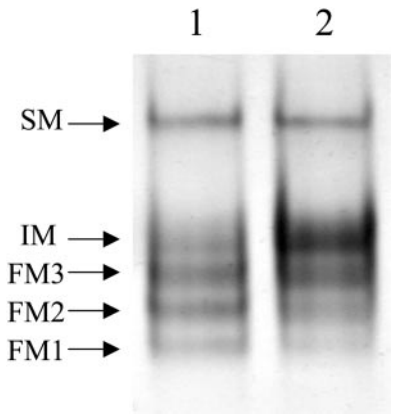

Fig. 3. Electrophoretic separation of isomyosins in the deep portion of vastus lateralis from control (*lane 1*) and trained (*lane 2*) rats. SM, slow isomyosin; IM, intermediate isomyosin; FM3, FM2, FM1, fast isomyosins.

kali MLC, the amounts of isomyosins composed of MLC_{3F}, i.e., FM1 ($P < 0.01$) and FM2 ($P < 0.05$), were remarkably decreased by training. This was especially true of the relative concentration of FM1, which amounted to only 1.8% in trained DVL, whereas control muscle comprised 9.6% (Table 1). In contrast, significant changes were not observed in either the slow isomyosin or FM3 content.

Two-dimensional electrophoresis. Nine FMs expressed in rodent fast-twitch muscle were designated by using the nomenclature of Termin and Pette (29) as FM1a–3a, FM1d–3d, and FM1b–3b. In control DVL, fast MHC isoforms contained in IM and FM were separated into seven spots by two-dimensional electrophoresis (Fig. 4*A*). On the basis of the previously reported differences in the electrophoretic mobilities of MHC isoforms and isomyosins (32, 36), they were identified as MHC isoforms comprised in FM3a, FM3d, FM2d, FM1d, FM3b, FM2b, and FM1b. In trained DVL, it was impossible to separate two MHC isoforms from FM3a and FM3d due to the increased amounts of MHC_{2a} and MHC_{2d} and to the subtle differences in their electrophoretic mobilities (Fig. 4*B*). Separating patterns indicate that the band of IM detected by PP-PAGE clearly represents a mixture of FM3a and FM3d and that FM3 and FM2 may be a mixture of FM2d and FM3b, and FM1d and FM2b, respectively. It is obvious that the amount of each spot of MHC in two-dimensional electrophoresis directly reflects the content of a single isomyosin. The large difference in the migration in the second dimension between MHC_{2b} and other fast MHC isoforms made it possible to evaluate the relative amounts of FM1b, FM2b, and FM3b within FMb. As can be seen in Fig. 5, the FM1b fraction, i.e., FM1b/(FM1b + FM2b +FM3b), was affected by training; its decrease from 24.2 ± 5.5% to 18.0 ± 4.3% was significant ($P < 0.05$). As mentioned above, the MLC_{3F} fraction, i.e., $MLC_{3F}/(MLC_{1F} + MLC_{3F})$, contained in FMb was calculated on the basis of the data on the distribution of FMb. Training-induced alterations in the FMb content resulted in significant reductions (44.9 ± 4.5 vs. 40.3 ± 3.2%, $P < 0.05$) in the MLC_{3F} fraction within FMb (Fig. 5).

DISCUSSION

The present findings on the expression of MHC isoforms are consistent with previous observations that endurance training brings about a decrease in MHC_{2b} together with an increase in MHC_{2a} in rodent fast-twitch muscles (1, 11, 21). According to the coexistence of specific MHC isoforms in transforming muscle, the exchange of MHC resulting from training appears to follow the sequence of $MHC_{2b} \rightarrow MHC_{2d} \rightarrow MHC_{2a}$ (24, 30). Bottinelli et al. (7) investigated in rat single fibers the cost tension, defined as the ratio between ATPase activity and isometric tension, and found that, on the average, type IIB fibers displayed the highest cost tension, type IID fibers were intermediate, and type IIA fibers were the lowest. Their data demonstrate that MHC-based fiber types possess different efficiency for the conversion of chemical to mechanical energy and imply that sequential transition from one MHC isoform to the next may be dictated by energy requirement. This assumption is supported by the observations by Ren et al. (26), who showed that administration of a creatine analog, which led to reductions in ATP concentration in muscle fibers, evoked changes in myosin expression in the direction of slower isoforms.

It was originally shown by Bárány (4) that a positive correlation exists between actin-activated myosin ATPase (mATPase) activity and the speed of muscle shortening. This observation was supported by subse-

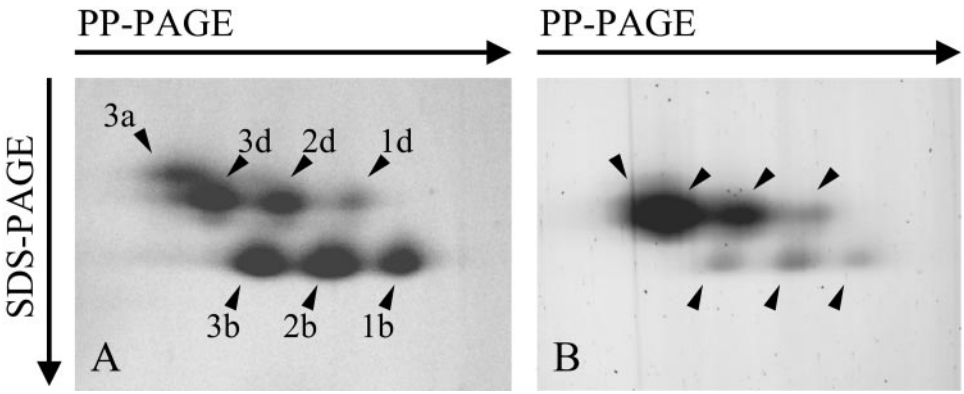

Fig. 4. Two-dimensional electrophoretic separation of MHC isoforms in the deep portion of vastus lateralis from control (*A*) and trained (*B*) rats. Proteins were separated by pyrophosphate (PP)-PAGE in the first dimension and then by SDS-PAGE in the second dimension. 3a, MHC_{2a} contained in FM3a; 3d, 2d, and 1d, MHC_{2d} from FM3d, FM2d, and FM1d, respectively; 3b, 2b, and 1b, MHC_{2b} from FM3b, FM2b, and FM1b, respectively.

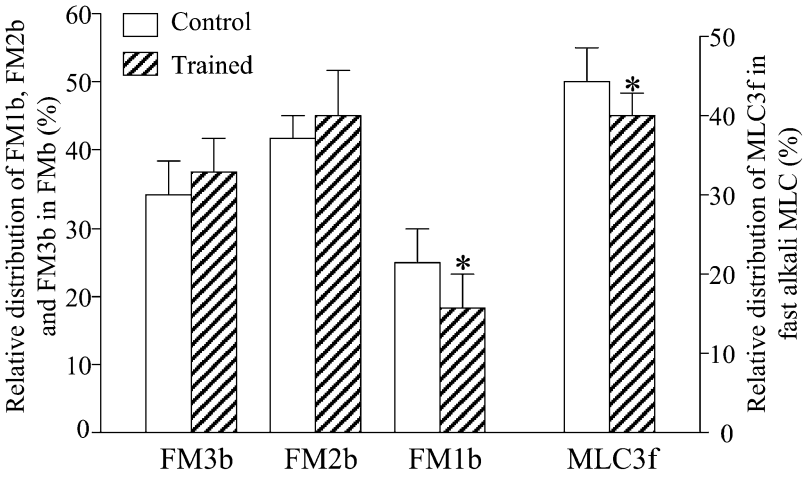

Fig. 5. Relative distribution of FM1b, FM2b, and FM3b and the fast alkali light chain 3F within isomyosins (FMb) consisting of MHC_{2b}. Values of the relative concentration of FM1b, FM2b, and FM3b in FMb (FM1b + FM2b + FM3b) were calculated by the results obtained by densitometric evaluation of two-dimensional electrophoresis for MHC separation. The MLC_{3F} fraction, i.e., $MLC_{3F}/(MLC_{1F} + MLC_{3F})$ was estimated from the data on the distribution of FMb. Values are means ± SD. $^*P < 0.05$, control vs. trained.

quent investigations showing that actin-binding and ATP-cleavage sites reside in the head region of MHC and that fibers containing slow MHC_1 display lower maximum velocity of shortening (V_{max}) than fibers consisting of any of three fast MHC isoforms (12, 25). On the assumption that V_{max} is determined primarily by mATPase activity, we attempted to estimate the changes in V_{max} of DVL that may occur with training from MHC isoform distribution presented here (Table 1) and the previous observations on mATPase activity of single fiber (7) and the relationship between V_{max} and mATPase activity (4, 7). The equation for the regression line of V_{max} vs. mATPase activity was obtained by plotting original data published by Bárány (4) or Bottinelli et al. (7). This estimation indicated that training-induced MHC transitions could result in a 7–10% decrease in V_{max} of whole muscle. Although the importance of MHC on contractile properties is also deduced from the observation that the mean values of both V_{max} and mATPase activity increase in the order of type IIA, IID, and IIB, the V_{max} of the three fast fiber types exhibits large variability with broad overlaps (7, 14, 16). A role of MLC in V_{max} was suggested by the findings that V_{max} is higher in fibers containing larger amounts of MLC_{3F} (6–8) and that type IIA fibers expressing MLC_{2S} exhibit a lower V_{max} than fibers lacking this MLC (18). The variability of V_{max} observed in type II fibers is interpreted to be attributable primarily to differences in the fast alkali MLC complements, i.e., MLC_{1F} and MLC_{3F}, in each fiber type because few type II fibers additionally comprise slow regulatory MLC (27, 28). In fact, our single fiber analysis demonstrated variations in the fraction of MLC_{3F} in each of three fast fiber types of the rat (36).

Similar to MHC, our results on the distribution of MLC also agree with data from the literature. For example, Wahrmann et al. (37) and Kirschbaum et al. (17) studied muscles exposed to endurance training and chronic electrostimulation, respectively, and found that MHC transitions in the order $MHC_{2b} \rightarrow MHC_{2d} \rightarrow MHC_{2a}$ were accompanied by decreases in the relative concentration of MLC_{3F}. Although, on the basis of these data, one can estimate the functional alterations evoked in whole muscle, taking into account that both MHC and MLC are involved in contractile properties and that the amounts of two fast alkali MLC isoforms expressed vary among type II fiber subtypes, additional data obtained by isomyosin-analysis seem to be necessary to gain insight into the V_{max} of fibers. In accordance with this study, one-dimensional electrophoretic study by Fitzsimons et al. (13) indicated that endurance training elicited a rise in IM and a reduction in FM2 in rat DVL. However, the fact that a separation of the three FM triplets by one-dimensional electrophoresis is incomplete in whole muscle containing more than a single fast MHC isoform (Figs. 3 and 4) indicates that most data derived from the use of this technique cannot accurately quantify changes in the content of a single FM. The two-dimensional electrophoresis method used in the present study made it possible to separate in whole muscle the triplet (FM1b, FM2b, and FM3b) of isomyosins (FMb) comprising MHC_{2b}. To our knowledge, this investigation is the first electrophoretic study that provides information concerning training-induced changes in the relative distribution of a single isomyosin within the triplet composed of the same fast MHC isoform. The results of two-dimensional electrophoretic analyses indicated that, not only in whole muscle but also within FMb, endurance training did evoke an appreciable decrease in the MLC_{3F} content (Fig. 5). In view of the above-mentioned role of fast alkali MLC in shortening velocity, it is conceivable that this change may result in a reduction in V_{max} in some type IIB fibers.

Bottinelli et al. (6) measured V_{max} and the MLC content of single fibers from rat fast-twitch muscle and found, in each of three fast fiber types, a positive correlation between V_{max} and the MLC_{3F} fraction expressed as MLC_{3F}/MLC_{2F}. The observed differences in the slope of the regression line suggest that the impact of fast alkali MLC isoforms on V_{max} is more pronounced in type IIB than in type IIA and IID fibers. Because of a molar equivalence between the MLC_{2F} contents and the sum of the MLC_{1F} and MLC_{3F} contents, the possible changes in V_{max} of 10-wk-trained type IIB fibers can be calculated on the basis of the

equation of the regression line [$y = 1.46 + 5.80x$, where y is V_{max} (in fiber length/s) and x is the MLC_{3F}/MLC_{2F} ratio] reported by Bottinelli et al. (6). This calculation revealed that the reduction in the MLC_{3F} content from 44.9 to 40.3% (Fig. 5) would cause a 6.4% decrease in the mean values of V_{max} in type IIB fibers (4.06 vs. 3.80 fiber length/s).

The finding that a reduction in MLC_3 may occur within IIB fibers is of interest with regard to the mechanisms that control the expression of fast alkali MLC. As shown in a study on electrically stimulated muscles of the rat, a decrease in the MLC content is more pronounced at the protein level than at the mRNA level (17), indicating an increased turnover of MLC_{3F}. It has been pointed out that this may be related to the rapid changes in fast MHC complement (3, 17). MHC_{2b} is characterized by a higher affinity for MLC_{3F} than MHC_{2a}; MLC_{3F} is bound to a larger degree to MHC_{2b} than to MHC_{2a} (29, 32, 33, 36). The replacement of MHC_{2b} by MHC_{2a} may, therefore, result in an increase in the amounts of free form of MLC_{3F}, which is more readily degraded than its bound form. The decrease in the relative content of MLC_{3F} within FMb would lead to additional increases in the free form of MLC_{3F} and suggests that the MLC_{3F} expression in muscles exposed to increased contractile activity may be regulated, not only by transitions in fast MHC isoforms, but also to some extent by the altered affinity of MHC_{2b} for MLC_{3F}.

In summary, the present study shows that training-induced changes in the distribution patterns of fast alkali MLC isoforms do occur within FMb composed of the fastest isoform MHC_{2b} as well as in whole muscle. It is accepted that the maximum shortening velocity correlates with not only MHC but also with MLC isoforms expressed in fibers. The alterations in fast alkali MLC within FMb shown here suggest that endurance training may exert the depressive effect on the contractile velocity of type IIB fibers and that a training-induced decrease in the contractile velocity of whole muscle may be caused by alterations in the fast alkali MLC patterns within a given fiber type as well as by transitions of MHC-based fiber populations. The question as to training-induced alterations in the alkali MLC pattern of isomyosins comprising MHC_{2a} and MHC_{2d} remains to be elucidated in further studies.

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