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Short communication

Co-Existence of myosin heavy chain I and IIa isoforms in human skeletal muscle fibres with endurance training

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ABSTRACT: The myosin heavy chain (MHC) composition of single fibres from m. vastus lateralis was analysed by one-dimensional electrophoresis and immunoblotting in three groups of young men with distinct difference in physical activity patterns. No major co-existence of MHC isoforms was found in the group with some daily physical activity. In the very sedentary group, however, 19±5% ($P<0.05$) of the fibres exhibited co-existence of MHC type IIa and IIb. Further, in the endurance trained group co-existence of MHC type I and IIa was manifested in 36±4% ($P<0.05$) of the fibres. Disuse and extreme usage of muscle both give rise to an elevation in co-expression of MHC isoforms in single muscle fibres but of markedly different combination of isoforms.

Key words: Human, myosin heavy chain, physical activity, skeletal muscle fibres.

INTRODUCTION

Cross innervation studies have revealed that skeletal muscle can alter its contractile characteristics; an alteration which can also be obtained by electrical stimulation using appropriate patterns of frequencies (7). On the molecular level this is associated with specific isoforms of the contractile proteins. The question has then arisen. Can a change in usage of muscle cause transformation of these isoforms? In rats a long-term, high intensity training schedule has induced an elevated relative number of histochemically identified type I fibres with concomitant changes in myosin light chains, SR-proteins and content of parvalbumin (5).

In man there are numerous reports on fibre type changes in connection with physical training based on histochemical staining of muscle biopsy material (for ref. see 8). Immunocytochemical techniques have also been used and the results support the notion that co-existence of myosin isoforms may exist in fibres of trained muscles, (8). Further support is available in the study by Baumann et al. (1) in which they based their conclusion on an electrophoretic identification of MHC isoform co-existence on pooled classified fibres.

Recently an electrophoretic technique has been developed which allows for characterization of MHC isoforms in a fragment of a single muscle fibre (4). This technique has now been used to evaluate the isoform pattern of MHC's in single fibres from a very highly trained muscle in endurance athletes which is compared with a less used muscle in control subjects, who had either been sedentary or were somewhat active in their daily life. The main finding is that co-existence of isoforms (I and IIa) is more common in the endurance trained muscles, whereas the most inactive subjects exhibit the highest frequency of fibres containing both IIa and IIb and only IIb isoforms.

SUBJECTS

Three groups of subjects were recruited for the present study, based on habitual physical exercise. One group (Sed) consisted of individuals with a minimum of physical activity in their daily lives (22±3 years old; n = 8; sedentary controls = Sed.). Another group were regularly active walking or bicycling as a mean of transportation. Occasionally they also did go out for a "jogg" (17±1 years old; n = 4; active controls = Act.). The third group were cross country skiers who for the last 2-4 years had prepared themselves to be selected for the national team (19±1 years old; n = 4; endurance athletes = End.). They trained 280-450 hrs per year and based on heart rate recording during the training the intensity was from 60 to 100% of their maximal oxygen uptake (74±2 ml x kg^{-1} x min^{-1}). Half of the training was either cross country skiing or roller-skiing, the other half was running. For all groups is valid that their activity pattern had not changed in recent years and was "as usual" the weeks prior to the muscle biopsy.

METHODS

Muscle tissue from the Sed. group was obtained from the midportion of the quadriceps femoris muscle (vastus lateralis = v.l.) as an open biopsy whereas the needle biopsy technique was used in the other groups. The same part of the muscle was sampled and suction was applied to secure 100-150 mg samples and pieces large enough for dissection of 2-4 mm long fibres.

One-dimensional electrophoresis and immunoblotting.

Single fibres from muscle specimens of the untrained controls (Sed.) were prepared and analyzed for MHC composition as previously described (4). Electrophoresis of the fibres from the other two groups (Act. and End.) was also performed in 6% SDS/PAGE gels, with the minor modification of using 37.5% glycerol (w/v) in the separating gel (6). Verification with monoclonal antibodies (mAbs) specific to human MHC type I (mAb BA-F8), IIa (mAb SC-71), and IIb (mAb BF-34) was performed (Figure 1 a). Immunoblotting with these three mAbs have previously demonstrated that they react selectively with rat skeletal muscles known to contain predominantly type I, IIa and IIb fibres, respectively, as determined by the histochemical reaction for myofibrillar ATPase (6). Procedures for electrophoretic transfer to nitrocellulose, incubation with mAb and detection of bound antibody by peroxidase-conjugated second antibody were as previously described (6). In average 25-35 fibres were analyzed in each subject from the Sed. group and 50-70 fibres in each subject of the other two groups. A total of 246, 212, 209 fibres were analyzed in the Sed., Act., and End. groups.

Statistics.

A one-way analysis of variance (ANOVA) was applied for statistical comparisons between the groups. The 0.05 confidence level was chosen for statistical significance.

RESULTS

As can be seen in Figure 1 a, b and c, lane (i), myosin purified from human m. vastus lateralis can be resolved into three bands with different migration rates in 6% SDS/PAGE gels with high glycerol content. As previously shown in humans (6) and demonstrated also in the present study with mAbs (Figure 1 a, lane (ii) to (iv)), these bands correspond to MHC type I, IIa and IIb. This is different from results of rat skeletal muscles which demonstrate that MHC type IIb migrates faster than MHC type IIa in 6% SDS/PAGE gels with high glycerol content (4). Therefore, the MHC isoforms seems to manifest species differences. Single fibres only containing MHC type I (Figure 1 b, lane (iv)), type IIa (lane (iii)) and type IIb (lane (ii)) could be identified. A large fraction (36%) of the fibres from the End. group showed co-existence of MHC type I and type IIa (Figure 2), nearly all with a major amount of MHC type I and minute amounts of MHC type IIa (Figure 1 c , lane (ii)). Co-existence of IIa and IIb MHC was most commonly observed in the Sed. group (Figure 1 c, lane (v)). This is in accordance with a recent study which demonstrated that 37% of the total fibre population from normal human v.l. muscle displays a co-existence of IIa and IIb MHC (2). The Sed. group had also the highest frequency of MHC type IIb. This isoform was completely absent in the End. group and only some few percent were observed in the Act. group. This latter group was also similar to the End. group in respect to the absence of fibres with co-existence of MHC IIa and IIb isoforms. However, they differed markedly in respect to the occurrence of co-existence of MHC I and IIa, which was quite low in the Act. as compared to the End. group, and thus similar to the Sed. group. The frequency of "pure" fibres with MHC IIa was quite the same in all three groups, whereas the frequency of MHC I was highest in the Act. group.

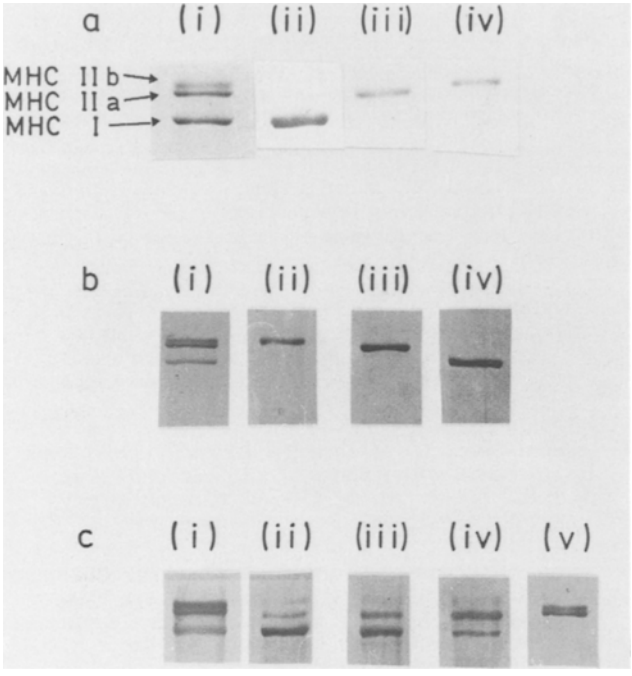

Figure 1.: Identification of human myosin heavy chain isoforms in single fibres of m. vastus lateralis by one-dimensional electrophoresis and immunoblotting. Panel a.: lane (i), purified myosin from human m. vastus lateralis after SDS/PAGE in 6% gels and silver staining; lane (ii) to (iv), myosin separated by SDS/PAGE on 6% gels were transferred to nitrocellulose filters and reacted with mAb BA-F8 (lane (ii)), specific to human MHC type I, mAb SC-71 (lane (iii)), specific to human MHC type IIa and mAb BF-34 (lane (iv)), specific to human MHC type IIb. Panel b.: lane (i), identical with lane (i) in panel a; lane (ii) to (iv), single fibres from m. vastus lateralis of the active control group containing only MHC type IIb (lane (ii)), type IIa (lane (iii)) and type I (lane (iv)) after SDS/PAGE in 6% gels and silver staining.
Panel c.: lane (i), identical with lane (i) in panel a; lane (ii) to (iv), single fibres from m. vastus lateralis of the endurance trained group showing co-existence of MHC type I and type IIa in varying proportions; lane (v) a single fibre from m. vastus lateralis of the active control group showing co-existence of MHC type IIa and IIb.

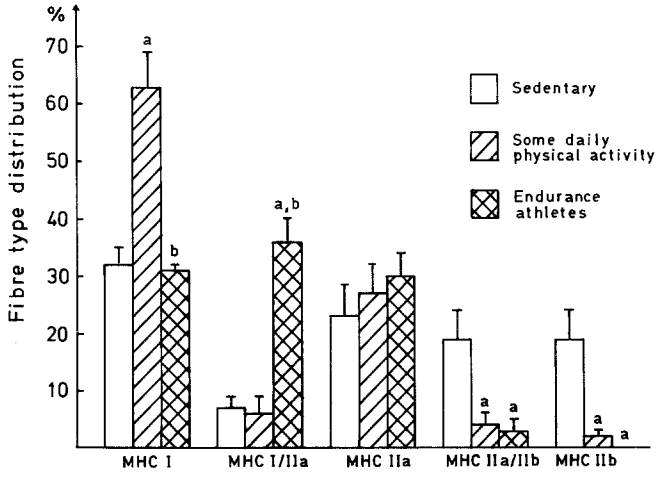

Figure 2.: Comparison of the fibre type composition in m. vastus lateralis of a group of sedentary men (22±3 years old, n = 8); a group of physically active men (17±1 years old, n = 4) and a group of very endurance trained men (19±1 years old, n = 4). Values are means ± S.E.M.. "a" indicates statistical significant difference ($P<0.05$) from the sedentary group and "b" from the physically active group. Single fibres was classified according to their content of myosin heavy chain isoforms after SDS/PAGE in 6% gels and silver staining. 246 fibres was analyzed in the untrained group, 212 fibres in the physically active group and 209 fibres in the endurance trained group.

DISCUSSION

The main finding of the present study is that the pattern of co-existence of myosin isoforms in single fibres of human skeletal muscle varied markedly in three groups with distinct differences in physical activity backgrounds. In the untrained muscles a high occurrence of MHC IIa and IIb co-existence was noted and in the very extremely endurance trained muscles as many as 36% of the fibres contained both MHC I and IIa. In contrast only a small fraction of fibres in the somewhat active muscles exhibited any

co-existence of MHC isoforms. A major problem in studies like this is the representativity of the muscle sample and in this case also whether the fibres dissected for electrophoretic analysis came close to represent the studied muscle. The total number of fibres examined in each group of subjects was quite substantial which constitutes a basis to suggest that the observed variation between groups reflects the relative occurrence of fibres, which contain co-existence of MHC. On the other hand, the site for the sampling of muscle tissue as well as the number of fibres studied in each subject do not allow for any conclusion of the relative occurrence of the various fibre types.

The conventional fibre typing based on the myofibrillar ATPase reaction seems to be determined by the type of MHC isoform (10). With co-existence of various isoforms in one fibre the dominant isoform are decisive, and with equal fractions intermediate staining may be obtained (IM-fibres or type IIc fibres). Since about 95% of the fibres in the End. group showing co-existence of MHC type I and IIa contained a major fraction of MHC type I (Figure 1 c, lane (ii)), these fibres would react as histochemical type I fibres. Thus, the histochemical appearance would indicate a predominance of type I fibres, which is consistent with previous histochemical studies of endurance trained athletes (8). Likewise, the almost complete absence of expression of MHC type IIb in the endurance trained group seems to be in accordance with earlier histochemical studies showing a very small proportion of type IIb fibres in endurance trained muscles (8). Of note is the demonstration of co-existence of MHC isoforms in single fibres, and the fact that it was the two most extreme groups in regard to activity who exhibited this co-existence, but of a different type. The question is then whether the regulatory mechanism behind this phenomenon is the same. A cross-sectional study gives limited basis for more definite statements. The fact that co-existence of MHC IIa and IIb is common in the ageing v.l. muscle (6) may speak in favour of that lack of regular activation of the type II fibres give rise to co-existence of these two MHC isoforms, whereas regular use favour the MHC IIa isoforms. It is also likely that it is a very pronounced usage of the type I and IIa fibres which is the cause of the high occurrence of co-existence of MHC type I and IIa.

Electrical stimulation studies in animals with low-frequency stimulation of fast muscles induces a transition process of histochemical fibre types from type IIb→ type IIa→ type I (7), also suggested to occur in man with training. In a recent study Staron et al. (9) used low-frequency stimulation of rabbit tibialis anterior muscles. The histochemical profile changed from ~95% type IIa and type IIb fibres to a predominance of type IIc fibres (~60%). Further stimulation resulted in a major population (~98%) of a fibre type termed "type I_t", which histochemically resembled type I fibres. This fibre type contained a major amount of MHC type I, but also minute amounts of a second MHC, migrating in the region of MHC type IIa. In accordance with these findings, our results could indicate that endurance training had induced a transition process to a histochemical type I fibre, containing major amounts of MHC type I and minor amounts of MHC type IIa.

In summary, the MHC composition of single fibres from m. vastus lateralis varied markedly between three groups with different physical activity patterns. A large proportion of fibres in the Sed. group showed co-existence of MHC type IIa and IIb or contained only MHC type IIb. On the contrary, nearly none of these fibres were found within the endurance trained group, but this group manifested a clear predominance of fibres showing co-existence of MHC type I and type IIa. About half of the histochemical type I fibres within the endurance trained group seems therefore to consist of fibres with major amounts of MHC type I and small amounts of MHC type IIa.

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