Meat Science 80 (2008) 1297–1303

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Simulation of giant fibre development in biopsy samples from pig *longissimus* muscle

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ARTICLE INFO

Article history:
Received 31 March 2008
Received in revised form 2 June 2008
Accepted 11 June 2008

Keywords:
Meat quality
Muscle fibre
Capillary
Histology
Electron microscopy

ABSTRACT

The incidence of hyper-contracted giant fibres in pig postmortem skeletal muscle is closely related to poor meat quality in terms of pale, soft, and exudative pork. Detection of a predisposition to develop giant fibres in live pigs could help to predict pork quality and to exclude affected pigs from genetic selection. The abundance and proportion of giant fibres in longissimus muscle were highest in Piétrain followed by Landrace, Large White, and Leicoma pigs of market weight. The postmortem development of giant fibres could be successfully simulated *in vitro* incubating biopsy samples from longissimus muscle at 37 °C for 60 min. For repeated measurements on three samples the intraclass correlation coefficient for the number of giant fibres/cm^2 was $\hat{\vartheta}_3 = 0.69$ for biopsy and $\hat{\vartheta}_3 = 0.87$ for carcass samples. "Simulated" giant fibres exhibited ultrastructural changes in plasma membrane, myofibrils, mitochondria, and sarcoplasmatic reticulum as shown previously for giant fibres in carcass samples.

1. Introduction

So-called giant fibres were first detected by Cassens, Cooper, and Briskey (1969) in pig muscle. Apart from the pig, giant fibres have also been found in skeletal muscle from poultry (Remignon, Zanusso, Albert, & Babile, 2000; Soike & Bergmann, 1998; Sosnicki, 1987), cattle (Sink, Mann, & Turgut, 1986) and horse (Weyermann & Dzapo, 1998). These fibres appear in histological muscle cross-sections as round-shaped, mostly above-average in size and are preferably situated at the edge of primary muscle bundles (Cassens et al., 1969; Hendrick, Lafferty, Aberle, Judge, & Forrest, 1971; Wegner & Ender, 1990). Giant fibres do not belong to a special metabolic or contractile type of fibres as shown by enzyme histochemistry (e.g. Dutson, Merkel, Pearson, & Gann, 1978; Handel & Stickland, 1986). The aetiology of this fibre anomaly is a subject of inconsistency and not yet fully clarified. For the majority, giant fibres are considered to arise from hypercontraction of individual fibres (e.g. Dutson et al., 1978; Sink et al., 1986; Sosnicki, 1987). Signs of myopathy have or have not been observed in giant fibres by Sosnicki (1987) and Handel and Stickland (1986), respectively. Others have characterized them as fibres being in a degenerative, pre-necrotic stage (Cullen, Appleyard, & Bindoff, 1979; Wegner & Ender, 1990; Wilson, Nieberg, Buhr, Kelly, & Shultz, 1990).

All reports on giant fibres have in common that these fibres have been detected exclusively in postmortem muscle. However, it is strongly suggested that animals, which develop the giant fibre syndrome during the conversion from muscle to meat, exhibit a respective predisposition *intra vitam* by changes in metabolic and/or structural features of myofibres. It has been clearly shown that both extreme fibre hypertrophy and the occurrence of giant fibres in pigs correlate with poor meat quality in terms of the pale, soft, exudative (PSE) condition (Essén-Gustavsson, 1995; Fiedler et al., 1999; Fiedler, Dietl, Rehfeldt, Wegner, & Ender, 2004) mainly resulting from enhanced and extended postmortem glycolysis (Scheffler & Gerrard, 2007). Therefore, the development of giant fibres must be considered in close association with postmortem energy metabolism. Accordingly, (Lahucky et al., 1993; Lahucky et al., 2002) were able to show that energy metabolism characterized by 31P NMR (nuclear magnetic resonance) spectroscopy in biopsy samples from pig muscle was closely related with postmortem metabolism and meat quality.

Pre-slaughter environmental conditions may contribute to the giant fibre syndrome as they affect postmortem muscle metabolism (Bee et al., 2006; Küchenmeister, Kuhn, & Ender, 2005; Shen et al., 2006). On the other hand, the heritability of the giant fibre percentage (h^2 = 0.2), its close genetic correlation with pork quality (r = 0.8) (Fiedler et al., 2004) as well as the detection of a QTL explaining 24% of the variability in this trait on chromosome number 12 (Wimmers et al., 2006) strongly suggest a genetic background of the giant fibre syndrome. Early detection of a predisposition to develop giant

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doi:10.1016/j.meatsci.2008.06.008

fibres in live pigs could therefore help to predict pork quality and to exclude affected pigs from breeding.

This study was conducted to investigate the abundance of giant fibres in pigs of four German breeds, and whether postmortem giant fibre development can be simulated in muscle biopsy samples *in vitro* as a prerequisite to detect a predisposition for the giant fibre syndrome in live pigs.

2. Materials and methods

2.1. Animals and samples

To quantify the occurrence and frequency of giant fibres in pigs of various German breeds, pigs of Large White ($n = 46$), Landrace ($n = 51$), Leicoma ($n = 44$), and Piétrain ($n = 44$) from a progeny test station (Jürgenstorf, Hybridschweine-Zuchtverband Nord/Ost e.V. Malchin, Germany) were examined. At slaughter, the pigs were 191 days of age and weighed 86 kg (hot carcass weight) on average. Samples from *longissimus thoracis et lumborum* were taken 24 h postmortem at the level of 13th/14th rib, snap-frozen in liquid nitrogen and stored at −70 °C until histological/histochemical analysis. At the same position the following characteristics of meat quality were measured as described in detail by Rehfeldt, Tuchscherer, Hartung, and Kuhn (2008): pH value at 45 min postmortem (pH_{45}), conductivity, impedance, colour, and drip loss at 24 h postmortem.

To simulate giant fibre development *in vitro,* samples from longissimus muscle were taken from 12 Piétrain pigs 5 min before slaughter by biopsy according to Wegner and Schöberlein (1984). Immediately after, three samples of 5 × 5 × 3 mm were cut by a razor blade and separately placed into pre-warmed Eppendorf tubes, which were closed and then incubated in a water bath at 37 °C for 60 min following the studies of Hennebach, Albrecht, Lengerken, and Pfeiffer (1980) and Lahucky, Fischer, and Augustini, (1982). Thereafter, transversal frozen sections of 12 μm were prepared and stained by HE and analyzed by image analysis (number of fibres and number of giant fibres per unit area) as described below. Samples from four Piétrain pigs were fixed in 4% glutaraldehyde in 0.1 M sodium phosphate buffer, washed in PBS, postfixed in 1% OsO4, dehydrated by acetone and embedded in epoxy resin Araldite for electron microscopy.

All procedures including use and treatment of the pigs were in accordance with the guidelines set by the Animal Care Committee of the State Mecklenburg–Vorpommern, Germany, based on the German Law of Animal Protection.

2.2. Muscle histology and histochemistry, microscopy

Serial transverse sections of 12 μm were cut in a cryostat (2800 Frigocut-N, Leica, Nussloch, Germany) and stained for cytoplasm and nuclei by haematoxylin/eosin (HE; Romeis, 1989) or for alkaline phosphatase (Gomori, 1952, described by Spannhof, 1967) to visualize capillaries. Further sections were exposed to a combined reaction for NADH-tetrazolium reductase (NADH-TR; Novikoff, Shin, & Drucker, 1961) and acid-preincubated ATPase at pH 4.2 (Guth & Samaha, 1970), which enables classification of slow-twitch oxidative (STO), fast-twitch oxidative (FTO) and fast-twitch glycolytic (FTG) muscle fibres modified according to Horak (1983). The abundance and cross-sectional area of the giant fibres and their classification as STO, FTO, and FTG types were determined using 300–350 muscle fibres per sample by image analysis (AMBA, IBSB, Berlin, Germany). For electron microscopy, ultrathin sections were prepared with an ultramicrotome Ultracut SWS (Leica) stained by uranyl acetate and lead citrate and studied in the transmission electron microscope EM 902A (Zeiss, Oberkochen, Germany).

2.3. Statistical analysis

Data were subjected to analysis of variance using the GLM procedure of SAS including the factors breed and gender and respective interaction. Significance of differences between least squares means was tested by the Student's *t*-test ($P < 0.05$). To evaluate the repeatability of the measures of the number of myofibres and of giant fibres/cm^2 within the animal, intraclass correlation coefficients (ICC) were obtained from one-way analysis of variance according to Rasch (1983), for which another example is given by Cerisuelo, Sala, Nürnberg, Baucells, and Rehfeldt (2007). The ICC ($\hat{\vartheta}$) was calculated as follows:

$$\hat{\vartheta} = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}^2} \quad (1)$$

where $\hat{\sigma}_a^2$ and $\hat{\sigma}^2$ are the variance between animals and the variance within the animal, respectively. The resulting ICC shows the repeatability of measurements in the case that only one sample is analyzed. The ICC $\hat{\vartheta}_n$ was then estimated in the case where more than one sample per animal ($n = 2$–4) is included in the analyses:

$$\hat{\vartheta}_n = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \frac{\hat{\sigma}^2}{n}} \quad (2)$$

3. Results

3.1. Appearance of giant fibres in histological sections

Giant fibres were found to be located primarily at the edge of, and at a lower frequency also within primary muscle bundles (Fig. 1). They showed a higher staining intensity after exposure to eosin compared with surrounding normal fibres (Fig. 2a). In addition, giant fibres did not exhibit a uniform type with both oxidative and glycolytic or fast and slow fibres appearing as giant fibres in cross-sections stained for ATPase and NADH-TR histochemistry (Fig. 2b and c). From the qualitative evaluation of sections stained for capillaries by alkaline phosphatase capillary density was lower around giant fibres as compared with the areas occupied by normal myofibres (Fig. 3).

To obtain information on the longitudinal appearance of giant fibres serial sections of a sample were cut over a distance of 7 mm. The appearance of the fibres varied from normal shape and hence not detectable as a giant fibre, over typical round–oval shape to partial or complete loss of myofibre structure over the longitudinal distance examined (Fig. 4). This suggests that giant fibres detected in cross-sections represent the result of partial myofibre hypercontraction and rupture.

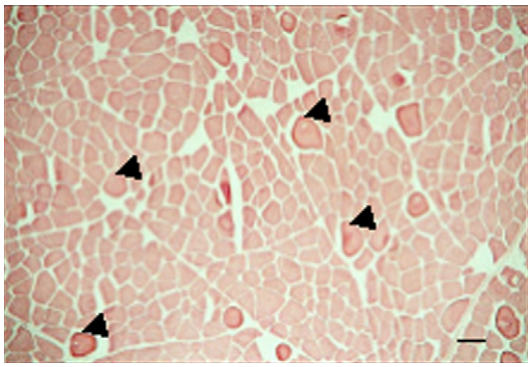

Fig. 1. Giant fibres (arrows) in *longissimus* muscle cross-section stained for haematoxylin–eosin staining (bar = 100 μm).