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Review Paper

ENZYME-IMMUNOHISTOCHEMICAL ASPECTS OF MUSCLE FIBER TYPE CLASSIFICATION IN MAMMALS

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Summary: Skeletal muscles are the most abundant and adaptable tissue in mammalians. They are composed of heterogeneous muscle fibers, in which distinct sets of structural proteins and metabolic enzymes are expressed. The percentages of different muscle fibers in the muscle define its morphological and functional characteristics. In this review, we summarize enzyme-immunohistochemical techniques to present muscle fiber type characteristics and their diversity in somatic skeletal muscles of various animal species. The principal methods to define myofiber properties on the tissue sections are based on the immunohistochemical determination of myosin heavy chain (MHC) isoforms and the myosin ATPase and metabolic enzyme histochemistry Four MHC isoforms (-I, -IIa, -IIx and -IIb) have been detected in somatic skeletal muscles of small mammals. Fibers that co-express more than one MHC isoform simultaneously are labeled as hybrid myofibers and are indicators of muscle fiber transition. The maximal shortening velocity of MHC fibers increases in the following order: -I < -IIa < -IIx < -IIb. On the basis of the myosin ATPase activity myofibers have been classified as types I, IIA, IIB and IIC. Type IIC fibers represent an intermediate type between MHC-I and type MHC-IIa fibers. Most large mammals do not possess fastest MHC-IIb isoform, although some recent studies in pigs and llamas have shown the existence of all three fast MHC isoforms in their skeletal muscles. Additional MHC isoforms are present transitorily during development, and in some highly functionally specialized muscles such as extraocular, laryngeal and masticatory muscles (MHC-extraocular, MHC-m). Embryonic and neonatal MHC isoforms are expressed during muscle development and regeneration. Slow MHC-I myofibers show high oxidative capacity, whereas fast MHC-II myofibers revealed entire spectrum of metabolic enzymes activity with large overlaps between contractile fiber types. Combining the contractile classification with metabolic enzymes activity, myofibers can be basically defined as slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast-twitch glycolytic (FG). In most cases enzyme and immunohistochemical techniques are not fully interchangeable, which makes combination of different techniques necessary to get a reliable classification of myofibers.

Key words: skeletal muscle; myosin heavy chain; muscle fiber type; histochemistry; mammals

Introduction

The principal muscle functional properties, such as contraction speed and fatigue resistance are mostly related to the proportions of myofiber types. Therefore, defining muscle fiber type composition became an essential step of any functional and applicative research in clinical and sports medicine as well as in animal muscle development and meat quality studies.

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In most mammals, skeletal muscle tissue represents about 55% of individual body mass and plays vital roles in locomotion, heat production and overall metabolism. In 19th century the French anatomist Louis Ranvier Antoine already observed that some muscles were darker and contracted more slowly during longer periods than paler muscles. This early observation was the basis for the distinction of red and white muscles, which was later found to be related to myoglobin content, an iron-containing oxygen transport protein in the muscle fibers (1). In the sixties and seventies of the last century, new histochemical procedures enabled to

distinguish muscle fibers on the basis of their contractile and metabolic properties. Furthermore, it was established that mammalian skeletal muscles were composed of different proportions of muscle fiber types, which define the properties of muscles as functional units. The proportions of the various myofiber types vary between muscles and between individuals for a given muscle (2). It is well known that endurance athletes have a greater proportion of slow-twitch oxidative fibers, whereas sprinters and weightlifters have more fast-twitch glycolytic fibers (3). Diverse myofiber type composition between individuals has been also reported in horses and dogs exhibiting different athletic abilities, as well as in different breeds of domestic pigs (4, 5).

On the cryosections the contractile properties of the myofibers are usually established either through immunohistochemical detection of the myosin heavy chain (MHC) isoforms or enzymehistochemical determination of myosin ATPase activity, while the energy metabolism is estimated on the basis of the histochemically demonstrated metabolic enzymes activity in the myofibers. Although all three techniques provide valuable data about the myofiber properties, the results could be sometimes erroneously interpreted, above all due to a lack of correspondence between myofiber classification systems within species and between species and because of antibodies immunoreactivity, which show certain diversity among various species (6).

Thus the main goal of this paper is to describe the cellular basis for the myofiber typing and present some particularities in the enzyme-immunohistochemical myofiber classification in different animal species.

Myosin heavy chain fiber type classification

The heterogeneity of mammalian skeletal muscle fibers is related to the diversity of myofibrillar proteins, predominantly the myosin heavy chain (MHC). Myosin is a large molecule composed of two myosin heavy chains: (200,000 kDa each) and four myosin light chains (MLC, app. 20 kDa) (7). MLC are divided into two alkali (essential) light chains and two regulatory light chains. The exact role of MLC in contraction is not fully established; however, it is assumed that they are involved in the regulation of shortening velocity of muscle fibers (8). The role of MHC is better established. It is both, a structural protein and an enzyme, which hydrolyses ATP and is therefore essential in determining excitation –contracting coupling and movement (9). It is well documented that MHC composition determines the force-velocity characteristics, making MHC composition a good tool to type myofibers functionally.

In mammalian skeletal muscles up to 9 MHC isoforms have been identified: -I, -IIa, -IIb, -IIx, -IIm, -neonatal, -embryonic, -extraocular and two cardiac. Each of them is encoded by a distinct gene and has its own myosin ATPase activity (10). They are grouped in clusters located in different chromosomes and forming distinct subfamilies. The subfamily of fast isoforms comprises genes coding for three fast isoforms (MHC-IIa, -IIx and -IIb) expressed in adult fast fibers of limb and trunk muscles and genes coding for extraocular, embryonic and neonatal isoforms. The subfamiliy od cardiac isoforms is composed of two genes, coding for slow (also β-cardiac) MHC expressed in cardiac muscle and in slow (type I) myofibers of skeletal muscles and for α-cardiac expressed in cardiomyocytes and in specialized skeletal muscles (masticatory, extraocular, laryngeal). Only the gene coding for masticatory (-IIm) MHC belongs to the third subfamily. This gene represents a autonomous subfamily because of the distinct chromosomal localization and also because sequence analysis carried out in cat, dog, and human shows a large diversity compared with all other MHC genes. (11).

The main MHC isoforms in adult locomotory skeletal muscle are -I, -IIa, -IIx and -IIb. MHC-I is a slow contracting isoform, while the three MHC-II isoforms are fast contracting; however, with different shortening speed. The polymorphism among adult MHC isoforms is functionally relevant as they determine not only myosin ATPase activity and fatigability, but the maximum shortening velocity of myofibers as well. Therefore, the existence of several MHC isoforms enables the skeletal muscles to fulfill different physiological demands. Studies on the isolated myfibers in rodents showed that the maximal shortening velocity increased in the following order: -I < -IIa < -IIx < -IIb (8, 12). Muscle fibers are capable of altering their phenotype under various conditions, such as altered neuromuscular activity, mechanical loading, hormonal profiles and aging. The changes in MHC isoforms follow a general scheme of reversible transitions from fast to slow and slow to fast in a order: MHC-I ↔ MHC-IIa ↔ MHC-IIx ↔ MHC-IIb (13, 14). The consequence of the MHC isoform tran-

sition scheme is that expression of two adjacent MHC isoforms in the same myofiber is possible. Such myofibers are designated as hybrid ones in contrast to so called pure myofibers, which contain only one MHC isoform. Recently, it has been established that different developmental and fast MHC isoforms could be co-expressed in the same muscle fiber during development, muscle regeneration and electrical stimulation, as well as in some highly specialized muscles such as extraocular, laryngeal and masticatory muscles (15).

Embryonic and neonatal MHC isoforms are typically expressed during muscle development and regeneration, yet they are also found in the intrafusal fibres (14). Masticatory (-IIm) MHC is phylogenetically ancient and confers high maximal muscle force and power. It is highly jaw-specific and is expressed in reptiles and fish. It is also found in several orders of mammals including carnivores, primates, chiropterans and diprotodonts.. In some species among listed mammals, masticatory myosin is replaced by some other isoform. It is postulated that during mammalian evolution, mastication of food became important, and in some yaw-closing muscles the masticatory myosin is replaced with α-cardiac, developmental, slow or fast limb isoforms to adapt to variety of diet (16, 17)

Extraocular MHC isoform has been shown in extraocular and some laryngeal muscles. The shortening speed of these muscles has proved to be even faster than that of masticatory ones (18, 19). Finally, α-cardiac MHC is fast contracting MHC isoform contained in cardiac muscle. It has been identified in adult human and rabbit masticatory muscles and is also temporally expressed during postnatal muscle development in pig and horse skeletal myofibers (20, 21) and in skeletal myofibers after chronic low frequency stimulation in rabbit (22). Currently, the shortening speed of adult MHC isoforms decrease in order -extraocular > -IIm > -IIb > -IIx > -IIa > α-cardiac > -I (23).

The expression of distinct MHC isoforms in myofibers, their function and the comparison of fiber type characteristics between different skeletal muscles and species has been studied using different procedures. Immunohistochemical detection with sets of monoclonal antibodies raised against different MHC is the most frequently used method to type myofibers. However, specifity of antibodies is still a problem in distinguishing between fast MHC isofoms in different species. Finally, *in situ* hybridization using nucleic acid probes for MHC isoforms and RT-PCR are used to analyze the expression of MHC at the mRNA level (24).

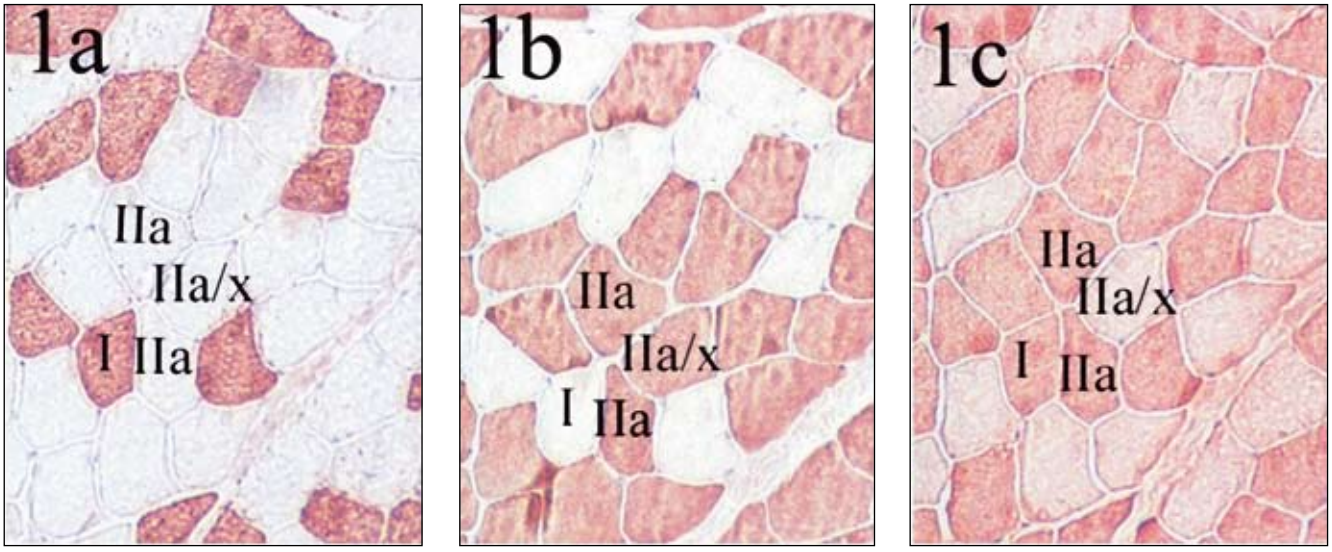

Figure 1: MHC isoforms expression in serial cross sections of canine triceps brachii muscle. MHC-I fibers were demonstrated using MHC-slow antibody (Fig. 1a). All myofibers that remained unstained were uniformly labeled with A4.74 antibody (fig 1b). The BF-35 antibody that recognizes all the MHC isoforms except -IIx, weakly stained a subpopulation of fast myofibers implying that they express the MHC-IIx isoform. Since these myofibers reacted positively with the A4.74 antibody, which is specific for MHC-IIa of rat, these fibers were first erroneously designated as hybrid co-expressing MHC-IIa and -IIx isoforms (MHC-IIa/x) (25). Further investigation showed that A4.74 antibody in canine muscles recognized both MHC-IIa and -IIx isoforms (26). Thus the BF-35 weakly stained myofibers were probably pure MHC-x myofibers, which were previously misclassified due to the nonspecific reaction of A,4.74 antibody

Myosin ATPase histochemistry

Barany (9) demonstrated that if myosin was extracted from skeletal muscle and activated in the presence of actin, the acto-myosin ATPase activity was directly proportional to the speed of shortening of the muscle from which the myosin was extracted. Since ATPase activity is ubiquitous in living organism, specific techniques to reveal myosin ATPase activity have been developed. They are all based on the precipitation of inorganic phosphate coming from the hydrolysis of ATP by myosin ATPase in the presence of Ca^{2+}. The staining procedure is performed on frozen unfixed sections, since fixation destroys enzyme activity. Subsequently, differences in the pH stability of myosin ATPase formed the basis for distinction of type I (slow) and type II (fast) myofibers (27). This method distinguished both fiber types at pH 9.4, because the fast type II fibers exhibited a much higher myosin ATPase activity at this pH than slow type I fibers. Further histochemical techniques based on propoperties of myosin ATPase activity revealed the presence of fast subtypes II fibers (28). Pre-incubation of serial cryosections in acid or alkali buffers before myosin ATPase staining could distinguish between type II fibers. Thus, three fast and one slow type could be demonstrated in small mammals (29, 30). The fast subtypes were shown to contain MHC-IIa, -IIx and -IIb, whereas the slow type contain MHC-I. These four types represent so called pure fibers containing only one MHC isoform. Alkali and acid stable type IIC fibers correspond to a hybrid myofiber population, containing both slow MHC-I and -IIa isoforms. Otherwise other hybrid fibers remain difficult to detect when using only myosin ATPase histochemisty. Their detection must be confirmed with complementary techniques such as immunohistochemistry. It should be stressed that interspecies differences exist for the pH stability/lability of myosin ATPase which makes identification of fiber types by myosin ATPase slightly different among species. (31).

Metabolic enzyme histochemistry

Another widely used method for determining the muscle fiber properties is histochemistry for selected enzymes of energy metabolism. Several metabolic enzymes have been chosen to represent metabolic pathways involve in either oxidative or glycolytic fuel utilization. Thus, different mitochondrial enzymes are markers of the potential oxidation of diverse substrates including fatty acids, carbohydrates and amino acids. Different enzymes of the glycolysis are used to determine the potential anaerobic catabolism of glycogen and glucose to lactate. In practice, succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (α-GPDH) are most frequently used to characterize oxidative and glycolytic potential capacities of myofibers, respectively (32, 33).

The combination of myosin ATPase with metabolic enzyme activities distinguishes, three basic muscle fiber types in mammalian muscles, i.e. slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast twich glycolytic (FG) (34). SO fibers are slow-contracting and are fatigue resistant. Structurally, they exhibit a small fiber diameter; possess a high mitochondrial and capillary density and a high myoglobin content. Energetically, these myofibers are rich in triglyceride droplets but have low level of glycogen and high energy creatine phosphate, which is usually used for explosive movements. Functionally, these fibers are used for aerobic activities like walking and maintaining posture. FOG fibers are fast contracting and resistant to fatigue. They have a high levels of mitochondria, capillary and myoglobine. They are rich in creatine phosphate and glycogen, moderately rich in triglycerides and exhibit an oxido-glycolytic metabolism. These myofibers are capable of prolonged anaerobic activity with a relatively high force output. FG fibers are fast contracting and very sensitive to fatigue. They have a low mitochondrial, capillary myoglobin and trigliceryde content (35) but exhibit high creatine phosphate and glycogen concentrations. FG fibers have large diameters and are used for short anaerobic activity with high force production such as galloping or jumping.

Slow type I myofibers are mostly oxidative and exhibit a rather uniform metabolic properties, whereas subtypes II fibers can be either oxido-glycolytic or glycolytic with large overlaps between subtypes. Moreover, MHC-IIb and -IIa fibers do not always correspond to FG and FOG fibers, respectively, and the discrepancy between myofiber classification becomes even more important when considering MHC-IIx myofibers. Therefore, the mixing of different classification systems can be misleading (36).

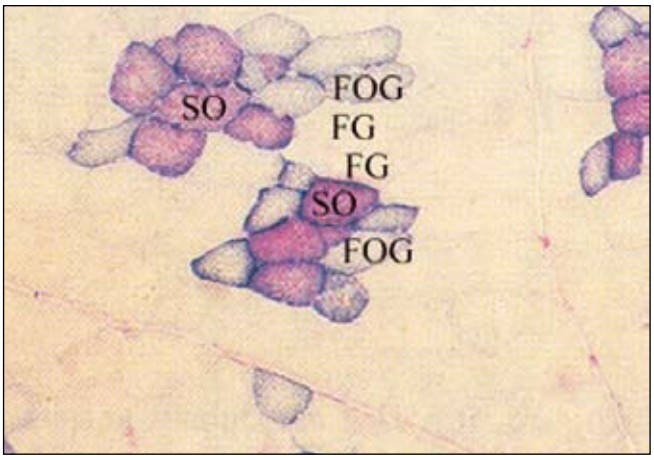

Figure 2: Pig longissimus dorsi muscle. Determination of slow-twitch oxidative (SO), fast-twitch oxido-glycolytic (FOG) and fast-twitch glycolytic (FG) fibers on a single cryosection using combine SDH histochemistry and immunolabeling with an anti-MHC-I (slow) isoform (41). A highly organized pattern and unique distribution of fibers composed of clusters of SO fibers, which are surrounded by FOG fibers and more external FG fibers can be observed

Because of its simplicity, muscle fiber classification into SO, FOG and FG is still widely used above all in studies in which basic information about contractile and metabolic properties of the muscles are required. To make the conventional enzyme-histochemical fiber typing more friendly to use, combined histochemical methods based on the successive staining of myosine ATPase and different metabolic enzymes such as SDH or NADH-TR on a single cryostat section have been developed (37, 38, 39). However, these techniques lead sometimes to unreliable fiber staining because of some incompatibilities in enzyme optimal conditions (40). More reliable results are usually obtained when combining successively metabolic enzyme staining and immunoistochemnical labeling of fibers (Fig. 2).

Patterns of muscle fiber type distribution in mammals

The distribution and proportions of fiber types vary between species and muscles. In most mammalian species, skeletal muscles exhibit a random spatial distribution of different fiber types. Fibers belonging to the same motor unit i.e. innervated by the same motorneurone, exhibit similar contractile and metabolic characteristics and are interspersed between fibers of other motor units. In small mammals, like rodents and lagomorphs, three fast MHC isoforms, -IIa, -IIx and -IIb are expressed in fast fibers (42, 43). On the contrary, MHC-IIa and -IIx isoforms are present in skeletal muscles of humans (44, 45), cats (46), dogs (25), cattle (47), goats (48), horses (49, 50) and brown bear (51).

The muscle fiber type composition depends on the specific function of a muscle and, furthermore, extends to species specific differences. From the comparison of fast MHC isoforms concentration between species it seems clear that the relative amount of MHC-IIb isoform decreases as body size increases, whereas that of MHC-IIa and -IIx increases. A possible explanation for such differences in muscle fiber composition between species could be that, that small animals with faster movements need faster twitch myofibers than larger animals which movements are slower. This hypothesis can explain why most large mammal species do not possess the fastest MHC-IIb isoform in their skeletal muscles. Such hypothesis was additionally confirmed in rabbit, where MHC-IIb isoform is more intensively expressed in young than adult animals. Decrease in the relative concentration of MHC-IIb isoform with increasing age possibly relates to the growth of the animal and changes in its locomotion pattern (24). MHC isoforms transformation associated with the process of growth was described also in large mammals, although they usually do not contain MHC-IIb isoform. In these species, the proportions of MHC-I and MHC-IIa myofibers increase, while that of MHC-IIx myofibers decreases during growth. In the early postnatal period the increased expression of MHC-I and -IIa isoforms is the consequence of a transition from developmental to adult MHC profile (52, 53). However, during later periods of growth some MHC-IIx myofibers obviously transform into MHC-IIa myofibers via hybrid MHC-IIa/x myofibers and into MHC I via hybrid MHC-I/IIa myofibers. Such transformations were observed in adolescent bears (51) and up to six years of age in different horse breeds (54, 55). With increasing age the percentage of hybrid fibers decreases, which supports their transitional role in muscle maturation. Taken together, the slower and more fatigue resistant characteristics of skeletal muscles with increasing age likely relate to a progressive adaptation to increasing body weight.

The lack of MHC-IIb isoform expression in most adult large mammals has been hypothesized to be related to body size and muscle fiber length. In large mammals the shortening of the fastest MHC-IIb isoform would produce such a high force that muscle could be injured (45). However, recent stud-

ies have shown the existence of all three fast MHC isoforms , including MHC-IIb, in adult pig longissimus (56) and llamas semitendinosus (57), which did not support hypothesis suggesting no expression of MHC-IIb in large mammals. In fact, gene coding for MHC-IIb isoform has even been discovered in humans; however, its expression in skeletal muscles remains to be confirmed (58). The reason why this isoform would be expressed only in pigs and llamas among large mammals is not known. Both species exhibit a so called type grouping distribution of the muscle fiber types with central clusters of MHC-I myofibers surrounded by MHC-IIa, then MHC-IIx and finally more external MHC-IIb myofibers (5, 56). In other species such fiber type grouping can be observed in relation to some neuromuscular disorders, whereas it is a normal spatial distribution in porcine skeletal muscle (59). Highly organized focal arrangement is supposed to be functionally relevant. Thus, central clusters of MHC-I myofibers would be most easily mobilized first for weak long-lasting contraction, whereas MHC-IIb fibers would be mobilized last for short-lasting forceful contraction. However, such a muscle fiber type distribution is not prerequisite, since most mammals exhibit random mosaic fiber distribution and are nevertheless fully functional. As well, it could be speculated that MHC-IIb isoform is expressed in some large glycolytic pig skeletal muscles as a result of intense selection for high muscularity and growth efficiency; however, further research is needed to test this hypothesis (60).

Correspondence between myofiber classification systems

It is well documented that maximal shortening velocity of muscle fibers expressing homologous MHC isoforms greatly decreases with increasing body size (61). Such functional diversity of the homologous fast MHC isoforms between species is likely related to different structural characteristics. This is probably one reason why MHC antibodies, which are usually raised against rat isoforms, can have diverse reactivity with homologous MHC in large mammals and why myosin ATPase histochemistry protocols must be adjusted to each animal species. Because of some important differences in the reactivity of MHC antibodies between species (Table 1), a set of different antibodies is usually used to avoid myofiber misclassification.

Table 1: Reactivity of commonly used antibodies raised against MHC isoforms in different species according to Smerdu at al. (6, 26, 51), Lefaucheur et al. (60) and Rivero et al. (49)

MHC isoforms \ Antibody		MHC-slow	A4.74	SC-71	F113, 15f4	BF-35	BF-F3
MHC-I	Rat	+	-	-	-	+	-
	Human	+	-	-	-	+	-
	Dog	+	-	-	-	+	-
	Bear	+	-	-	-	+	-
	Horse	+	-	-	-	+	-
	Pig	+	-	-	-	+	-
MHC-IIa	Rat	-	+	+	+	+	-
	Human	-	+	+	+	+	-
	Dog	-	+	+	+	+	-
	Bear	-	-	-	+	+	-
	Horse	-	+	+	+	+	-
	Pig	-	+	+	+	+	-
MHC-IIx	Rat	-	-	-	+	-	-
	Human	-	±	±	+	+ or -	-
	Dog	-	+	+	+	-	-
	Bear	-	+	+	+	-	-
	Horse	-	-	-	+	-	-
	Pig	-	-	±	+	-	-
MHC-IIb	Rat	-	-	-	-	+	+
	Human	-	-	-	+	+	-
	Dog	-	-	-	+	+	-
	Bear	-	-	-	+	+	-
	Horse	-	-	-	+	+	-
	Pig	-	-	-	+	+	+

(- = negative reaction, +/- = weak reaction, + = positive reaction)

The specificity of MHC-slow antibodies is unambiguous because they revealed type I myofibers in skeletal muscles of all species (25, 48, 49, 60), suggesting that the slow MHC-I isoform is highly conserved among species. On the opposite, fast MHC isoforms can show different antigenic properties between species. Thus, both A4.74 and SC-71 antibodies are specific to MHC-IIa isoform of rat, but cross-react with MHC-IIx in human, dog, pig, goat (26, 48, 60, 62, 63). In bear skeletal muscles both antibodies actually recognize MHC-IIx and not MHC-IIa isoform (51). This was confirmed with antibody BF-35, which reveals all MHC exept IIx in rat (42). Similar problems were

observed with the antibody F113.15F4, which recognizes MHC-IIa, -IIx and -IIb in rat, and only MHC-IIa and -IIx in dog and bear (25, 51). Some misclassification of myofibers between species also occurs using myosin ATPase histochemistry. The staining pattern of fibers depends upon the lability of myosin ATPase to pH preincubation and is related to the MHC isoforms content within a single myofiber. When two isoforms are expressed in the same myofiber, the staining pattern of the myosin ATPase is ambiguous and can lead to misclassification of the muscle fiber type, especially of fast type II fibers. In the past myosin ATPase based classification led to some contradictory reports on fast fiber sub-types in large mammals. In some studies of canine muscles only type IIA and IIC myofibers were found (64, 65, 66, 67). On the contrary, other authors claimed that type IIB myofibers are present even in dogs, although they were slightly less acid-labile than type IIB in other species (68). The immunohistochemical labeling of MHC isoforms demonstrated that strongly acid-stable subclass of canine fast fibers, which were dark after preincubation at pH 4.6 and would thus correspond to type IIB myofibers of other species, actually expressed MHC-IIa isoform, and the more acid labile sub-class, which were named as IIDog fibers (69, 70) actually corresponded to MHC-x fibers (26). Such integrated use of both myosin ATPase and immunohistochemical labeling of MHC isoforms demonstrated that type IIB fibers have been misclassified in numerous previous studies based upon traditional myosin ATPase histochemistry in other large mammals as well (71). Myosin ATPase can also lead to fiber misclassification because of partial denaturation of the enzyme. Thus, the rapid postmortem acidification combined with increased muscle temperature encountered in some glycolytic muscles of stress susceptible pigs can lead to irregular and altered myosin ATPase staining these PSE (pale, soft and exudative) muscles (Figure 3). In such case, the use of antibodies against MHC isoforms is a far more reliable technique to type myofibers (72).

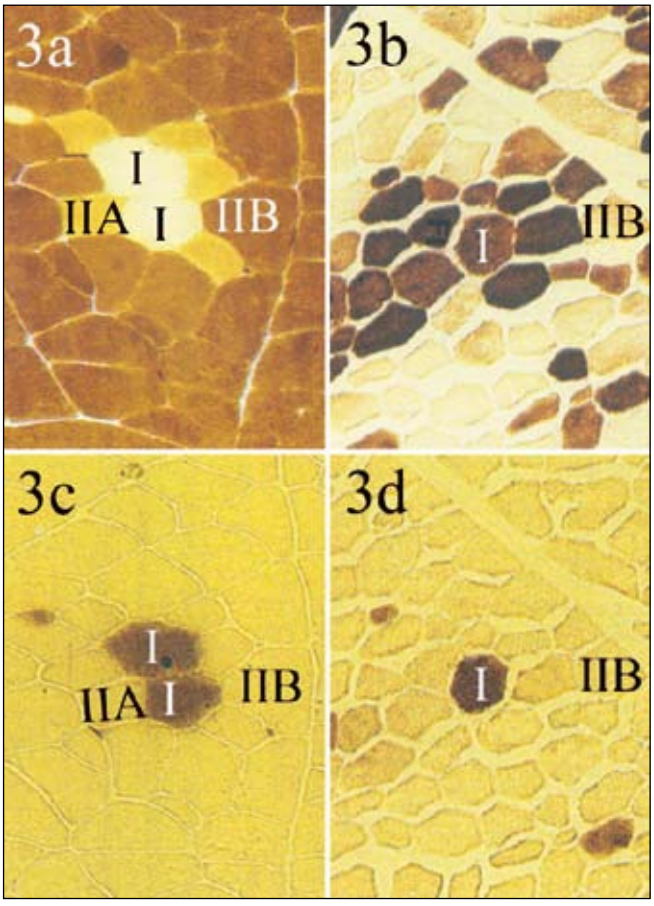

Figure 3: Alkali stable myosin ATPase activity (a, b) (73) and myosin ATPase activity pH 4.3 preincubation (c, d) of a normal (a, c) and PSE (b, d) pig longissimus dorsi muscle. Three different fiber types are distinctly recognized in normal muscle (I, IIA, IIB); whereas, the staining pattern of the alkali stable myosin ATPase is altered in PSE muscle. The PSE condition mostly inactivated the alkali stable myosin ATPase activity in peripheral fast-twich glycolytic IIB fibers

Conclusion

Big differences in muscle fiber type composition exist between muscles and species, and between individuals within species. It is well documented that skeletal muscles is a highly adaptable tissue which can be influenced by many intrinsic and extrinsic factors, such as age, altered neuromuscular activity and mechanical loading. The principal methods to type myofibers on the tissue cryosection are the immunohistochemical detection of MHC isoforms and the myosin ATPase and metabolic enzyme histochemistry. Overall, it must be stressed that immunohistochemical and enzyme histochemical classifications are not always fully interchangeable between and even within species, suggesting that different techniques often have to be combined to get a reliable myofiber typing.

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ENCIMSKO-IMUNOHISTOKEMIČNI VIDIKI RAZVRŠČANJA TIPOV MIŠIČNIH VLAKEN PRI SESALCIH

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Povzetek: Skeletne mišice so pri sesalcih tkivo, ki ga je v telesu največ in je tudi najbolj prilagodljivo. Sestojijo iz mišičnih vlaken, ki se razlikujejo po vsebnosti strukturnih proteinov kot tudi po aktivnosti presnovnih encimov. Zato številčni deleži tipov vlaken v mišici določajo njene morfološke in funkcionalne značilnosti. V članku so predstavljene osnovne encimsko-imunohistokemične tehnike, na osnovi katerih prepoznavamo značilnosti posameznih tipov mišičnih vlaken kot tudi njihovo raznovrstnost v skeletnih mišicah različnih živalskih vrst. Na tkivnih rezinah razvrščamo mišična vlakna na osnovi imunohistokemičnega določanja vsebnosti izoform težkih miozinskih verig (MHC), aktivnosti miozinske ATP-aze in aktivnosti presnovnih encimov. V somatskih skeletnih mišicah manjših sesalcev so dokazane štiri različne izoforme težkih miozinskih verig (MHC-I, -IIa, -IIx in –IIb). Glede na vsebnost izoforme raste hitrost krčenja mišičnih vlaken v naslednjem zaporedju MHC: -I < -IIa < -IIx < -IIb. V t. i. hibridnih vlaknih sta izraženi dve izoformi MHC in sta pokazatelj preobrazbe mišičnih vlaken. Na osnovi aktivnosti miozinske ATP-aze vlakna razvrščamo v tipe I, IIA, IIB in IIC. Vlakna tipa IIC predstavljajo prehodni tip med vlakni MHC-I in MHC-IIa. Mišice pri večini velikih sesalcev ne vsebujejo najhitrejše MHC-IIb izoforme, vendar pa so zadnje študije pokazale prisotnost vseh treh hitrih MHC izoform tudi pri domačem prašiču in lami. Zrkelne, grlne in žvekalne mišice, ki se tako funkcijsko kot razvojno razlikujejo od somatskih, vsebujejo tudi t. i. ekstraokularno (MHC-ekstraokularna) oz. mastikatorno (MHC-m) izoformo, med razvojem in regeneracijo pa so v mišicah prisotne še razvojne izoforme (MHC-embrionalna, MHC-neonatalna). Počasna MHC-I vlakna kažejo veliko oksidativno presnovno zmožnost, v hitrih MHC-II vlaknih pa je aktivnost presnovnih encimov zelo različna. Na osnovi krčljivostnih lastnosti mišičnih vlaken in ob upoštevanju njihove presnovne aktivnosti jih lahko razvrstimo v tri osnovne tipe: počasi krčljiva oksidativna (SO), hitro krčljiva oksidativno-glikolitična (FOG) in hitro krčljiva glikolitična (FG). Razvrščanje vlaken na osnovi samo ene od opisanih metod je večkrat problematično, zato je za natančno in zanesljivo določitev lastnosti mišičnih vlaken potrebno sočasno uporabiti različne encimsko-imunohistokemične tehnike.

Ključne besede: skeletna mišica; težka miozinska veriga; tip mišičnega vlakna; histokemija