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Original Research Paper

CHANGES IN HISTOCHEMICAL PROPERTIES OF MUSCLE FIBRES IN DEVELOPING CANINE SKELETAL MUSCLES

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Summary: In the study changes in muscle fibres of canine skeletal muscles were observed during development from perinatal period to 6 months of age. Emphasis was put on the histochemical fibre type classification and general morphological properties. In neonates muscle fascicles contained one centrally located primary fibre which in some cases still retained a central space as seen in developing myotubes. These fibres started to stain differently from surrounding secondary fibres on foetal day 55. The classification of muscle fibres according to the myosin ATPase (mATPase) method was possible after third week post partum; prior to this the majority of fibres seemed to be undifferentiated. Between the third and the sixth week 7 different fibre phenotypes were found and in two-month-old dog the usual adult composition of muscles with 4 fibre types was first noted. The glycolytic and oxidative capacities were weak in neonates but increased gradually with age. The metabolic differentiation between fibres was first noted at the third week. The diameter of fibres was increasing constantly. The number of muscle fibres assessed by ratio between primary and secondary fibres increased in perinatal period.
When compared to data in the literature, we ascertained that dog skeletal muscles are relatively immature at birth. There were parts of muscles which developed even more slowly and still had a myotubal morphology in neonates. Some muscle-dependent differences were noted: the diaphragm developed faster and an early distinction between slow (m. rhomboideus) and fast muscles (m. extensor carpi radialis and m. tibialis cranialis) was observed. Mature morphology with a random distribution of fibre types inside muscle fascicles and a defined metabolic profile was observed in all muscles in two-month-old dogs. The standard mATPase method became applicable to determine fibre types by this time.

Key words: anatomy, veterinary; muscle, skeletal; muscle fibres - growth and development; myosin ATPase; dogs

Introduction

Canine skeletal muscles have been studied mainly with histochemical methods. On the basis of muscle fibre type numbers and distribution one can presuppose the muscle predominant function and the state of activity. Fibre types according to the mATPase reaction found in dogs were slow type I, hybrid IIC and fast IIA. Instead of the conventional type IIB rather an unique fast subtype of fibres was described (1), labelled as type IIDog by Lattore et al. (2). These fibres strongly express the myosin heavy chain (MHC) isoform IIx, a protein expressed only in some muscles (3, 4). Type IIDog fibres could be therefore also named IIX fibres. Another dog peculiarity is a high degree of oxidative activity in the muscle fibres suggesting that dog muscles are adapted to endurance activity (1, 4, 5).

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During development fibre types are more ambiguous. It is well known that different MHC isoforms are present in the fibres during development, hence the fibre types do not comply with the traditional (adult) classification (6). New fibre types were proposed, such as IB and IC. However, in developing canine muscles a high proportion of IIC fibres was reported by several authors (7, 8). These fibres retain a high mATPase activity in alkaline and acid preincubations. IIC fibres in adults are hybrid fibres since they contain fast and slow MHC isoforms. It is obvious that developing muscle fibres are hybrid as well, but co-expressing developmental MHC isoforms, i.e. embryonic and neonatal (MHC-emb, MHC-neo). These developmental isoforms are replaced by adult MHC isoforms, before and/or after birth, depending on the length of gravidity and subsequently adult fibre types are established. More appropriate designation for darkly stained fibres according to the mATPase method in developing muscles is therefore "undifferentiated fibres" (7, 9, 10).

The degree of muscle fibre differentiation or muscle maturity at the time of birth is correlated with the length of gravidity and general maturity of neonates, which reflects animal's physiological needs right after birth (9, 11). While most of the studies were performed on laboratory animals (including cats), some information is available for domestic animals as well. In cattle the total number of muscle fibres is fixed at foetal day 230. Primary generation consists of slow fibres as in other animals and humans, except of purely fast muscle m. cutaneus trunci, in which primary myotubes expressed fast isoform from the beginning. In mid-gestation various types of secondary fibres were observed, differentiating to slow or fast fibres. Only the third generation of fibres was still undifferentiated just before birth. The precocity of differentiation was muscle-type dependent (12, 13). Also in m. tibialis cranialis of neonate sheep all primary fibres were slow. Secondary fibres started to express adult fast isoforms in mid-gestation - some of them only transiently since they transformed into slow fibres by day 20 post partum (14). In newborn horses a lot of fibres seem to be type IIX and transformed into IIA in the next 48 weeks. By week 10 after birth all fibres differentiated (15, 16). Pig muscles are unique in the way that the central location of slow fibre remains visible throughout the adult life and is accompanied by a rosette of secondary slow fibres that are established in the third postnatal week. A third generation of small diameter fibres was noted only after birth. Dramatic changes were described in the first postnatal week in piglets: the disappearance of undifferentiated fibres (decrease in developmental MHC), formation of proper type I and II fibres (increase in MHC- I and MHC-IIa) and remodelling of energy metabolism (17, 18).

Although immunohistochemistry and immunoblotting provide additional and less ambiguous information on the fibre composition, the enzyme-histochemistry, namely the mATPase fibre type classification, oxidative and glycolitic capacity still present quick, cost-efficient and species-universal methods in quick diagnostics of muscle pathology, regeneration or training efficiency. Postnatal changes in canine skeletal muscles were histochemically assessed in the past, but one study concentrated on a single muscle (10) while in the other the glycolitic capacity assessment of the fibres was lacking and an interval between postnatal weeks 5 and 12 was not studied (7). The aims of our study were therefore to compare several different skeletal muscles in perinatal and postnatal period, determine the time of muscle maturation with regard to morphological characteristics of muscle fibres, and compare the fibre type classification in young dogs to other animals. The muscle characteristics of prenatal and pubertal dogs were also assessed for the first time.

Material and methods

Muscle samples were obtained from 6 fetal dogs after histerectomy or cesarean section on foetal days 50, 55 and 60 (F50, F55, F60; gestational period is on average 63 days) and from 16 puppies with an age range between 1 day and 6 months (1, 3, 5, 11, 15, 22, 28, 42, 60 and 180 days) which died of natural causes or were euthanised due to severe trauma. The puppies had no apparent neuromuscular deficiencies. Five adult dogs were included in the study for comparative purposes. All dogs were of medium size (pure-breeds or mongrels with known parents). The samples were frozen in liquid nitrogen and stored at -80°C. The middle portions of the following muscles were extracted: m. rhomboideus (p. capitis), m. longissimus dorsi (at the level of the last rib), the diaphragm, m. triceps (c. longum), m. extensor carpi radialis, m. sartorius (p. cranialis), m. semitendinosus, m. rectus femoris, m. tibialis cranialis and m. masseter. Transverse serial cryosections (10 μm) were cut on Leica CM 1800 cryostat at -17°C, mounted on APES-covered slides and air-dried.

To determine fibre types in dog skeletal muscles the sections were processed for the mATPase reaction following some of the procedures described by Latorre et al. (2). The sections were incubated either in 0.1M Na-acetate at pH 4.3 and 4.35 or in 0.2M Na-acetate at pH 4.4, 4.5 and 4.6 for 5 minutes at room temperature. For the alkaline preincubation the solutions of 0.1M $CaCl_2$, 0.07M Na-acetate and 0.075M Na-barbital adjusted to pH 9.8 and 10.2 were used (15 min, RT). Sections were then incubated in medium containing 0.1M $CaCl_2$, 0.07M Na-acetate and 0.075M Na-barbital, pH 9.65 and ATP 1.5 mg/ml for 60 min folloving the acid preincubation or 30 minutes folloving alkaline preincubation, both at 37°C. After washing in 0.2M $CaCl_2$ visualization was performed by incubation in a 2% (w/v) cobalt chloride solution (5 min), followed by fresh 1% (w/v) ammonium sulphide solution for 30 seconds.

The diameter of muscle fibres was measured by Lucia M imaging software (Optoteam Wienna). Minimum diameter was selected as a measure of fibre diameter to avoid errors due to possible section obliquity.

To estimate fibres' basic metabolic profile the presence of active oxidative enzyme succinate dehydrogenase (SDH) and glycolytic mitochondrial menadion-linked α-glycerophosphate dehydrogenase (α-GPDH) was demonstrated as previously described by Nachlas et al. (1957) and Dubowitz and Brooke (1973), respectively.

The age-dependant differences in enzyme activities was followed by biochemistry. Frozen muscle samples were cut on microtome and homogenised (Ultra-turrax, IKA-Werke) in 20 volumes of 100 mM KPO_4, 5 mM EDTA and 5 mM EGTA (pH 7.4) while kept on ice. The homogenate was sonicated on ice to further disrupt mitochondrial membranes and frozen. The procedure was repeated and after second thawing the samples were further diluted with ice-cold 100 mM KPO_4, 5 mM EDTA and 5 mM EGTA (pH 7.4) to achieve final dilution 1:20 (wet tissue mass : buffer volume). Citrate synthase (CS) activity was determined by the standard method of Srere (19) and lactate dehydrogenase (LDH) by Bergmeyer and Bernt (20) spectrophotometrically at 25°C using UV/VIS Spectrofotometer Perkin Elmer Lambda 12.

The appearance of CoA-SH was measured at wavelength 412 nm to asses CS activity in the following reaction: acetyl-CoA + oxaloacetate + H_2O ↔ citrate + CoA-SH + H^+ (side reaction CoA-SH + DTNB → mercapptide ion). Aliquots of the diluted homogenate were used for the assay in duplicates. They were kept on ice just prior to the analysis and then submerged in water bath at 25°C to increase reaction kinetics. 975 µl of 100 mM Tris (pH 8,1) used as a buffer was dispersed into 1.5 ml quartz cuvette and the following reagents added: 75 µl 3 mM acetyl-CoA, 150 µl 1 mM DTNB, 150 µl oxaloacetate and 150 µl of sample homogenate.

The LDH activity was determined by the rate of oxidation of NADH (pyruvate + NADH + H^+ ↔ lactate + NAD^+) as decrease in extinction at 340 nm. To 1.5 ml of buffer-pyruvate solution (50 mM phosphate, pH 7.5 and 0.63 mM pyruvate), 25 µl of reduced NADH (ca. 11.3 mM β-NADH obtained by dissolving 14 mg NADH-Na_2 and 15 mg $NaHCO_3$ in 1.5 ml distilled water) and 50 µl of sample homogenate ere added. Readings were taken at 20-s intervals and plotted against time. Enzyme activities were calculated from the rate of change of assay absorbance at the maximal linear slope and expressed as micromoles per minute per gram (wet mass) of tissue.

Results

In foetuses and newborns all muscles were composed of fascicles which had one centrally located primary myotube/myofibre, surrounded by secondary fibres with smaller diameter. In the diaphragm myotubes were transforming into myofibres already on F50, but m. rectus femoris and m. triceps brachii were still composed solely of myotubes on F50. Between fascicles and individual fibres of foetuses (F50, F55) there were wide intercellular spaces with loose connective tissue that diminished just before birth (F60). Fibres were more or less rounded compared to typical polygonal morphology of mature fibres. In other muscles myotubes (seen as rings or crescents) remained visible in some parts of the muscle in neonates while other parts of the same muscle were maturing faster. The tubal morphology of primary fibres was therefore noted up to postnatal day 5 and that of the secondary fibres up to postnatal day 1 in majority of the muscles. Typical changes in myofibre morphology are shown in Fig. 1. In some parts of m. rhomboideus, m. rectus femoris, m. triceps brachii and m. semitendinosus tubal morphology was noted up to postnatal day 11 (Fig. 2).

No distinction between primary and secondary fibres could be made on the basis of the mATPase staining method on fetal days F50 or F55 (se panels A and B in Fig. 1). On F60 and in newborns two different fibre types were observed according to mATPase staining method: primary fibres had a low mATPase activity and secondary fibres retained a high mATPase activity after both, alkaline and acid preincubation (for example of acid preincubation see Fig. 1C). On the third day post partum a few intermediately stained fibres occurred in some muscles. The rhomboideus muscle had the highest number of intermediately-stained fibres on postnatal days 11 and 22 after acid preincubation at 4.4 (Fig. 2 and Table 1). By the third week a mosaic appearance in mATPase staining with acid preincubation was noted in most muscles (Fig. 1E).

A spectrum of staining intensities was observed between weeks 3 and 6 and seven different fibre phenotypes could be established in the majority of the muscles. They did not completely comply with the standard classification of fibre types in adults, but the destination of differentiation could be proposed (Table 2). Fibres with the smallest diameter retained a high mATPase activity after all preincubation media used and they were referred to as undifferentiated fibres. M. rectus femoris and m. triceps brachii had more undifferentiated fibres than the other muscles. Proper type I fibres were

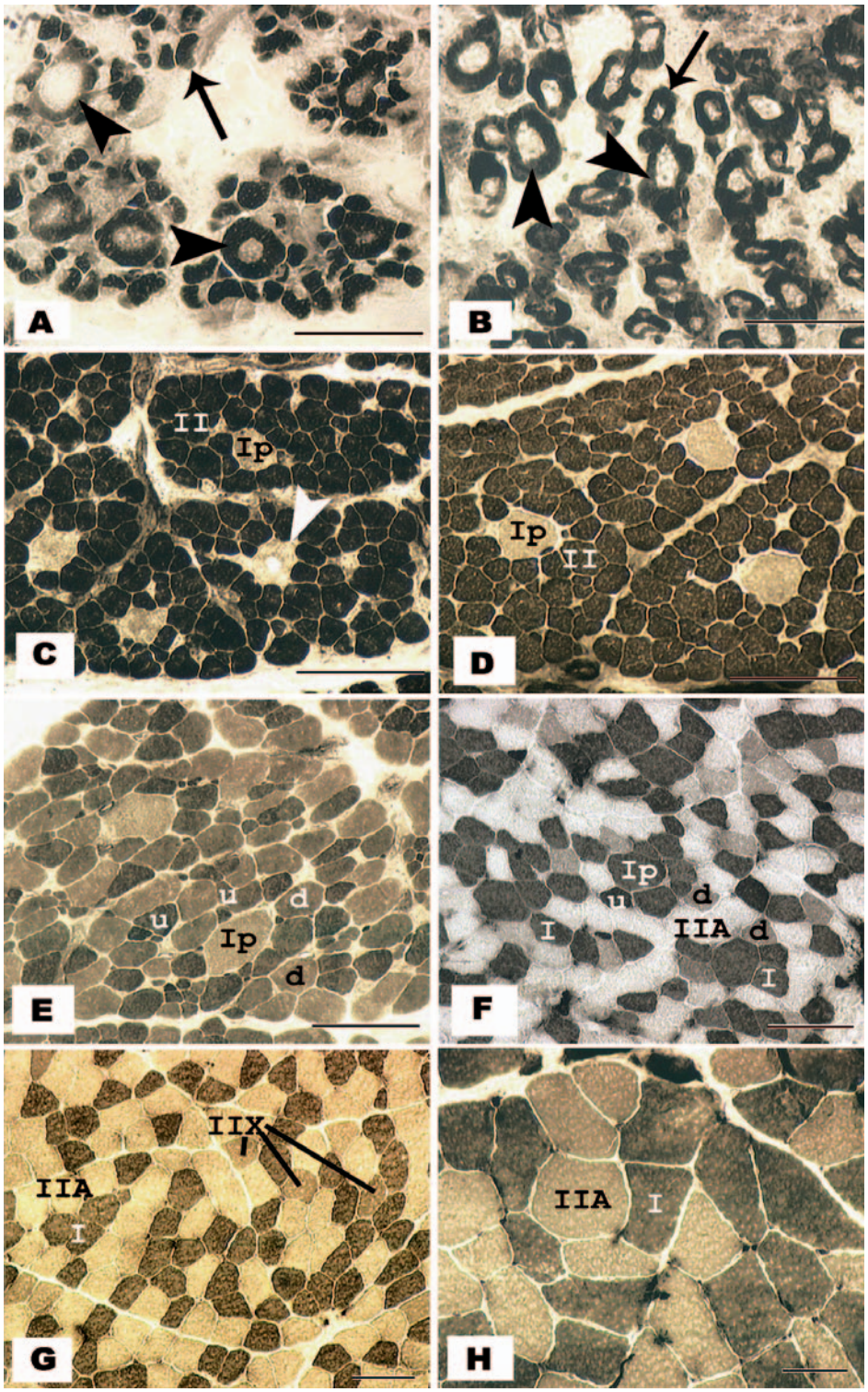

Figure 1: Canine skeletal muscle fascicles in m. sartorius (A) and in m. triceps brachii (B) on foetal day 50 (F50); m. sartorius on F60 (C), on postnatal day 11 (D), the third week (E), the sixth week (F), the second month (G) and in adult dog (H) according to the mATPase reaction at pH 4.4. Few days before birth big primary myotubes (arrowheads) and smaller secondary myotubes (arrows) can be seen clearly. After birth, there is a centrally located primary myofibre (Ip), surrounded by smaller secondary ones (II). The number of secondary fibres increased after birth. After the third week undifferentiated (u) and differentiating (d) fibres can be observed and adult types start to appear (I, IIA). IIX fibres were first noted at two-month-old dog. By postnatal week 6 the inversion of the staining properties of primary slow fibres (Ip) occurred (compare panels C, D and E with F). Scale bars = 50 μm.

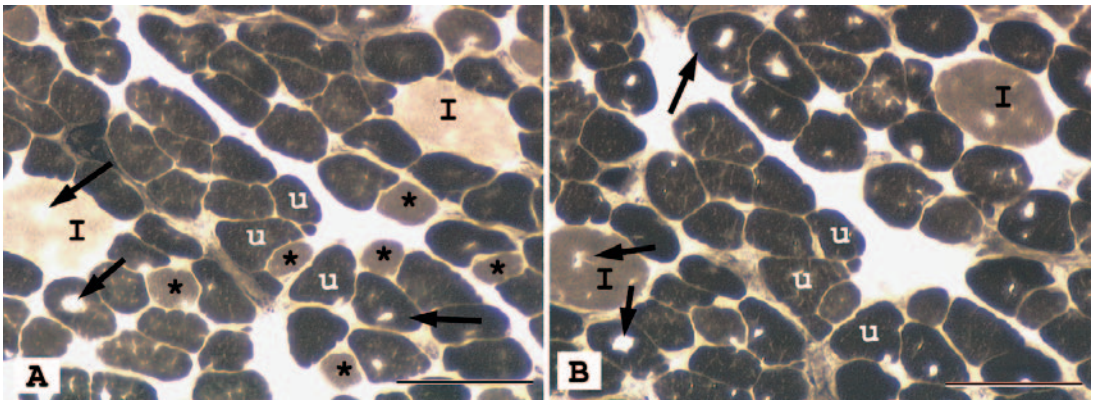

Figure 2: The mATPase demonstration of fibre types in serial sections of m. rhomboideus on postnatal day 11; acid preincubation at pH 4.4 (A) and 4.6 (B). After acid preincubation at pH 4.4 (and 4.3, not shown) about 11% of the fibres stained with intermediate intensity (*). They possibly represent the future slow fibres but stain dark at 4.5 and higher, the same as the rest of secondary fibres, classified as undifferentiated (u). At this age big type I fibres (I) are weakly stained after 4.2 - 4.4 pH values of preincubation media (also in alkaline, not shown) and intermediately in pH 4.5 (and 4.6). Some fibres still have a tubal morphology (arrows). Scale bar = 50 μm.

Table 1: Three different dog fibre phenotypes established by the mATPase method after foetal day 55 and up to the third postnatal week. Gray to black circles represent the staining intensity of muscle fibres. Big primary fibres had different staining properties than any adult fibre type. Intermediately stained fibres (differentiating) occurred in some parts of m. extensor carpi radialis, m. tibialis cranialis and m. rhomboideus at postnatal day 3, but in most of the other muscles at postnatal day 11, except in m. triceps brachii, m. semitendinosus and m. rectus femoris, where only big fibres and undifferentiated fibres were seen.

developing muscles between postnatal days 1 and week 3			
	preincubation		
fibre type	alkaline **10.2**	acid **4.5**	acid **4.3**
big primary fibres	○	◉	○
differentiating	◉	◉	●
undifferentiated	●	●	●

Table 2: General staining scheme of seven different fibre phenotypes established by the mATPase method on weeks 3, 4 and 6 in investigated canine muscles excluding the masseter. The undifferentiated fibres had the same staining properties as adult IIC fibres but were the smallest in the diameter. With the three fibre types which did not fall into normal adult category a proposed differentiating direction is given. Primary fibres failed to comply with proper type I until the second month of age as well as proper IIDog were not found during this period.

developing muscles between postnatal weeks 3 and 6			
	preincubation		
fibre type	alkaline **10.2**	acid **4.5**	acid **4.3**
primary slow (big type I)	○	●	●
secondary slow (small type I)	○	●	●
undifferentiated (IIC)	●	●	●
differentiating IIC → I	◉	●	●
differentiating IIC → IIA	●	●	◉
differentiating IIC → IIDog (IIX)	●	◉	●
IIA	●	◉	○

observed between weeks 3 and 6 (Fig. 1 E and F). They retained a high mATPase activity after the acid preincubation (dark stain) and had lost it after alkaline preincubation (no stain), however, the big primary fibres stained slightly after alkaline preincubation until week 6. In m. rhomboideus there was already 43% of type I fibres in six-week-old dog, while other muscles had between 12 and 19% of type I fibres. In adults m. rhomboideus was the slowest muscle, with the ratio between fast and slow fibres about 1:1.

At two months of age the muscle fibre type composition resembled those found in adult animals (Fig. 1 G). Fibre types I, IIA, IIC and IIX (IIDog), irregularly distributed inside muscle fascicles, were found (except in m. extensor carpi radialis, see below), and their proportions were similar to previously established patterns in adult animals (3). Type IIM fibres in m. masseter, as seen in adults, strongly resemble the undifferentiated fibres: they stained dark after acid and alkaline preincubations.

The number of fibres per muscle fascicle was increasing. The ratio between primary and secondary fibres increased in average from 1:9 on F55 to 1:25 on F60 and to 1:44 at day 5 post partum in most of the muscles. In the masseter these numbers were lower, in average 1:5 fibres on F50, 1:15 on F60 and 1:19 on day 5. M. rectus femoris and m. triceps brachii had slightly higher number of fibres in one muscle fascicle after postnatal day 5; in average ratio between primary and secondary fibres was 1:47.

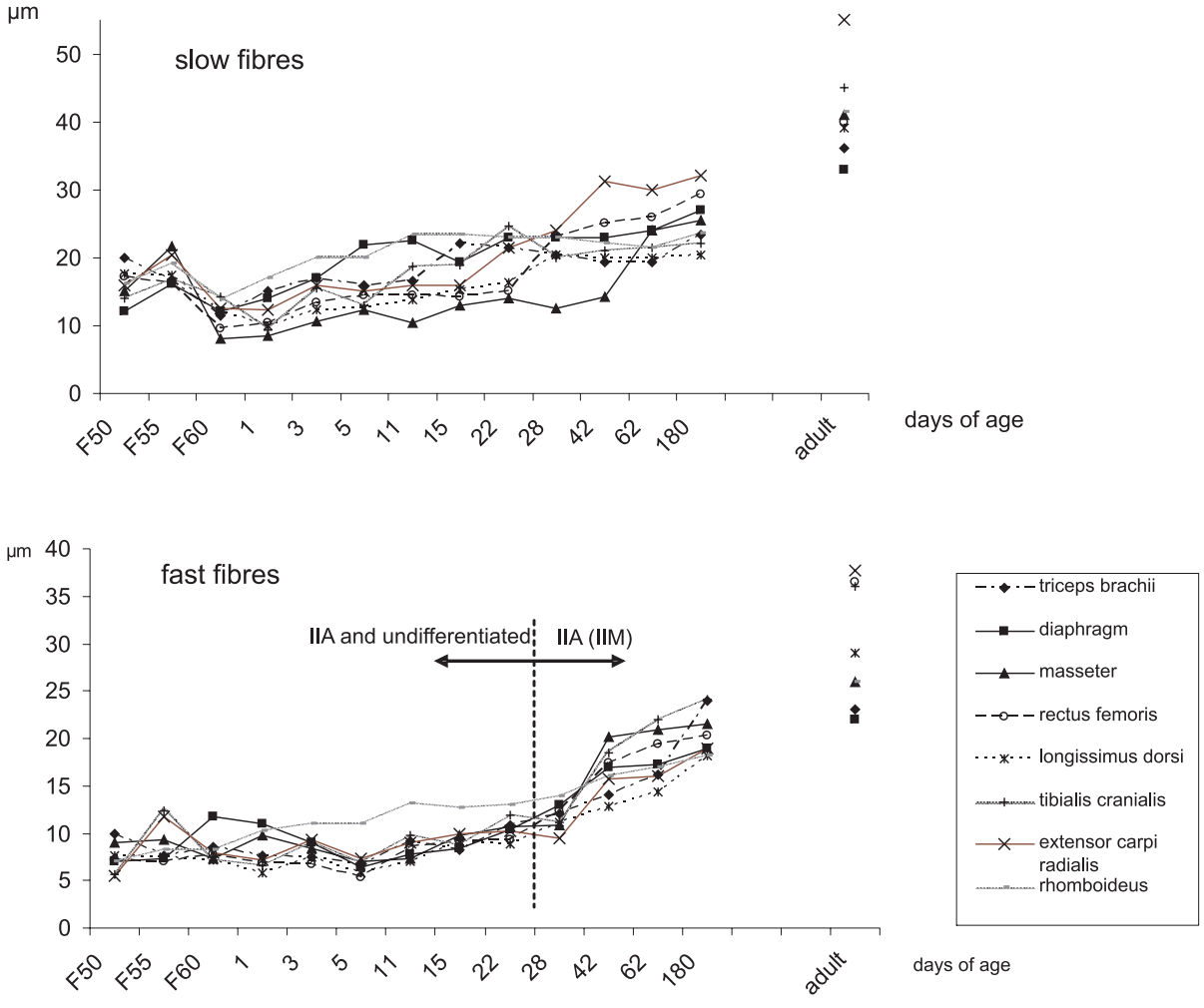

Figure 3: Average diameter of slow and fast muscle fibres. Up to postnatal day 28 all of the secondary fast fibres were measured for the fast fibres. In older dogs, only the dia-meter of type IIA fibres (in m. masseter the IIM fibres) is shown as these fibres are the most numerous ascendant of secondary fibres. IIX (IIDog) fibres, where present, had in average 30% bigger diameter than IIA fibres.

Fibre's diameter gradually increased in the postnatal period, as shown in Fig. 3. Primary fibres were on average twice as big as secondary fibres in the same muscle until six weeks of age. While the central space of primary myotubes closed in neonates, the average diameter transiently decreased. A slight decrease of the average diameter was also noted in secondary fibres between F55 (F60 in the diaphragm) and postnatal day 5. For comparison, type I fibres in adults were 40 - 100% bigger than type I fibres in a pubertal dog (6 months of age) with the exception of diaphragm in which an increase of only 22% was noted. As for type IIA fibres an increase of average fibre diameter between 20 and 80 % (as much as 100 % in m. extensor carpi radialis) was determined in adults compared to six month-old dog. The type IIM fibres in m. masseter had only 15% bigger diameter in adults compared to pubertal dog.

Extensor carpi radialis was the "fastest" muscle in our study. In adults it was composed in average of 11 % of slow fibres and 85 % of fast fibres. The ratio between fast type IIA and IIX (IIDog) fibres varied greatly between the individuals but it was always in favour of IIA fibres. In general the muscle fascicles retained their foetal fibre type distribution - the central position of slow fibre surrounded by fast fibres remained visible in adult muscle. In some superficial fascicles no type I fibres were detected in pubertal and adult dogs (Fig 4), although all fascicles had a slow primary fibre in neonates and young dogs. Similar composition, but only with higher number of slow fibres had m. tibialis cranialis.

The enzyme-histochemical reactions demonstrating the activity of metabolic enzymes SDH and α-GPDH were relatively weak in prenatal and neonatal muscles with the reaction for α-GPDH being just slightly more intense (Fig. 5A and B). The staining in both methods intensified thereafter and the first differences in α-GPDH staining intensity among muscle fibres were noted in the diaphragm at the third week and in m. extensor carpi radialis and m. tibialis cranialis at the fourth week (Fig 5C and D). In other muscles slight differentiation in SDH reaction and stronger in α-GPDH was noted by sixth week (Fig. 5 E and F). At two months of age a prominent differentiation between glycolytic and oxidative fibres was observed, the same as at six-month-old (Fig. 1G) and adult dogs (Fig.1H). All fibres had relatively strong activity of oxidative enzyme without a distinct differentiation between type I and II fibres except in the m. tibialis cranialis and m. extensor carpi radialis. Glycolytic activity was much more prominent in type IIA and IIDog fibres.

Increase of enzyme activities was confirmed by biochemistry. As shown in Fig.6, the activities of LDH (glycolytic metabolism) and CS (oxidative metabolism) measures in m. semitendinosus significantly increased in a similar fashion. The LDH and CS activities were maximal by fourth week and sixth week, respectively, and these values were similar to adult dogs. However, in a samples of two-month-old and six-month-old dog the values were significantly lower.

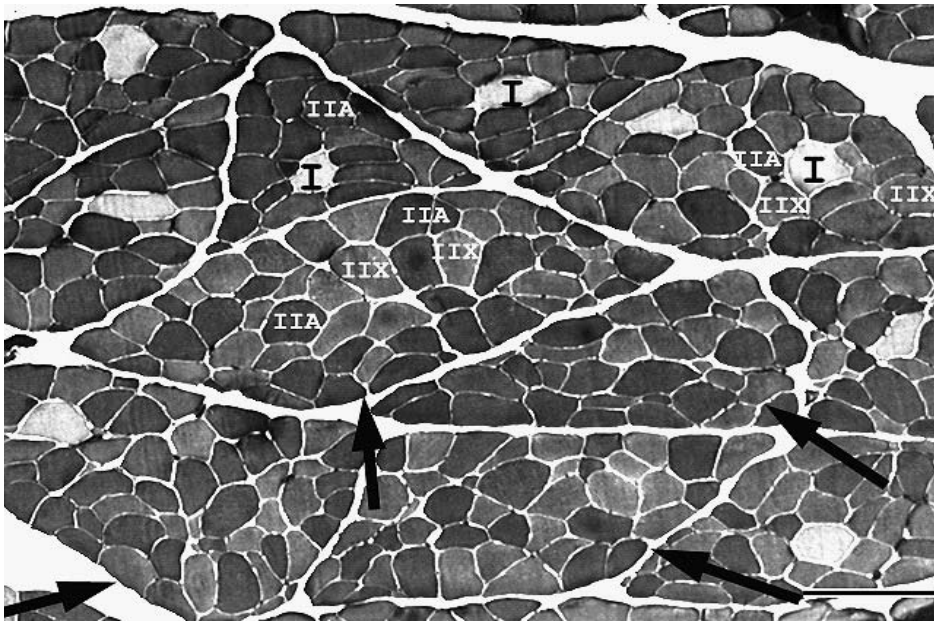

Figure 4: M. extensor carpi radialis in pubertal (6-month-old) dog; mATPase method at pH 4.6. Most of the muscle fascicles retained their developmental distribution of fibre types - i.e. one centrally located slow fibre (I), surrounded by fast fibres (IIA and IIX). However, some fascicles contained no slow fibres at all (arrows), such fascicles were located superficially in the muscle belly. Scale bar = 100 μm.

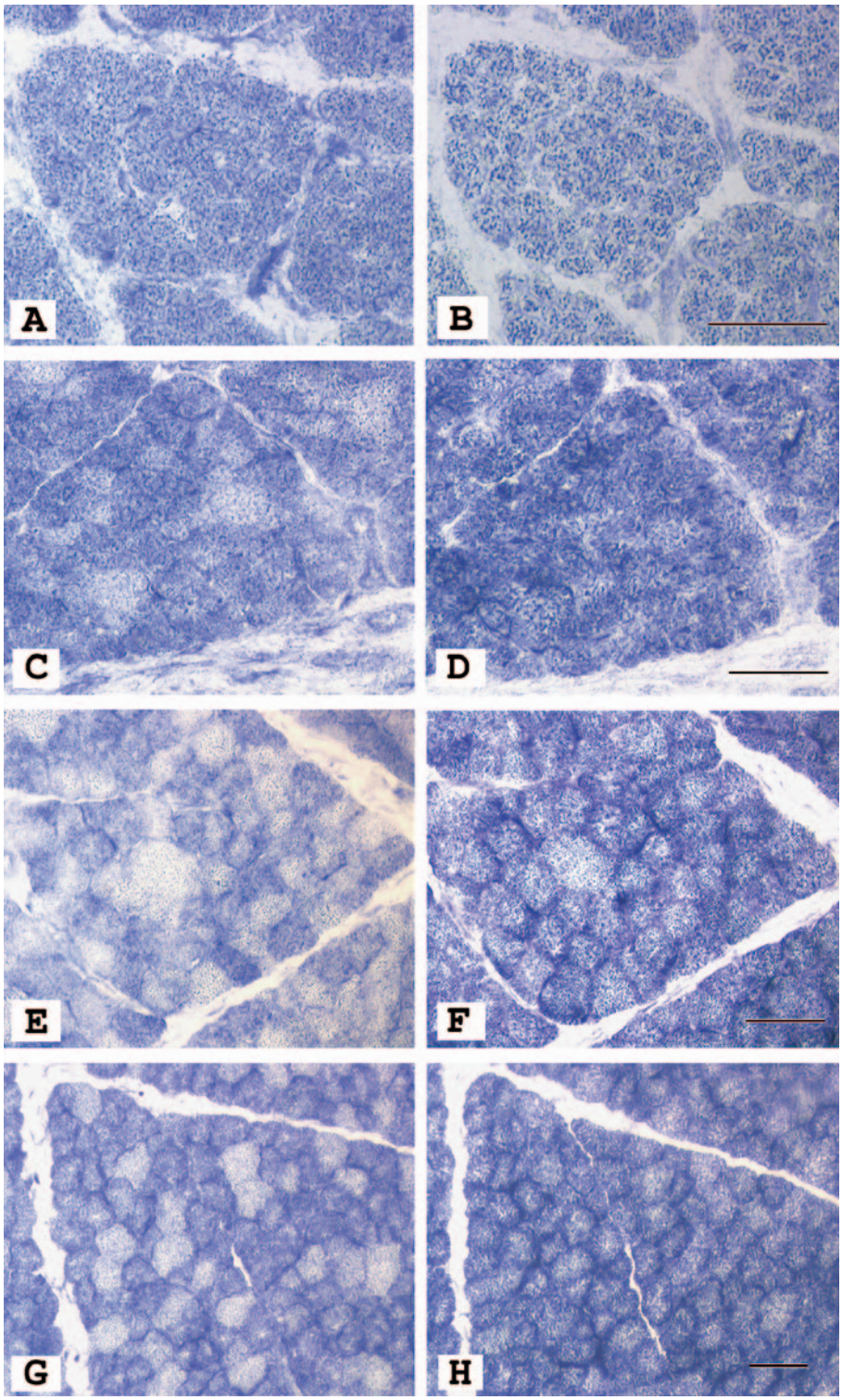

Figure 5: Demonstration of glycolytic enzyme α-GPDH (left column) and for oxidative SDH (right column) in serial sections of m. semitendinosus on fetal day 60 (A, B), 6-week-old dog (E, F) and 6-month-old dog (G, H), and m. tibialis cranialis in a 4-week-old dog (C, D). In prenatal, neonatal and early postnatal period the staining was uniform among fibres and the staining intensity was increasing with age. First glycolytic differentiation was noted in diaphragm at the third week and in m. extensor carpi radialis and m. tibialis cranialis at the fourth week (second row), while in other muscles this was notable by the sixth week (third row). At two months and later the differentiation was the same as in the adult animals - i.e. slow fibres had lower glycolytic activity but all fibres had a high oxidative activity. Scale bars = 50 μm.

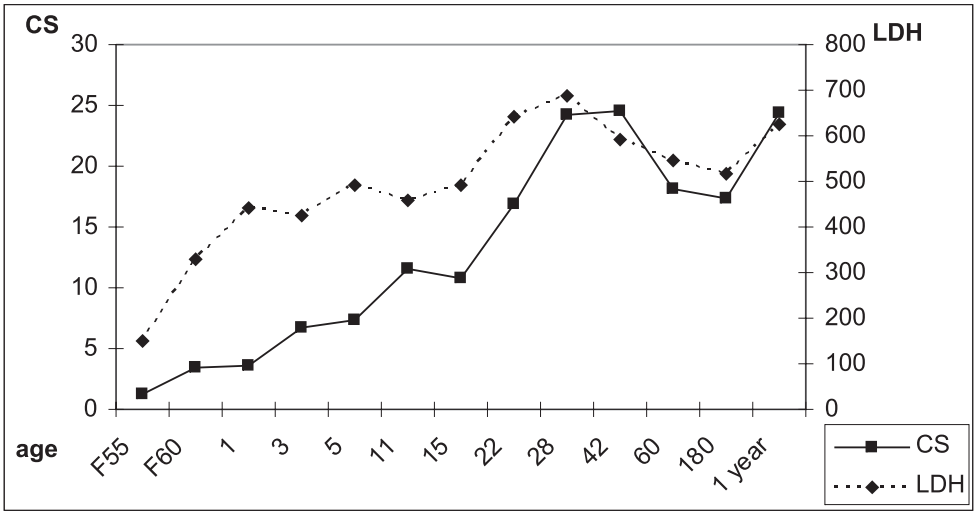

Figure 6: Increase of maximum enzyme activities in m. semitendinosus with age. Cytrate synthase (CS) and lactate dehydrogenase (LDH) activities in μmol per minute per g wet mass of muscle tissue.

Discussion

The described central organisation of skeletal muscle fascicles and the presence of fibres with internal nuclei or central perinuclear space (tubal morphology) in neonatal dogs speaks of relative immaturity at the time of birth. However, a quick maturing was noted in perinatal period: most of the muscle fascicles were loose and composed of myotubes few days before birth but the majority of them transformed into organised and more tightly packed units by postnatal day 5. Just in some parts of the muscles myotubes were still seen after birth. It seems that the functional parts of the muscle mature faster in neonates. The general maturation was also slightly delayed in m. rhomboideus, m. triceps brachii, m. rectus femoris and m. semitendinosus.

Fibre types in neonate canine muscle are different from adult fibre types. The majority (91-97%) of fibres was undifferentiated; their staining properties were comparable to the adult type IIC. Only one fibre per muscle fascicle, located in the centre of the fascicle was the slow type fibre. They were classified as primary type I in contrast to secondary (normal) type I. Primary fibres didn't obtain the typical mATPase staining properties untill sixth week post partum. Another, intermediate type of fibres started to appear few days after birth but it was not up to postnatal weeks 3 and 4 that 7 universal fibre phenotypes were established. The early appearing differentiating or "intermediate" fibres were ambiguous since they appeared in functionally different muscles i.e. they were the most numerous in m. rhomboideus which had a high number of type I fibres 3 weeks later, but they appeared early also in m. tibialis cranialis and m. extensor carpi radialis which became typical fast muscles with IIA fibres predominance in next four weeks. Although these differentiating fibres resembled the slow type staining, it is unlikely that there was an early differentiation into slow type in fast muscles since the established transition of MHC isoforms goes in direction from developmental → IIa → I (6, 22). The staining properties of early differentiating fibres obviously only reflected the loss of developmental components but their intended adult profile has not been acquired yet.

Some muscle-dependant differences were noted in establishing the metabolic profile as well. Optical density of SDH and α-GPDH staining technique increased with age and was at first uniformly distributed among fibres. Increasing enzyme activity was confirmed by biochemistry – the CS and LDH activities were increasing up to fourth and sixth week, respectively, but were again lower in a two-month-old and six-month-old dogs. This might be explained by prominent differentiation between oxidative and glycolytic fibres on tissue sections which occurred by sixth week and coincided with appearance of different mATPase fibre types. As a consequence the joint enzyme activity in the whole muscle could decrease. It might, however, also represent the individual or breed-dependant peculiarity and

more samples of different breeds from this period (2 – 6 months of age) would be needed before conclusions could be made. Precocity of metabolic maturation was again observed in the diaphragm, m. tibialis cranialis and m. extensor carpi radialis. The differentiation was more prominent with the α-GPDH. This reflects the adult profile where all fibres have relatively high oxidative capacity (1, 2, 3).

If we compare our results of time-dependant transformations with data known for cats (11, 21) we can conclude that the two species develop similarly. In cats the most prominent transformations occurred between days 30 and 40 which is comparable to weeks 4 and 6 in our research. After this period the maturation of dog muscles seems to be slower, i.e. the appearance of IIX fibres occurred later, i.e. in two-month-old dog, most likely because on average the puberty in dogs develops later in life than in cats.

Compared to bigger domestic animals, in perinatal period the dog muscles are morphologically and functionally immature. The morphology of neonate puppy muscles resemble the situation found at mid-gravidity in cattle. A neonate calf is able of standing and walking and its muscle fibres types are randomly dispersed with only traces of developmental isoforms expressed (12, 13). A high content of IIX fibres was reported in neonate foals (15, 16), while in our research they did not appear until the second month. Piglet muscles seem to be much less developed at birth if compared to calf, foal or lamb, but still more mature than that of the dog. The classification of fibre types according to mATPase method in pig was applicable already few days post partum (17). On the other hand, small mammals like rodents and rabbits have almost foetal morphology of skeletal muscles in perinatal period but quickly undergo dramatic changes. In rabbits mature morphology of muscle fibres was established relatively early, at postnatal day 40 (23, 24, 25).

The results of our study indicate that the number of dog muscle fibres is not definite at the time of birth. Such late formation of muscle fibres was described in mice and rats, where myogenesis is completed in the first week post partum (26). In bigger animals and humans the myogenesis is supposed to conclude in foetuses but there are reports of a late-forming third generation of fibres, at least in bigger muscles (12, 27). In sheep and pig the third generation of fibres with small diameter and expression of developmental isoforms was noted only after birth (17, 28, 29). We observed undifferentiated fibres with very small diameter in dog m. triceps brachii, m. longissimus dorsi and m. rectus femoris muscles as late as in 2 and 6 month-old dogs. The fibres with small diameter in dogs were described before (30), nevertheless, the formation of the third generation of myofibres in dogs remains to be established.

The apparent decrease in an average fibre diameter as seen in Fig. 3 can be explained. First, in the case of primary fibres, this happens in neonates when myotubes close completely and transform into myofibres. Second, in the case of secondary fibres, this happens by postnatal day 5 which coincided with the noted increase of secondary to primary fibres ratio. The average diameter obviously decreased due to the appearance of new fibres with a very small diameter.

While reading previous studies on developing canine muscle we got an impression that enzyme-histochemical methods are suitable for assessing the development of fibre types in the early postnatal period (7, 10). However, on closer inspection it was obvious that it was almost impossible to classify the fibres before the tenth week and becomes reliable after the twelfth week. In our study the classification became quite reliable in two-month-old dog (week 9) through there was still a certain number of the undifferentiated fibres. The mATPase method is also inappropriate to follow the development of m. masseter since the IIM fibres strongly resemble the undifferentiated fibres. Using this method it was impossible to detect transformation from undifferentiated to the adult state of the masseter muscle. It is not likely that muscle fibres were undifferentiated for so long since studies of masticatory muscles of other animals showed an active transformation on the basis of the MHC isoform content (21, 24) and similar was reported on canine pharyngeal muscles, which share the embryonic origin with the masticatory muscles (8).

We conclude that in developing canine muscles there is an active transformation from undifferentiated foetal into directed developmental stage in the first three weeks post partum. Between the third and the sixth week an active differentiation of fibre types takes place and by the second month mATPase fibre classification technique becomes applicable. Some early differences among muscles were seen i.e. faster maturation of fully active muscle – the diaphragm and fast muscles (m. extensor carpi radialis and m. tibialis cranialis). Fast and slow muscles show fibre-type differences relatively early, by the third week post partum. M. triceps brachii, m. longisimus dorsi

and m. rectus femoris retained a certain proportion of undifferentiated fibres longer than other muscles studied. It is to be expected that all muscles with big diameter are likely to retain differentiation and growth capacity up to the pubertal age.

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SPREMEMBE HISTOKEMIČNIH ZNAČILNOSTI MIŠIČNIH VLAKEN V PASJIH SKELETNIH MIŠICAH V POROJSTVENEM RAZVOJU

M. Štrbenc

V raziskavi smo proučili spremembe v pasjih skeletnih mišičnih vlaknih med razvojem od prerojstvenega obdobja do šestih mescev starosti. Poudarek je bil na histokemičnih metodah določanja tipov mišičnih vlaken in osnovnih morfoloških značilnostih. Pri novorojenih živalih smo v mišičnih snopih opisali eno centralno ležeče počasno vlakno, ki v nekaterih primerih vsebuje centralni prostor, kar je značilno za razvijajoče se miotube. Primarna vlakna so se na podlagi dokazovanja aktivnosti miozinske ATPaze pričela ločevati od sekundarnih 55. dan brejosti. Do tretjega tedna po rojstvu so bila vsa vlakna neizdiferencirana, po tem času pa je bila možna omejena klasifikacija mišičnih vlaken. Med tretjim in šestim tednom starosti smo določili 7 fenotipov mišičnih vlaken na podlagi metode z miozinsko ATPazo, pri dvomesečnem psu pa so imele mišice večinoma zrel profil s štirimi običajnimi tipi vlaken. Glikolitična in oksidativna kapaciteta je bila v vlaknih novorojencev šibka, je pa zlagoma naraščala in prve razlike med vlakni glede glikolitične kapacitete smo opazili tretji teden po rojstvu. Premer mišičnih vlaken je naraščal ves čas porojstvenega razvoja in še po šestem mesecu. Tudi skupno število vlaken, ocenjeno kot razmerje med primarnimi in sekundarnimi vlakni, se je v obporodnem obdobju povečalo.

Če primerjamo razvoj mišičnih vlaken pri psu s podatki o razvoju pri drugih domačih živalih, lahko ugotovimo, da so pasje mišice ob rojstvu relativno nezrele. Nekateri deli mišic so se razvijali še celo počasneje od ostalih, saj so imela vlakna cevkasto strukturo še pri novorojencih. Opazili smo tudi določene razlike med mišicami. Trebušna prepona (diafragma) se je kot polno dejavna mišica razvijala hitreje od ostalih, relativno zgodaj pa smo ugotovili tudi razlike med počasno (m. rhomboideus) in hitrima mišicama (m. extensor carpi radialis in m. tibialis cranialis). Zrelo morfologijo mišic z naključno razporejenimi različnimi mišičnimi vlakni znotraj snopov in izoblikovanim presnovnim profilom smo ugotovili v vseh mišicah pri dveh mescih starosti, v tem obdobju pa je tudi že možno uporabljati klasično metodo ugotavljanja aktivnosti mATPaze in presnovnih encimov za določanje mišičnih tipov.

Ključne besede: anatomija, veterinarska; mišica, skeletna; mišična vlakna - rast in razvoj; miozinska ATPaza; psi