MECHANICAL PROPERTIES OF TYPE I AND TYPE IIB SINGLE MUSCLE FIBRES

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Background

Muscles are primarily composed of three fibre types (type I, type IIA and type IIB), which differ in metabolic and contractile properties. On the basis of fibre type distribution, muscles can be divided into red and white muscles. Red muscles are mainly composed of oxidative type I fibres, whereas white muscles are mainly composed of glycolytic type IIB fibres. Type IIA fibres are both glycolytic and oxidative. The variation in tenderness between muscles has often been correlated to their metabolic properties, as determined by the fibre type distribution. Thus, the rate of proteolysis has been reported to occur faster in bovine white muscles (*M. longissimus dorsi*, *M. biseps femoris and M. semitendinosus*) than in the red *M. psoas major* (Olson et al., 1976; Koohmaraie et al., 1988). Mutungi et al. (1996) investigated the effect of post-mortem storage at refrigerated temperatures on the mechanical properties of porcine single muscle fibres. The results showed that post-mortem storage for 11 days resulted in a marked decrease in the breaking stress and strain of single muscle fibres. The changes in strength were more marked in muscle fibres isolated from the red *M. iliocostalis* than in those from *M. longissimus dorsi* (LD). However, the contribution of individual fibre types to meat tenderness was not measured.

The objective of this study was to determine the mechanical properties of type I and IIB single muscle fibres. Furthermore, to investigate the effect of post-mortem storage on the mechanical properties of single muscle fibres at the individual fibre type level.

Methods

LD and *M. vastus intermedius* (VI) were obtained from two slaughter pigs 24 hours post-mortem (p.m.). Muscle samples (weighing approximately 80 g) were cut, vacuum packed and either immediately frozen in liquid nitrogen or stored at 4°C for 7 days post-mortem before freezing in liquid nitrogen. Samples were stored at -80°C until use. The procedure for tensile test of single muscle fibres broadly followed the methodology of Mutungi et al. (1996). Small strips of muscle was carefully dissected from the frozen muscle samples and placed in a solution containing 50 mM MES (pH 5.6), 100 mM KCl, 280 mM Mannitol and 0.2 mM EGTA. Single fibres were isolated under a binocular dissecting microscope at room temperature and rapidly transferred onto aluminium templates so they extended across a gap (2-3 mm) cut in the centre of the plate. The muscle fibres were attached to the aluminium plate by gluing the fibre ends onto the plate with cyanoacrylate adhesive. Care was taken not to stretch the fibre during the transfer process. The free length of the fibre was measured using vernier calipers and the diameter was measured using a Leica DMIRB microscope. The aluminium plate with the fibre glued onto it was then attached between two screw-up clamps, one attached to a motor and the other to an isometric force transducer of a small mechanical testing device (Lewis & Purslow, 1989). The side-pieces of the aluminium plate bridging the gap was carefully cut leaving the fibre hanging between the two plate ends. Fibres were stretched at a constant rate of 13.8 µm/s until fracture. Breaking loads and extensions were monitored using a 16 bit A/D converter (National Instuments) running under LabView data acquisition software and breaking stress and strain values were calculated. Breaking stress is defined as maximum load per unit cross-sectional area of each fibre. Breaking strain is taken as the strain at maximum stress.

After tensile testing the remaining of each fibre were collected and transferred into sample buffer (4 M urea, 1 M thiourea, 0.025 M Tris(hydroxymethyl)-aminomethane, pH 6.8, 1.5% SDS and 1% DTT). Myosin heavy chain isoforms in the individual solubilised fibre samples were located by performing dot-blot. Muscle fibre types were determined by identification of myosin heavy chain isoforms (MHC-2042, MHC-2043 and MHC-2047; American Type Cell Culture). MHC-2042 recognises type IIA and IIB fibres, MHC-2043 recognises type I fibres and MHC-2047 recognises type I and IIA fibres.

Results and Discussion

Table 1 show the effect of fibre type and post-mortem storage on the tensile breaking stress and strain of single muscle fibres isolated from porcine LD and VI. Type IIB fibres isolated from LD 1 day p.m. were significantly ($P < 0.001$) weaker than type I fibres. No significant difference in breaking stress and breaking strain values between type I fibres isolated from VI (red muscle) and type I fibres isolated from LD (white muscle) 1 day p.m. were observed. The diameter of type IIB fibres (98.7 ± 3.1 m) isolated from LD 1 day p.m. were significantly greater ($P < 0.001$) than the diameter of type I fibres (54.1 ± 1.7 m). However, changes in muscle fibre diameter between type IIB fibres and type I fibres are proportionately smaller than the observed changes in breaking stress, suggesting that some additional fibre type-related effect must be involved. Storage for 8 days at refrigerated temperatures led to a significant decrease ($P < 0.001$) in the breaking stress of type I fibres isolated from both LD and VI compared to type I fibres isolated 1 day p.m. The breaking strain of type I fibres isolated from VI decreased ($P < 0.001$) significantly after 8 days of storage. In contrast, the breaking strain values of type I fibres isolated from LD did not change during p.m. storage. Storage of LD samples for 8 days did not result in a significant decrease in the breaking stress of type IIB fibres compared to 1 day p.m., indicating that a majority of the weakening of type IIB fibres had already occurred within the first 24 hours after slaughter. However, the breaking strain of type IIB fibres isolated from LD decreased ($P < 0.01$) significantly after 8 days of storage, as compared to day 1 fibres.

These results strongly suggest that the mechanical properties of single muscle fibres differ between fibre types (type I and IIB fibres) within a muscle. Furthermore, the mechanical properties of a given fibre type does not seem to be muscle type dependent, i.e. breaking stress of type I fibres does not change even though the environment surrounding the fibres, such as the fibre type distribution, are markedly different. The fact that type IIB fibres are weaker than type I fibres within LD may suggest that the mechanical properties of the protein matrix differ between fibre types. Additionally, type IIB fibres may be more susceptible to early proteolysis.

Conclusions

- Type IIB fibres are significantly weaker than type I fibres in *M. longissimus dorsi*.
- The mechanical properties of type I fibres does not differ between muscles.

References

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Table 1. Breaking stress and breaking strain values of type I and type IIB fibres isolated from porcine *M. longissimus dorsi* and *M. vastus intermedius* on day 1 or day 8 post-mortem. Values are expressed as means ± SE of a minimum of 30 fibres (except for type I fibres isolated from LD day 8 post-mortem where n=6).

Trait	Fibre type	Day 1		Day 8	
		VI	LD	VI	LD
Breaking stress (kPa)	I	311.0 ± 11^{a}	320.2 ± 16^{ax}	176.3 ± 12^{b}	192.5 ± 29^{bx}
	IIB		121.3 ± 7^{ay}		100.6 ± 19^{ay}
Breaking strain (%)	I	53.4 ± 2.4^{a}	53.6 ± 3.2^{ax}	33.7 ± 2.8^{b}	55.0 ± 8.3^{ax}
	IIB		33.8 ± 2.2^{ay}		19.9 ± 2.2^{by}

[a,b] Within trait and rows, means with the same letter does not differ ($P > 0.05$)
[x,y] Within trait and columns, means with the same letter does not differ ($P > 0.05$)