FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 42, No. 3, 2004
pp. 181-190

Characteristics of myosin profile in human vastus lateralis muscle in relation to training background

B. Zawadowska[1], J. Majerczak[2], D. Semik[1], J. Karasinski[1], L. Kolodziejski[3], W. M. Kilarski[1], K. Duda[3] and J. A. Zoladz[2]

[1]Department of Cytology and Histology, Institute of Zoology, Jagiellonian University,
[2]Department of Muscle Physiology, Academy of Physical Education,
[3]Cancer Institute, Cracow, Poland

Abstract: Twenty-four male volunteers (mean ± SD: age 25.4±5.8 years, height 178.6±5.5 cm, body mass 72.1±7.7 kg) of different training background were investigated and classified into three groups according to their physical activity and sport discipline: untrained students (group A), national and sub-national level endurance athletes (group B, 7.8±2.9 years of specialised training) and sprint-power athletes (group C, 12.8±8.7 years of specialised training). Muscle biopsies of *vastus lateralis* were analysed histochemically for mATPase and SDH activities, immunohistochemically for fast and slow myosin, and electrophoretically followed by Western immunoblotting for myosin heavy chain (MyHC) composition. Significant differences ($P<0.05$) regarding composition of muscle fibre types and myosin heavy chains were found only between groups A (41.7±1.6% of MyHCI, 40.8±4.0% of MyHCIIA and 17.5±4.0% of MyHCIIX) and B (64.3±0.8% of MyHCI, 34.0±1.4% of MyHCIIA and 1.7±1.4% of MyHCIIX) and groups A and C (59.6±1.6% of MyHCI, 37.2±1.3% of MyHCIIA and 3.2±1.3% of MyHCIIX). Unexpectedly, endurance athletes (group B) such as long-distance runners, cyclists and cross country skiers, did not differ from the athletes representing short term, high power output sports (group C) such as ice hockey, karate, ski-jumping, volleyball, soccer and modern dance. Furthermore, the relative amount of the fastest MyHCIIX isoform in *vastus lateralis* muscle was significantly lower in the athletes from group C than in students (group A). We conclude that the myosin profile in the athletes belonging to group C was unfavourable for their sport disciplines. This could be the reason why those athletes did not reach international level despite of several years of training.

Key words: Myosin - Muscle - Training - Exercise - Sport

Introduction

Skeletal muscles of mammals are heterogeneous in composition of muscle fibres and adaptable - these properties ensure functional diversity in performing complex movement patterns. During the last two decades it has been established that the phenotype of muscle fibres and their contractile and metabolic proteins as well as fibre cross-sectional area may change extensively in response to different work regimes [42, 43]. This ability of muscle tissue may have practical implication for both sportsmen and patients.

Skeletal muscle fibres are classified into two major groups, the slow-twitch and fast-twitch fibres. Their contractile properties are determined by myosin heavy chain (MyHC) isoforms: the slow MyHCI and the fast types MyHCIIA and MyHCIIX [6, 31, 40]. According to Staron and Pette [43], there is a direct link between MyHC composition in muscle fibres and their mATPase activity. Slow muscle fibres express predominantly the slow type MyHCI isoform with some proportion of fast type MyHCIIA [5, 6, 15, 16, 36, 40, 45]. Slow fibres rely more on aerobic metabolism, whereas the fast fibres depend more on anaerobic metabolism. Thus, slow fibres are important for endurance activities and sports such as long-distance running, cycling and swimming, whereas fast fibres are key to power pursuits such as weight lifting and sprinting [4]. Moreover, IIX fibres are superior to IIA fibres in respect to maximal power output and are crucial for sprint strength disciplines [37]. Human slow muscle *soleus* contains an approximately equal amount of type I and type IIA myosin isoforms, while type IIX isomyosin is not expressed in this muscle [21]. In contrast, human fast muscle such as *vastus*

Correspondence: B. Zawadowska, Dept. Cytology and Histology, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland; e-mail: zaw@zuk.iz.uj.edu.pl

lateralis expresses all three types of myosin isoforms at variable proportions [21, 27].

It is well known that *vastus lateralis* of untrained, physically active subjects contains approximately 50% of type I and 50% of type II muscle fibres. For example in the studies by Andersen and Aagaard [1], Andersen *et al.* [4] and Klitgaard *et al.* [26] the *vastus lateralis* possessed approximately 50% slow type I, 40% type IIA and 10% IIX myosin isoforms. On the other hand, world class marathon runners and ultra-endurance athletes have 95% type I fibres [4, 22, 35, 37], whereas sprinters, power weight lifters and throwers undertaking an anaerobic type of strength training contain predominantly IIA and IIX fibres [2, 3]. The latter athletes possess the proportion of type I fibres as low as 20% in the muscles concerned [38]. Hence, skeletal muscles can express different myosin isoforms depending on the type of training regime. Another example of plasticity of the mature skeletal muscles is that long-term endurance training induces significant conversion from fast to slow muscle fibres [24, 41, 46]. For example, 8-week training decreased by 21%-70% type II fibre population in 3 of 4 subjects [7, 25]. Furthermore, MyHC isoform shift relies on production of a new polypeptide of slow MyHC in fibres, which are still classified as type IIA fibres. These changes at the molecular level in type IIA fibres mark the beginning of their transition towards the slow type I fibres [7]. Exercise-induced transformation in the reverse direction (type I to IIA) appears much more difficult to be achieved and requires rigorous exercise regimen [4]. According to Esbjörnsson *et al.* [14] and Jansson *et al.* [23], sprint training may cause such transformation in human *vastus lateralis* muscle. On the other hand it has recently been shown that each kind of training (endurance as well as power activities) decreases the expression of type IIX MyHC isoform [1].

Although top class endurance athletes contain predominantly type I fibres and the muscles of power athletes contain predominantly type II fibres [13, 18, 34], it is still not known to what extent the distribution of muscle fibre types in sportsmen is the effect of inherited, genetic predisposition and environmental influences such as different training regime. Furthermore, literature on this subject usually describes myosin profile in muscles of world class sportsmen but little is known about myosin characteristic in muscles of national and sub-national level athletes. Those athletes, despite of many years of training, can not reach international level. We postulate that one of the reasons may be improper myosin profile in their locomotor muscles crucial for the excellence in their sport.

Hence, the aim of the present study was to analyse the composition of muscle fibre types and myosin heavy chains in a thigh muscle *vastus lateralis* of physically active but untrained students and of national and subnational level sportsmen, representing different sport disciplines.

Materials and methods

Subjects. Twenty-four male volunteers (mean ± SD: age 25.4±5.8 years, height 178.6±5.5 cm, body mass 72.1±7.7 kg) of different training background were investigated and classified into three groups according to their physical activity and sport discipline. Group A contained 7 physically active but untrained students of the Academy of Physical Education in Cracow. Group B included 9 endurance athletes (7.8±2.9 years of specialised training): long distance runners, cross country skiers and cyclists. Group C consisted of 8 athletes (12.8±8.7 years of specialised training) performing short term, high power output sports such as ski jumping, karate, ice-hockey, soccer, modern dance, volleyball and handball. Sportsmen of group B and C represented national level in their sport disciplines. All volunteers were subjected to detailed medical interview and check-up. The Local Ethical Committee approved the investigations.

Muscle biopsy. After local anaesthesia (2 ml of 1% lignocaine), percutaneous muscle biopsy samples were taken from the middle part of *vastus lateralis m. quadricipitis femoris* 15 cm above the upper margin of patella using ∅ 5 mm Bergström needles [8]. Each biopsy was transversally divided into two parts. One part was assigned for histochemical and immunohistochemical analyses and the other part was used for SDS-PAGE and Western immunoblotting.

Histochemistry and immunohistochemistry. The biopsies were covered with a mixture of Tissue Tek (TAAB laboratories, UK) and a talcum powder and rolled into pig colon submucosa. Such specimens were frozen in isopentane cooled with liquid nitrogen and stored until use. Serial, 10 μm thick cryosections were treated for mATPase after alkaline (pH 10.4) and acid (pH 4.35 and 4.6) preincubations [10, 19], and for SDH activity [33]. For immunohistochemistry sections were incubated overnight with mouse monoclonal antibody (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK) against fast myosin heavy chain (NCL-MHCf, 1:100), slow myosin heavy chain (NCL-MHCs, 1:150), neonatal myosin heavy chain (NCL-MHCn, 1:50) or developmental myosin heavy chain (NCL-MHCd, 1:60) diluted in Tris buffered saline pH 7.2 (TBS). For visualization of immunoreactions Biotin-Streptavidin kit (DAKO, Denmark) was applied according to kit instruction (DAKO LSAB 2 System, Peroxidase). After final washes, sections were mounted in glycerine jelly, viewed and photographed in Axioskop light microscope (Zeiss, Oberkochen, Germany). The point counting method with A121 square lattice test system was used [48] to establish the numerical density of the muscle fibre types in relation to the investigated biopsies. Muscle fibres of type I, IIA+IIX and IIA/I were counted in sections stained for mATPase activity after alkaline preincubation. Fibres of type I were also counted in sections immunostained for slow myosin heavy chain while hybrid fibres expressing isomyosins I/IIA and fast fibres IIA + IIX were counted in sections immunostained for fast myosin heavy chain.

SDS-PAGE and Western immunoblotting. Muscle biopsies were cryosectioned and extracted with 62.5 mM Tris, 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, pH 6.8. SDS-PAGE of myosin extract was performed according to Carraro and Catani [12] with 3% stacking and 6% separating gels containing 37.5% glycerol at 60 V for 30 min followed by 180 V for 3 h. For immunoblotting, samples were transferred onto immobilon-P transfer membrane (Millipore Corporation, Bedford, USA) at 30 V for 12-15 h [47]. Membrane was blocked for 1 h in 10% non-fat dry milk in TBS, 0.1%Tween 20 (dilution buffer) and then incubated for 1 h with primary monoclonal antibody against fast myosin diluted 1:200, or against slow myosin diluted 1:100 in dilution buffer. Bound primary antibody was detected with goat anti-mouse alkaline phosphatase conjugate (Pierce Chemical Co., Rockford, IL, USA), diluted 1:2500, followed by BCIP/NBT (5-bromo-4-chloro-3-indol phosphate/ nitroblue tetrazolium) treatment. The relative amounts of slow and fast MyHC

resolved by SDS-PAGE and fast isoforms MyHCIIA and MyHCIIX analysed by Western immunoblotting were assessed by densitometric analysis of polyacrylamide gels and PVDF membranes, using CCD camera (Fotodyne Incorporated) and a Gel Pro Analyser software.

Statistical analysis. All values are reported as mean±standard deviation (SD). Data was examined by MANOVA and tested for differences by Tuckey test. A confidence level of $P<0.05$ was chosen to indicate statistical significance.

Results

Histochemistry and immunohistochemistry

Histochemical and immunohistochemical examination of 24 muscle biopsies allowed to classify muscle fibres into four types, I, I/IIA, IIA/I and IIA+IIX, according to their MyHC isoform content. Fibres of type I contained pure slow myosin and were negative in reaction for mATPase activity after alkaline preincubation (Fig.1 a). Type II fibres - fast fibres were considered as both IIA+IIX fibres. They were positive (dark) after alkaline and negative (white) after acid preincubations (Fig.1 a, b, c). Both IIA + IIX fibres displayed intense immunostaining with antibody against fast myosin (Fig.1d) and were negative after immunostaining with antibody against slow MyHC (Fig.1e). Hybrid muscle fibres I/IIA were detected after immunoreaction against fast myosin and displayed an intermediate staining intensity between the fast and slow type fibres (Fig.1d). The I/IIA fibres were recognized in 22 investigated biopsies (Table 1). Another hybrid muscle fibres IIA/I were discerned after reaction for mATPase activity with alkaline preincubation as the fibres of brown colour in contrast to the black coloured fibres of type II (Fig.1a). Fibres IIA/I were present only in four biopsies (Table 1). The metabolic profile of the particular types of muscle fibres was evaluated visually on the basis of colour density of diformazan deposits in reaction for SDH activity. Both hybrid types of muscle fibres, I/IIA and IIA/I, showed high oxidative activity similar to type I fibres, while type II fibres displayed colour density from medium to none (Fig. 1f). Immunohistochemical analysis of frozen sections from biopsies of *vastus lateralis* with monoclonal antibodies against neonatal and developmental myosin revealed that none of 24 muscle biopsies contained neonatal or developmental myosin (not shown).

The mean percentages of slow type I, fast type II and hybrid (I/IIA + IIA/I together) muscle fibres in *vastus lateralis* from students (group A), endurance athletes (group B) and athletes training sprint strength disciplines (group C) are summarised in Table 1 and displayed in Figure 2. Significant differences in muscle fibre type content existed only between group A and the two other groups for slow and fast fibres ($P<0.02$), and between group A and group C for hybrid fibres ($P<0.03$).

SDS-PAGE and Western immunoblotting

Electrophoretic analysis of myosin heavy chains in polyacrylamide gel revealed the presence of the two most abundant isoforms of MyHC in each of 24 biopsies of *vastus lateralis*. The fastest migrating band represents slow MyHCI isoform and the slower moving band refers to fast MyHCIIA isoform. The third isoform, MyHCIIX, with the slowest electrophoretic mobility was detected in all biopsies from untrained students (group A), in 3 biopsies from endurance athletes (group B), and in 4 biopsies from athletes performing short term, high power output sports (group C). An example of MyHC patterns representative for each group is shown in Figure 3. Densitometric analysis of slow and fast isoforms of MyHC resolved in polyacrylamide gels showed that *vastus lateralis* from the group of students contained almost equal amount of slow and fast myosin heavy chains, while in endurance athletes and athletes of group C slow MyHCI was more abundant than both MyHCIIA and MyHCIIX (Fig. 4). Differences in MyHC content were statistically significant at $P<0.02$ only between the group of students and the other two groups of sportsmen.

To evaluate more accurately the relative amount of MyHCIIA and MyHCIIX protein in muscle biopsies, myosin heavy chains resolved by SDS-PAGE were transferred onto PVDF membrane and probed with monoclonal antibody against fast myosin. A typical example of immunoblot representative for the investigated groups is shown in Figure 5. Densitometric analysis of MyHC protein bound to PVDF membrane revealed that MyHCIIA dominated over MyHCIIX in all investigated biopsies (Fig. 6). However, statistically significant differences existed only between group A and the two other groups for MyHCIIA at $P<0.01$, and for MyHCIIX at $P<0.03$. There was no difference between group B and C for MyHCIIA or MyHCIIX. The results of SDS-PAGE and Western immunoblotting analyses for all 24 subjects are summarised in Table 1.

To evaluate the relative proportions of MyHCI, MyHCIIA and MyHCIIX in biopsies from *vastus lateralis*, data from Western blotting was expressed as percentages of total MyHCII estimated by SDS-PAGE. Therefore, in group A there was 41.7±1 .6% of MyHCI, 40.8±4.0% of MyHCIIA and 17.5±4.0% of MyHCIIX, in group B: 64.3±0.8% of MyHCI, 34.0±1.4% of MyHCIIA and 1.7±1 .4% of MyHCIIX, and in group C: 59.6±1.6% of MyHCI, 37.2±1.3% of MyHCIIA and 3.2±1.3% of MyHCIIX.

Analysis of correlation

Three different methods were used for examining MyHC protein expression in human *vastus lateralis* muscle - myofibrillar ATPase assay, immunohistochemical labelling with monoclonal antibodies and

Table 1. Histochemical, immunohistochemical and electrophoretical characteristics of muscle biopses from vastus lateralis of 24 volunteers

Biopsy number and sport discipline	Percentage of muscle fibre types (mean ± SD)						Percentage of MyHC (mean ± SD)			
	mATPase			Antibodies			SDS-PAGE		Western blotting	
	I	IIA / I	IIA + IIX	I	I / IIA	IIA + IIX	I	IIA + IIX	IIA	IIX
1. student	33.1 ± 1.8	0	66.9 ± 4.7	26.2 ± 2.2	1.2 ± 0.6	72.6 ± 3.4	16.1 ± 7.5	83.9 ± 7.5	62.6 ± 2.6	37.4 ± 2.6
2. student	44.4 ± 2.2	0	55.6 ± 1.8	41.1 ± 3.0	3.1 ± 0.9	55.8 ± 3.6	44.3 ± 0.5	55.8 ± 0.5	59.1 ± 4.3	40.9 ± 4.3
3. student	48.1 ± 2.1	0	51.9 ± 5.7	48.3 ± 3.8	0	51.7 ± 4.2	49.3 ± 0.9	50.8 ± 0.9	67.1 ± 19.2	32.9 ± 19.2
4. student	46.9 ± 3.9	0	53.1 ± 4.6	41.6 ± 3.9	1.2 ± 4.4	57.2 ± 6.0	38.8 ± 2.6	61.3 ± 2.6	77.2 ± 0.3	22.8 ± 0.3
5. student	49.6 ± 2.2	0	50.4 ± 5.4	46.9 ± 2.6	0.9 ± 0.9	52.2 ± 4.4	43.3	56.7	71.0 ± 13.2	29.0 ± 13.2
6. student	64.5 ± 3.6	0	35.5 ± 4.0	51.2 ± 4.7	1.5 ± 0.8	47.3 ± 4.4	46.0	54.0	81.3 ± 7.7	18.7 ± 7.7
7. student	63.3 ± 3.9	0	36.7 ± 2.7	55.6 ± 3.8	9.5 ± 2.2	34.9 ± 3.2	53.9	46.1	71.9	28.1
Mean percentage (group A)	**50.0 ± 2.8**	**0**	**50.0 ± 4.3**	**44.4 ± 3.4**	**2.5 ± 1.4**	**53.1 ± 4.2**	**41.7 ± 1.6**	**58.4 ± 1.6**	**70.0 ± 6.8**	**30.0 ± 6.8**
8. distance runner	66.5 ± 3.5	0	33.5 ± 4.6	64.4 ± 5.1	1.5 ± 1.9	34.1 ± 3.0	66.0 ± 0.1	34.0 ± 01	100	0
9. distance runner	45.3 ± 3.4	0	54.7 ± 3.9	47.9 ± 3.6	2.8 ± 1.0	49.3 ± 5.0	70.2 ± 2.7	29.8 ± 2.7	100	0
10. distance runner	62.9 ± 3.7	0	37.1 ± 3.1	65.4 ± 4.3	0.7 ± 1.1	33.9 ± 2.9	79.2 ± 2.1	20.8 ± 2.1	90.0 ± 14.1	10.0 ± 14.1
11. cyclist	61.3 ± 3.8	0	38.7 ± 3.5	64.4 ± 5.0	1.7 ± 0.7	33.9 ± 2.2	52.7	47.3	73.7 ± 12.1	26.3 ± 12.1
12. distance runner	51.4 ± 6.6	0	48.6 ± 4.0	44.6 ± 5.5	5.4 ± 3.1	49.9 ± 5.0	39.6	60.4	94.1 ± 8.3	5.9 ± 8.3
13. cyclist	61.2 ± 2.0	0	38.8 ± 2.5	65.9 ± 4.9	3.6 ± 1.1	30.5 ± 3.2	64.9	35.1	100	0
14. cyclist	75.1 ± 4.4	5.0 ± 0.5	19.9 ± 1.0	68.7 ± 5.1	1.1 ± 0.5	30.2 ± 1.1	55.3	44.7	100	0
15. cross country skier	79.3 ± 2.9	0	20.7 ± 2.8	61.7 ± 7.4	15.2 ± 4.3	23.1 ± 2.5	68.3	31.7	100	0
16. cross country skier	86.5 ± 2.7	0	13.5 ± 1.3	81.3 ± 6.0	6.1 ± 1.7	12.6 ± 1.1	82.6 ± 2.3	17.5 ± 2.3	100	0
Mean percentage (group B)	**65.5 ± 3.7**	**1.7 ± 0.06**	**33.9 ± 3.0**	**62.7 ± 5.2**	**4.2 ± 1.7**	**33.0 ± 2.9**	**64.3 ± 0.8**	**35.7 ± 0.8**	**95.3 ± 3.8**	**4.7 ± 3.8**
17. modern dancer	71.8 ± 4.5	5.8 ± 1.9	22.4 ± 2.2	71.0 ± 3.9	4.3 ± 0.8	24.7 ± 2.8	71.6 ± 1.1	28.5 ± 1.1	100	0
18. karate fighter	42.0 ± 3.2	0	58.0 ± 4.7	46.9 ± 3.2	0	53.1 ± 4.3	41.2 ± 5.1	58.8 ± 5.1	92.1 ± 11.7	7.9 ± 11.7
19. soccer player	66.2 ± 4.6	0	33.8 ± 2.0	52.9 ± 4.9	13.5 ± 3.4	33.5 ± 2.7	45.6	54.4	78.4 ± 8.5	21.6 ± 8.5
20. volleyball player	56.5 ± 3.2	5.7 ± 1.2	37.8 ± 2.0	55.9 ± 4.2	2.8 ± 1.3	41.3 ± 2.5	57.7	42.3	95.3 ± 6.6	4.7 ± 6.6
21. ski-jumper	70.0 ± 3.1	0	30.0 ± 1.9	61.4 ± 3.8	5.6 ± 1.5	33.0 ± 5.6	61.1	38.9	100	0
22. hockey player	81.3 ± 2.9	0.8 ± 0.4	17.9 ± 1.9	69.0 ± 6.0	7.7 ± 0.9	23.3 ± 1.7	71.7 ± 2.8	28.3 ± 2.8	100	0
23. hockey player	58.9 ± 2.3	0	41.1 ± 3.0	66.5 ± 4.9	11.3 ± 2.1	22.2 ± 3.9	69.7 ± 2.3	30.4 ± 2.3	100	0
24. handball player	79.5 ± 3.4	0	20.5 ± 1.9	70.0 ± 5.5	4.8 ± 2.3	25.2 ± 2.7	57.9 ± 1.4	42.1 ± 1.4	69.8	30.2
Mean percentage (group C)	**65.8 ± 3.4**	**1.5 ± 0.4**	**32.7 ± 2.5**	**61.7 ± 4.5**	**6.2 ± 1.5**	**32.0 ± 3.3**	**59.6 ± 1.6**	**40.5 ± 1.6**	**91.9 ± 3.3**	**8.1 ± 3.3**

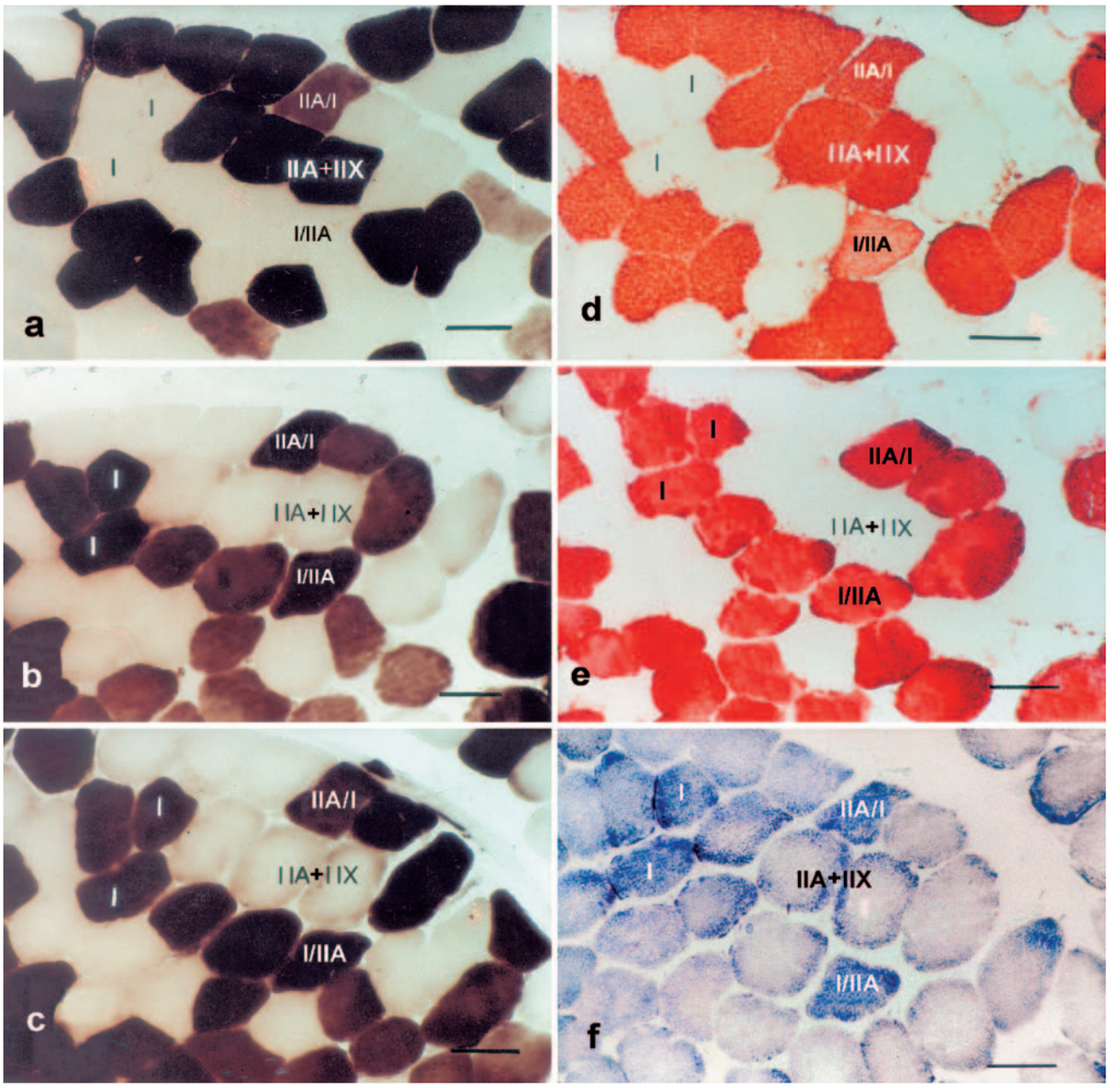

Fig. 1. Histochemical and immunohistochemical analysis of slow I, fast IIA+IIX, and hybrid I/IIA, IIA/I muscle fibres in serial frozen sections from human *vastus lateralis* muscle. Myosin ATPase activity after preincubations in buffers at pH: (**a**) 10.4, (**b**) 4.35, (**c**) 4.6. Immunostaining with monoclonal antibodies against: (**d**) fast and (**e**) slow myosin isoforms. Histochemical staining for SDH activity to depict aerobic capacity of muscle fibres (**f**). Bar = 50 μm.

SDS-PAGE followed by Western immunoblotting. There was a significant positive correlation at $P<0.001$ between: the relative amount of slow and fast MyHC resolved by SDS-PAGE, and the number of slow and fast muscle fibres assayed by mATPase ($r=0.74$), the number of slow and fast muscle fibres demonstrated by monoclonal antibodies and assayed for mATPase ($r=0.95$), and the relative amount of slow and fast MyHC resolved by SDS-PAGE, and the number of slow and fast muscle fibres demonstrated by monoclonal antibodies ($r=0.84$) (Fig. 7). High values of correlation coefficient point out a strong correlation between the specific parameters estimated with these methods.

Discussion

The main purpose of this study was to characterise myosin profile of thigh muscle *vastus lateralis* in national and subnational level athletes representing different sport disciplines in relation to a control group of physically active but untrained students. This main locomotor skeletal muscle was chosen because it is well

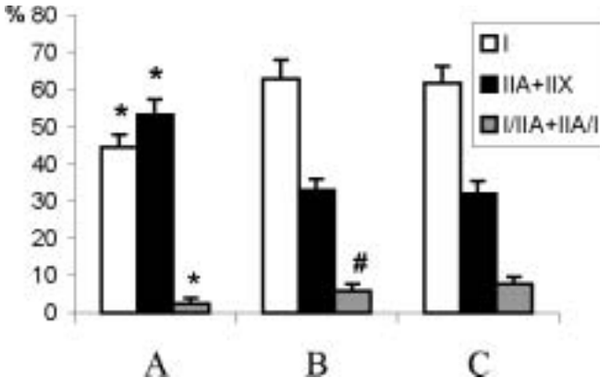

Fig. 2. Graphic demonstration of the mean percentage of slow I, fast II, and hybrid I/IIA + IIA/I fibres in biopsies of *vastus lateralis* muscle of untrained students (A), athletes of group B and athletes of group C. Significant differences (*) exist between group A and the two other groups for slow and fast fibres (P<0.02), and between groups A and C for hybrid fibres (P<0.03). There were no significant differences (#) between groups A and B and between B and C for hybrid fibres.

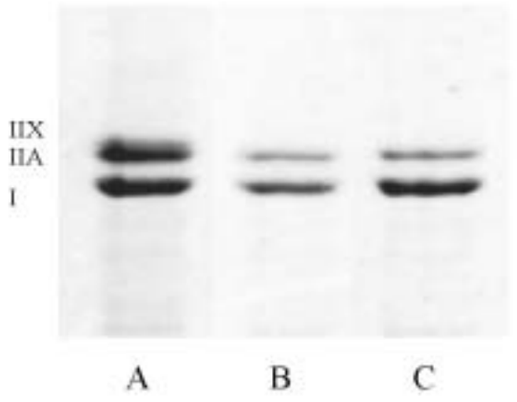

Fig. 3. SDS-PAGE analysis of myosin heavy chain content in biopsies from *vastus lateralis* representative for untrained students (A), athletes of group B and athletes of group C. I, IIA and IIX indicate the positions of MyHCI, MyHCIIA and MyHCIIX in polyacrylamide gel, respectively.

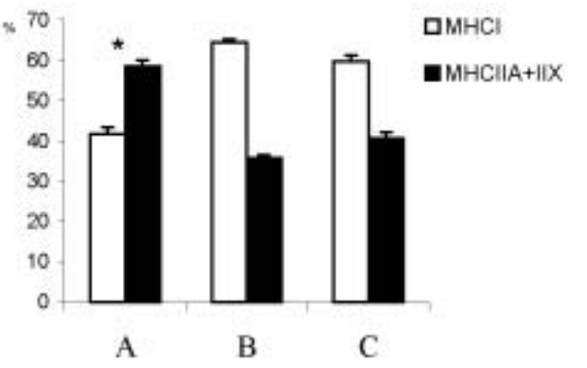

Fig. 4. Graphic illustration of the mean percentages of slow MyHCI and fast MyHCII resolved by SDS-PAGE in biopsies of *vastus lateralis* muscle from untrained students (A), athletes of group B and athletes of group C. Significant differences (*) exist between group A and the two other groups at P<0.02.

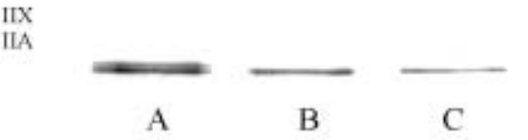

Fig. 5. Western immunoblot of fast isoforms MyHCIIA and MyHCIIX from biopsies of *vastus lateralis* representative for the group of untrained students (A), athletes of group B and athletes of group C. PVDF membrane was probed with monoclonal antibody against fast myosin. IIA and IIX indicate the positions of MyHCIIA and MyHCIIX in the membrane, respectively.

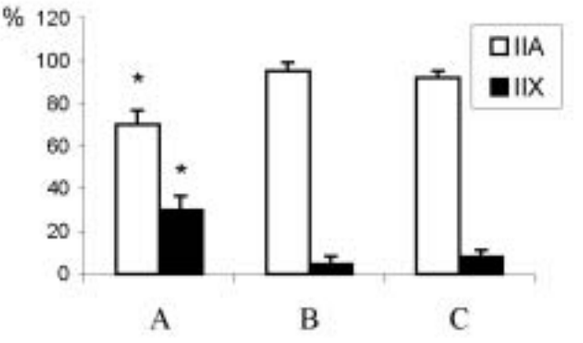

Fig. 6. Graphic demonstration of the mean percentages of fast isoforms MyHCIIA and MyHCIIX in biopsies of *vastus lateralis* from untrained students (A), athletes of group B and athletes of group C. Significant differences (*) exist between group A and the two other groups for MyHCIIA at P<0.01, and for MyHCIIX at P<0.03.

characterised physiologically and biochemically and its phenotype clearly reflects any adaptive changes taking place during a variety of physiological conditions such as different types of training regime [2, 3, 28, 42, 44, 49]. It was reasonable to expect qualitative or quantitative differences in the composition of muscle fibre types and myosin heavy chain content in this muscle between students and national/subnational class athletes, and between athletes representing endurance and short term, high power output sport disciplines. Characteristics of muscle biopsies were done by combining histochemical mATPase activity, immunohistochemical labelling with monoclonal antibodies against fast and slow myosin, and protein electrophoresis followed by Western immunoblotting. High values of correlation coefficient be-

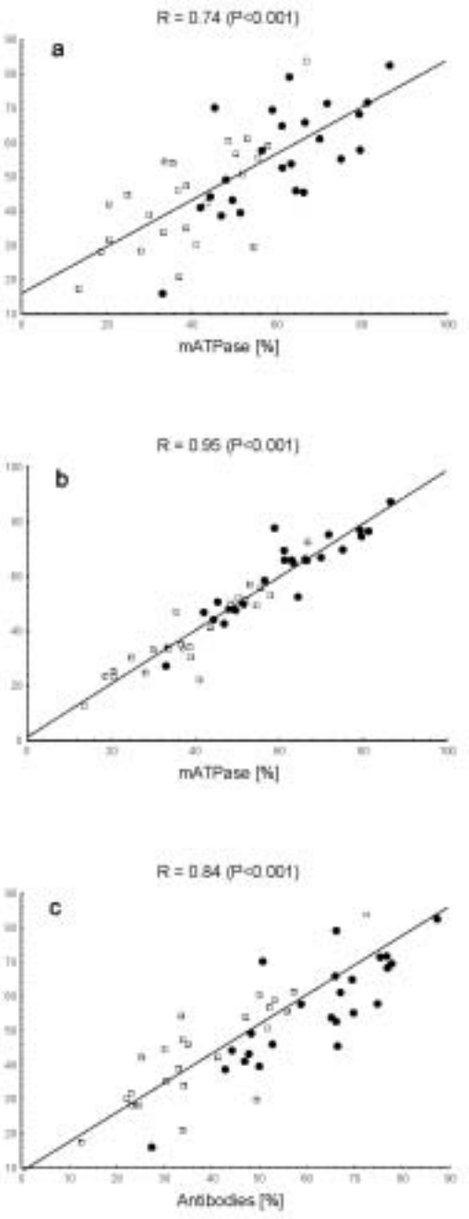

Fig. 7. Scatter plots of the relative amount of slow and fast MyHC resolved by SDS-PAGE and the number of slow and fast muscle fibres assayed by mATPase (**a**), the number of slow and fast muscle fibres analysed with monoclonal antibodies and assayed for mATPase (**b**), and the relative amount of slow and fast MyHC resolved by SDS-PAGE and the number of slow and fast muscle fibres analysed with monoclonal antibodies (**c**). The correlation coefficients (r) are given in the scatter plots. Critical value at $P<0.001$ is $r=0.465$. Open squares and solid circles represent slow and fast types, respectively, of MyHC and muscle fibres.

tween the results obtained with these methods point out a strong correlation between myosin heavy chain content and muscle fibre types composition in human *vastus lateralis* muscle and thus emphasize good accuracy in biopsy characteristics (Fig. 7). This assumption is in agreement with the results of Staron and Pette [43] who showed the direct link between MyHC composition and mATPase activity, and with the report of Rivero *et al.* [36] on correlation analysis between four different methods, which gave the clear proof that myosin heavy chains represent the best possible marker of muscle fibre type characteristics.

The major and unexpected finding of the present study was that endurance athletes and athletes representing high power output sport disciplines have not revealed any significant differences in the composition of the slow and fast muscle fibres and myosin heavy chains in their *vastus lateralis* muscle (Fig. 2, 4, 6). Muscle phenotype in both groups of these athletes was similar with low amount of MyHCIIX isoform and marked predominance of slow type muscle fibres and slow myosin heavy chain isoform MyHCI. Thus, this phenotype was adapted rather for long lasting and sustainable activities typical for endurance disciplines than for fast disciplines requiring high power output during short time. Furthermore, the relative amount of the fastest MyHCIIX isoform in *vastus lateralis* muscle was significantly lower in athletes training sprint strength disciplines than in students. However, it is difficult to establish to what extent the expression of this slow phenotype was inborn or was the result of training volume overload because the myosin profile before the beginning of the training process was unknown. In any case, lack of an appropriate myosin profile such as that in muscles of top class athletes of sprint events [4] might be one of the reasons that did not allow them to achieve international competition level.

Many studies on conversion of muscle fibres found that repeated training overload induces fast to slow transformation in myofibrillar protein isoforms and fibre type composition [4, 17, 28, 30]. During resistance or running exercise, IIX fibres are converted to the slower but more oxidative IIA fibres. However, it was found by Andersen *et al.* [4] that during the rest period, rather than simply returning to the pre-training level, the relative amount of MyHCIIX isoform roughly doubled three months into detraining. Moreover, the default gene hypothesis suggests that when the muscle is not subjected to stretch or force generation, the fast myosin heavy chain gene is expressed by default [17]. For this reasons an "overshoot" phenomenon of the expression of the fast myosin MyHCIIX isoform, reported by Andersen *et al.* [1, 4], should be considered in the case of athletes performing short term, high power output sport disciplines to whom fast IIX fibres are crucial. A program of vigorous weight training supplemented with other forms of anaerobic exercise was proposed by Andersen *et al.* [4] for sprinters, as this training regime converts not only type IIX fibres to IIA but also type I fibres to IIA.

In group B, there was a national level athlete with high amount of slow myosin heavy chain (82.6% MyHCI, 17.4% MyHCIIA and 0% MyHCIIX) who won the Polish championship, while in group C there was a sportsman (61.1% MyHCI, 38.9% MyHCIIA, 0% MyHCIIX), who in spite of long-lasting training did not achieve international results. It is worth mentioning that untrained students possessed on average 41.7% MyHCI, 40.8% MyHCIIA and 17.5% MyHCIIX isoforms. Our study shows that the choice of sport discipline not always corresponds to favourable myosin profile. Hence, the best criterion of such choice would be to evaluate type of skeletal muscle myosin before training, which directly results from individual genetic predispositions of athletes.

According to Picard *et al.* [32], histochemical and immunohistohemical classifications of muscle fibres well agree for so called "pure" fibres of type I, IIA, and IIX but present some differences for the classification of hybrid fibres. Hybrid muscle fibres I/IIA possessed more slow myosin than fibres IIA/I that expressed the greater amount of fast myosin. Hybrid fibres are thought to represent an intermediate stage in the transformation process of fast to slow and from slow to fast fibres [1, 4, 11, 20]. In the present study, four fibre types were detected in human *vastus lateralis* muscle using mATPase assay and immunohistochemistry with monoclonal antibodies: "pure" slow type I and fast type II considered as IIA+IIX fibres, and hybrid fibres classified as type I/IIA and type IIA/I. There was significantly more hybrid fibres in *vastus lateralis* of athletes representing short term, high power output sports than in the same muscle of untrained students, which suggests that the presence of hybrid fibres was the result of intensive physical training of this muscle. Hence, these results well agree with the current concept on the origin of hybrid fibres [1, 4, 11, 20].

As expected, significant differences in slow and fast muscle fibres and myosin heavy chain content in *vastus lateralis* were found between physically active but untrained students and both groups of national class athletes. On the average, the untrained students' muscles expressed almost equal levels of fast (type IIA+IIX) and slow (type I) phenotype. However, in this group we encountered students with slow fibre percentage as low as 33% and as high as 64% in the *vastus lateralis* muscle assayed for mATPase activity. The same students contained 37% and 18% of MyHCIIX isoform, respectively, in their *vastus lateralis* analysed by Western immunoblotting. Therefore, judged by these criteria, they have a similar possibility to achieve national level in sport performance as the subjects from groups B and C.

As discussed above, the highest expression of MyHCIIX isoform was found in the group of untrained students. This is in agreement with the recent study of Andersen and Aagaard [1] showing overexpression of MyHCIIX after detraining process as well as with the findings showing overexpression of MyHCIIX in immobilised muscles [for review see 9, 29]. Surprisingly, the subjects from group C in which one would expect the highest expression of MyHCIIX, did not differ in this respect from subjects of the group B.

It is well established that the muscles containing higher proportion of type IIX fibres possess greater maximal velocity of shortening, which is the key factor in maximal power generating capability [for review see 39], required for successful performance in the sports trained by the subjects from group C. According to the experimental data, not force but the maximal velocity of contraction plays the key role in producing maximal power output. Training-induced decrease in the number of IIX fibres may decrease maximal power output. On the other hand, higher proportion of MyHCIIX isoform found in untrained students, without a training of muscle force, does not guarantee high power generating capabilities in their muscle. According to the power-velocity relationship of different muscle fibres [39], low proportion of the fastest MyHCIIX in the muscles of the sportsmen from group C, when compared to group A, may limit the generation of maximal power output needed for successful performance in their sport disciplines.

We conclude that the myosin profile in the athletes belonging to group C is unfavourable for their sport disciplines. This could be the reason why those athletes, despite of several years of training, did not reach international level.

Acknowledgements: The authors wish to thank Prof. E. Pyza for critical reading of the manuscript, Drs. A. Fiertak and G . Tylko for excellent assistance in statistical analysis, and Drs. A. Duda and P. Pierzchalski for densitometric analysis of MyHC. We thank Aventis Pharma for sponsoring of printing colour illustration. This study was supported by the National Committee for Scientific Research (KBN), grant no. 4505D 058 17, and by funds from the Academy of Physical Education, Cracow, Poland for statutory research in 2003.

References

[1] Andersen JL, Aagaard P (2000) Myosin heavy chain IIX overshoot in human skeletal muscle. Muscle Nerve 23: 1095-1104

[2] Andersen JL, Klitgaard H, Saltin B (1994) Myosin heavy chain isoforms in single fibres from *m. vastus lateralis* of sprinters: influence of training. Acta Physiol Scand 151: 135-142

[3] Andersen JL, Mohr T, Biering-Sorensen F, Galbo H, Kjaer M (1996) Myosin heavy chain isoform transformation in single fibres from *m. vastus lateralis* in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES). Pflügers Arch 431: 513-518

[4] Andersen JL, Schjerling P, Saltin B (2000) Muscle, genes and athletic performance. Sci Am 283: 48-55

[5] Baldwin KM (1996) Effects of altered loading states on muscle plasticity: what we have learned from rodents? Med Sci Sports Exerc 28: S101-S106

[6] Baldwin KM, Haddad F (2001) Effects of different activity and inactivity paradigms on myosin heavy chain gene expression in striated muscle. J Appl Physiol 90: 345-357
[7] Baumann H, Jäggi M, Soland F, Howald H, Schaub MC (1987) Exercise training induces transitions of myosin isoforms subunits within histochemically typed human muscle fibres. Pflügers Arch 409: 349-360
[8] Bergström J (1975) Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 35: 609-616
[9] Bottinelli R (2001) Functional heterogeneity of mammalian single muscle fibres: do myosin isoforms tell the whole story? Pflügers Arch 443: 6-17
[10] Brooke MH, Kaiser KK (1970) Muscle fiber types: how many and what kind? Arch Neurol 23: 369-379
[11] Cadefau J, Casademont J, Grau JM, Fernandez J, Balaguer A, Vernet M, Cusso R, Urbano-Marquez A (1990) Biochemical and histochemical adaptation to sprint training in young athletes. Acta Physiol Scand 140: 341-351
[12] Carraro U, Catani CA (1983) Sensitive SDS-PAGE method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. Biochem Biophys Res Commun 116: 793-802
[13] Costill DL, Daniels J, Evans W, Fink W, Krahenbuhl G, Saltin B (1976) Skeletal muscle enzymes and fiber composition in male and female track athletes. J Appl Physiol 40: 149-154
[14] Esbjörnsson M, Hellsten-Westing Y, Balsom PD, Sjodin B, Jansson E (1993) Muscle fibre type changes with sprint training: effect of training pattern. Acta Physiol Scand 149: 245-246
[15] Fitts RH, McDonald KS, Schluter JM (1991) The determinants of skeletal muscle force and power: their adaptability with changes in activity pattern. J Biomech 24, Suppl 1: 111-122
[16] Fitts RH, Widrick JJ (1996) Muscle mechanics: adaptations with exercise-training. Exerc Sport Sci Rev 24: 427-473
[17] Goldspink G (1998) Selective gene expression during adaptation of muscle in response to different physiological demands. Comp Biochem Physiol B Biochem Mol Biol 120: 5-15
[18] Gollnick PD, Armstrong RB, Saubert CW, Piehl K, Saltin B (1972) Enzyme activity and fiber composition in skeletal muscle of untrained and trained man. J Appl Physiol 33: 312-319
[19] Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. Exp Neurol 28: 365-367
[20] Harridge SDR (1996) The muscle contractile system and its adaptation to training. In: Human muscular function during dynamic exercise. Marconnet P, Saltin B, Komi P, Poortmans J [Eds], Karger, Basel, pp 82-94
[21] Harridge SD, Bottinelli R, Canepari M, Pellegrino M, Reggiani C, Esbjörnsson M, Balsom PD, Saltin B (1998) Sprint training, *in vitro* and *in vivo* muscle function, and myosin heavy chain expression. J Appl Physiol 84: 442-449
[22] Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831-838
[23] Jansson E, Esbjörnsson M, Holm I, Jacobs I (1990) Increase in the proportion of fast-twitch muscle fibres by sprint training in males. Acta Physiol Scand 140: 359-363
[24] Jansson E, Sjodin B, Tesch P (1978) Changes in muscle fibre type distribution in man after physical training. A sign of fibre type transformation? Acta Physiol Scand 104: 235-237
[25] Jostarndt-Fögen K, Puntschart A, Hoppeler H, Billeter R (1998) Fibre-type specific expression of fast and slow essential myosin light chain mRNAs in trained human skeletal muscles. Acta Physiol Scand 164: 299-308
[26] Klitgaard H, Bergman O, Betto R, Salviati G, Schiaffino S, Clausen T, Saltin B (1990) Co-existence of myosin heavy chain I and IIa isoforms in human skeletal muscle fibres with endurance training. Pflügers Arch 416: 470-472
[27] Larsson L, Moss RL (1993) Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. J Physiol 472: 595-614
[28] Linossier MT, Denis C, Dormois D, Geyssant A, Lacour JR (1993) Ergometric and metabolic adaptation to a 5-s sprint training programme. Eur J Appl Physiol Occup Physiol 67: 408-414
[29] Majerczak J, Duda K, Zoladz JA (2001) The effect of innervation, hormonal and mechanical factors expression of myosin isoforms in human skeletal muscle. Folia Med Cracov 42: 89-104
[30] Pette D (1998) Training effects on the contractile apparatus. Acta Physiol Scand 162: 367-376
[31] Pette D, Staron RS (1990) Cellular and molecular diversities of mammalian skeletal muscle fibers. Rev Physiol Biochem Pharmacol 116: 1-76
[32] Picard B, Duris MP, Jurie C (1998) Classification of bovine muscle fibres by different histochemical techniques. Histochem J 30: 473-479
[33] Pool CW, Diegenbach PC, Scholten G (1979) Quantitative succinate-dehydrogenase histochemistry. A methodological study on mammalian and fish muscle. Histochemistry 64: 251-262
[34] Prince FP, Hikida RS, Hagerman FC (1976) Human muscle fiber types in power lifters, distance runners and untrained subjects. Pflgers Arch 363: 19-26
[35] Ricoy JR, Encinas AR, Cabello A, Madero S, Arenas J (1998) Histochemical study of the *vastus lateralis* muscle fibre types of athletes. J Physiol Biochem 54: 41-47
[36] Rivero JL, Talmadge RJ, Edgerton VR (1999) Interrelationship of myofibrillar ATPase activity and metabolic properties of myosin heavy chain-based fibre types in rat skeletal muscle. Histochem Cell Biol 111: 277-287
[37] Saltin B, Gollnick PD (1983) Skeletal muscle adaptability: significance for metabolism and performance. In: Handbook of Physiology: Skeletal Muscle. Peachy LD, Adrian RH, Geiger RS [Eds], Williams and Wilkins, Baltimore, pp 555-631
[38] Saltin B, Henriksson J, Nygaard E, Andersen P, Jansson E (1977) Fiber types and metabolic potentials of skeletal muscles in sedentary man and endurance runners. Ann NY Acad Sci 301: 3-29
[39] Sargeant AJ (1998) The determinants of anaerobic muscle function during growth. In: Pediatric anaerobic performance. Van Praagh E [Ed], Human Kinetics Publishers, Champaign III, pp 97-117
[40] Schiaffino S, Reggiani C (1996) Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev 76: 371-423
[41] Simoneau JA, Lortie G, Boulay MR, Marcotte M, Thibault MC, Bouchard C (1985) Human skeletal muscle fiber type alteration with high-intensity intermittent training. Eur J Appl Physiol Occup Physiol 54: 250-253
[42] Staron RS (1991) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in single human muscle fibers. Histochemistry 96: 21-24
[43] Staron RS, Pette D (1986) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. Histochemistry 86: 19-23
[44] Stienen GJ, Kiers JL, Bottinelli R, Reggiani C (1996) Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. J Physiol 493: 299-307
[45] Talmadge RJ (2000) Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. Muscle Nerve 23: 661-679
[46] Tesch PA, Karlsson J (1985) Muscle fiber types and size in trained and untrained muscles of elite athletes. J Appl Physiol 59: 1716-1720

[47] Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350-4354
[48] Weibel ER (1979) Stereological methods. In: Practical methods for biological morphometry. Academic Press, London
[49] Zhou MY, Klitgaard H, Saltin B, Roy RR, Edgerton VR, Gollnick PD (1995) Myosin heavy chain isoforms of human muscle after short-term spaceflight. J Appl Physiol 78: 1740-1744

Accepted April 29, 2004