Session 4 Muscle biology and biochemistry

L 1 ROLE OF FIBER TYPES IN MEAT QUALITY

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Introduction

The observation that skeletal muscles varied in appearance dates at least back to Lorenzini (1678) who noted that rabbit muscles were not all identical in color. Ranvier (1874) observed that the color of the muscle correlated with the speed of contraction. As the cellular nature of muscle became more clear and histological techniques improved, it was soon recognized that individual muscle cells had different properties and that muscles were usually mixtures of varying proportions of the different cell types. An early review by Needham (1926) summarized differences that had been observed between red and white muscle. Fiber typing is an extremely complex problem because of the different perspectives scientists have brought to the matter. Anatomists have described muscle as red or white, physiologists talk about fast and slow contraction speeds, and biochemists think in terms of metabolism types such as primarily glycolytic or oxidative. While the red muscles usually have slower contraction speed and are driven by oxidative metabolism and the white muscles shorten more rapidly and are fueled by glycolysis, there are enough exceptions and intermediate states to cause generalized confusion. Several types of fiber type nomenclature have been instituted, but there is no current agreement on a single system. The purposes of this review are two fold: (1) to summarize the different systems and nomenclature used to describe types of skeletal muscle fibers and (2) to review past and current work on fiber types as they affect meat quality attributes. This review is not intended to be all-inclusive, and the primary emphasis is placed on the fiber type properties of the pig. Additional information can be found in previous reviews (Cassens and Cooper, 1971; Karlsson et al. 1999).

Historical

Dubowitz (1960) was the first to classify muscle fibers into type I and type II. The former had high oxidative enzyme concentrations while the latter had a greater glycolytic enzyme profile (Dubowitz and Pearse, 1960). It soon became apparent that this simple division was inadequate. Staining for mitochondial enzymes or phosphorylase yielded a variety of intermediate fibers. Others described muscle fibers as red, white, and intermediate. A more extensive classification was developed by Brooke and Kaiser (1970) that was based on the ATPase activity of myosin after pre-incubation at acid pH. Type I fibers were stable with treatment while type II fibers were inactivated at various pH values. Thus human type IIA fibers were inactivated after pre-incubation at pH 4.9 while type IIB fibers required a pH below 4.5 to affect their activity. A demonstration of the different effects of pH on the activity in the pig is shown in Figure 1. Note that the activity in all the fibers remains higher if the pre-incubation is at 4.7. Three major intensity gradations can be distinguished after pH 4.7 ot 4.6 pre-treatment but only two groups after pH 4.4 pre-incubation. The darkest stained fibers at all these pH values are type I. Fibers with type IIA ATPase typically had a similar high mitochondrial enzyme content as the type I fibers; this resulted in other investigators grouping fibers as β, α, and αβ (Yellin and Guth, 1970) or β-red, α-red, and α-white (Ashmore and Doerr (1971). Peter and coworkers (1972) referred to these fiber types as "slow-twitch-oxidative" [SO], "fast-twitch-oxidative-glycolytic" [FOG], and and "fast-twitch-glycolytic" [FG]. Unfortunately the methods developed for fiber typing in one species did not apply exactly to another; for example (1) the pH sensitivity for the ATPase reaction was different for rat and rabbit versus human muscle (Brooke and Kaiser, 1970) and (2) the IIA fibers of horse Gluteus medius muscle possess higher oxidative potential than the type I fibers due to evolutionary adaptation to locomotion performance (Rome at al., 1990). In addition several of the major nomenclature systems have persisted to the present time. Table 1 attempts to identify the approximate equivalency among the various nomenclature schemes.

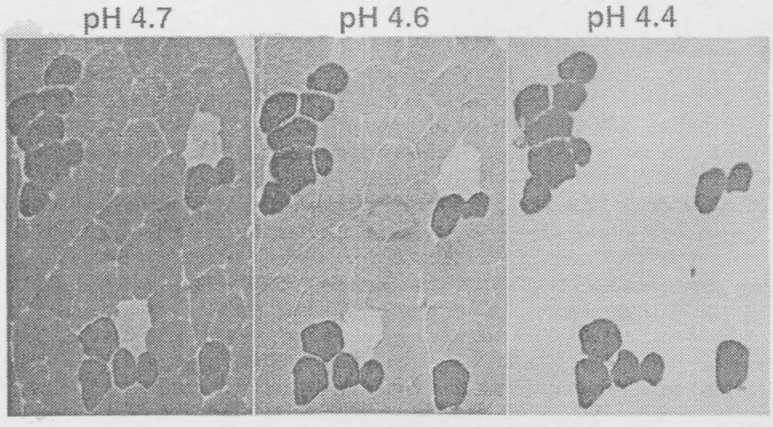

Figure 1. Histochemical myosin ATPase in the pig Longissimus after pre-incubation at different pH values

Newer methods of fiber type classification have been developed using monoclonal antibodies that recognize specific myosin isoforms. Schiaffino and coworkers by used monoclonal antibodies to classify fibers containing myosins identified as type I, type IIA, type IIB, and type IIX (Schiaffino et al. 1989). The type IIX stained fibers did not closely fit any of the previous typing classifications. Independently Termin and coworkers (1989) described individual muscle fibers containing a unique myosin that they termed type IID. It was subsequently verified that the type IIX and type IID myosins are identical. Although this method of fiber typing held great promise, it has become increasingly recognized that many fibers contain multiple myosin isoforms and were thus hybrids. Type IIC fibers, identified by showing partial ATPase activity with pre-incubations as low as 3.9 (Brooke and Kaiser, 1970),

were later shown to contain mixtures of type I and type II myosin. Studies in which the speed of contraction was compared with the histochemical and immunochemical pattern have shown that the shortening speed progresses from type I to type IIA to type IIX to type IIB. Hybrid fibers most commonly consist of pairs of myosins along this continuum, i.e. I+IIA, IIA+IIX, or IIX+IIB (Pette and Staron, 2000).

Table 1. Nomenclature systems for muscle fiber types

Investigators	Type I	Type IIA	Type IIX/D	Type IIB
Brooke & Kaiser, 1970	Type I	Type IIA	Type IIB	Type IIB
Yellin & Guth, 1970	β	αβ	α	α
Ashmore & Doerr, 1971	βR	αR	αW	αW
Peter et al., 1972	Slow, oxidative (SO)	Fast, oxidative, glycolytic (FOG)	Fast glycolytic (FG)	Fast glycolytic (FG)
Larzul et al., 1997	Type I	Type IIA	Type IIBr	Type IIBw
Brocks et al., 2000	Type I	Type IIA	Type IIBr	Type IIBw
Gil et al., 2001	Type I	Type IIA	Type II*	Type IIB

Fiber Types in the Pig

The earliest studies on fiber types in the domestic pig were conducted by Beecher and coworkers (1965). They found that pig muscle fibers had variations in histochemical oxidative enzyme profiles similar to those observed previously in humans and laboratory animal species. Moody and Cassens (1968) showed that the fiber type proportions were different between the Longissimus and the trapezius with the former having high phosphorylase activity and low NADH teterazolium reductase (a mitochondrial enzyme) and the latter having opposite patterns. Suzuki and Cassens (1980) examined the pH sensitivity of the ATPase reaction and found evidence for at least three different intermediates between the type I and type II. Interest developed concerning whether the development of the Pale, Soft, Exudative (PSE) condition was related to fiber types. Studies by Sair and coworkers (1972) and Swatland and Cassens (1973) suggested that there were somewhat higher proportions of glycolytic fibers in the animals that developed PSE meat. Sosnicki (1987) found that pigs with poorer meat quality typically had a lower proportion of βR and a higher proportion of αW fibers. More recent studies (Essen-Gustavsson et al., 1992) suggested that there were no differences in the fiber type proportion (determined by histochemical ATPase) between pigs with different halothane genotypes. In contrast Larzul and coworkers(1997) demonstrated a significant correlation (0.55) between the lightness of Longissimus color and percentage of type IIBw fibers (the latter were identified as being negative for succinic dehydrogenase and weakly positive staining for ATPase after pH 4.35 pre-incubation). These workers also found highly significant relationships between type IIBw content with pH at 30 minutes postmortem and ultimate pH. Ruusunen and Puolanne (1997) compared fiber type proportions between different pig breeds and animals found that there were more differences within a breed than between breeds. Studies by Henckel and coworkers (1997) demonstrated significant correlations between the percentage of type IIA fibers (ATPase) and tenderness. Candek-Potokar and coworkers (1999) compared histological profiles (percentages of αR,βR,αW) with various meat properties and concluded that the correlations were low. Feidler and coworkers (1999) demonstrated that animals that were homozygote for the malignant hyperthermia mutation had significantly more fast twitch glycolytic fibers than normal. Depreux and coworkers (2000) used ELISA tests along with a series of monoclonal antibodies to compare patterns of pig Longissimus myosin amounts in

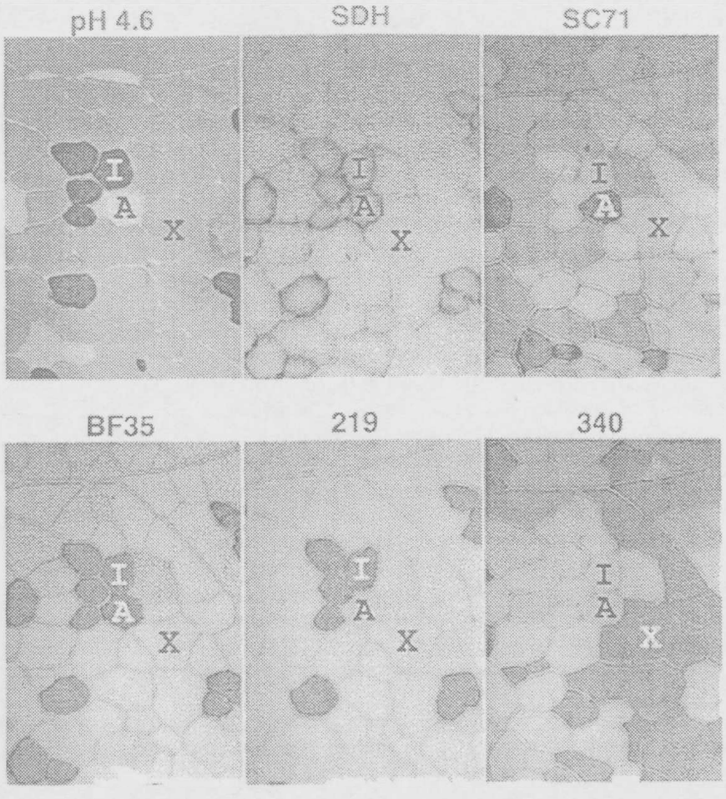

Figure 2. Serial sections from pig Longissimus stained with ATPase, succinic dehydrogenase, and monoclonal antibodies. I=type I; IIA=type IIA; X=type IIX or IIX/IIB hybrids.

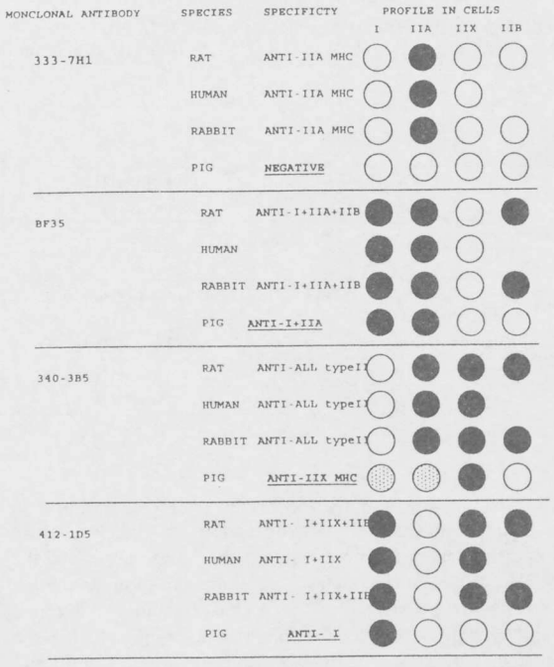

Figure 3. Species variation in response to different myosin monoclonal antibodies

halthane positive and halothane negative pig Longissimus muscle. They found that the halothane positive pigs had significantly less type I and IIA myosin and more type IIB (using ELISA methods) than their normal counterparts. These workers used a series of monoclonal antibodies for the myosin and fiber type identification. However, one of the antibodies used in this study was SC-71, a monoclonal that stains myosin IIA fibers exclusively in the rat. Depreux and coworkers used in situ hybridization with a pig myosin IIX antisense riboprobe to conclude that SC-71 reacts mainly with myosin IIX in the pig. Thus myosin specific antibodies in one species may not react in identical fashion in other species. There may also be the possible danger that a monoclonal may behave differently between histochemical and ELISA methods. Further examples of potential antibody problems are illustrated in Figures 2. The pattern of staining depends on the method used. Note that the pH 4.6 pre-incubation and monoclonal anti-myosin 219 show identical dark staining of type I fibers. The type I and type IIA have opposite patterns with ATPase but identical staining with succinic dehydrogenase. Note the variety of staining intensities with SC-71; the darkest stained fibers appear to be type IIA. This result does not agree with the Depreux et al. (2000) findings. Further examples of different staining patterns between species are illustrated in Figure 3. Monoclonal 333-7H1 (Sant'Ana Pereira et al., 1995) reacts with type IIA fibers in rat, human, and rabbit muscle but does not react with any pig fiber types. Monoclonal BF35, developed by Bottinelli and coworkers (1991), reacts with types I, IIA, and IIB in rats and rabbits but only types I and IIA in pigs (see Figure 2). Monoclonal 340-3B5 reacts with all type II myosins in rats and rabbits and is negative for type I myosins in the same species, but the same antibody reacts strongly with type IIX, weakly with type I and IIA, and negatively with type IIB in the pig. Finally, monoclonal 412-1D5 gives strong reaction with types I, IIX, and IIB in rats and rabbits but only with type I myosin in pigs. Needless to say, these anomalies may cause serious difficulties in both proper fiber type identification and the defining of the relationship between fiber type and meat quality.

The situation with hybrid fibers also complicates our understanding of fiber type effects on meat quality. Lefaucheur and coworkers (1998) used conventional histochemical fiber type methods in combination with in situ hybridization with probes for myosin types I, IIA, IIX, and IIB. Fibers that have been previously identified as IIB based on ATPase and mitochondrial enzyme patterns (such as the medium stained fibers in Figure 1, pH 4.6 pre-incubation of this review) were found to be 18% pure type IIX, 31% IIX/IIB hybrids, and 51% pure type IIB in the Longissimus (Lefaucheur et al., 1998). In the red portion of the semitendinosus virtually all fibers with conventional IIB classification are really type IIX.

Summary and Conclusions

Attempts to divide muscle fibers into discrete classes remain difficult even with newer methods relying on monoclonal antibody and molecular biological techniques. How should the hybrid fibers be classified? What are the detection limits in fibers to determine if they are hybrid or pure; i.e. is a fiber that is really 95% type IIB and 5% type IIX classified as a hybrid or pure IIB? If fiber composition is really a continuum, then trying to define groups depends on the border decisions. A further problem is that many of the monoclonal myosin antibodies that have been developed using non-pig myosins either do not react in the pig or react with multiple myosins. Although results to date suggest that meat quality is related to fiber type and most specifically to the proportion of type IIB fibers, new antibodies either need to be developed or characterized that allow better identification of this fiber type in the pig. Accurate fiber typing will assist in implementation of a balanced genetic improvement in the pig that includes cost of production (i.e, growth rate and feed conversion ratio), carcass lean percentage and meat quality.

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