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

C. Rehfeldt[1*], I. Fiedler[1], A. Schubert-Schoppmeyer[1], G. Nürnberg[1], L. Jonas[2], K. Ender[1] & S. Maak[1]

[1]Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany.
[2]Institute of Pathology, University of Rostock, 18057 Rostock, Germany.
*E-mail: rehfeldt@fbn-dummerstorf.de

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. Early 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 (n=44) followed by Landrace (n=51), Large White (n=46), and Leicoma (n=44) pigs of market weight. To study, whether the *postmortem* development of giant fibres can be simulated *in vitro*, samples from *longissimus* muscle of 12 Piétrain pigs were taken by shot biopsy, placed into pre-warmed tubes and incubated in a water bath at 37°C for 60 min. Additional muscle samples were obtained from carcasses after slaughter (24 h *postmortem*). The development of giant fibres could be successfully simulated in muscle biopsy samples. For repeated measurements on three samples the intraclass correlation coefficient for the number of giant fibres/mm² was $\hat{\vartheta}_3$ = 0.69 for biopsy and $\hat{\vartheta}_3$ = 0.87 for carcass samples. "Simulated" giant fibres exhibited ultra-structural changes in plasma membrane, myofibrils, mitochondria, and sarcoplasmatic reticulum as shown previously for giant fibres in carcass samples. (supported by the H. Wilhelm Schaumann Stiftung).

Introduction

So-called giant fibres have been detected in skeletal muscles from pigs, poultry, cattle, and horse. In histological muscle cross sections they appear as round-shaped, mostly above-average in size and preferably situated at the edge of primary muscle bundles. The aetiology of this fibre anomaly is a subject of inconsistency and not yet fully clarified. Of the majority, giant fibres are considered to arise from hypercontraction of individual fibres, while others have characterized them as fibres being in a degenerative, pre-necrotic stage. Giant fibres have been detected exclusively in *postmortem* muscle. In pigs their incidence is closely related to poor meat quality in terms of pale, soft, and exudative pork, wherefore the development of giant fibres must be considered in close association with *postmortem* energy metabolism. Early detection of a predisposition to develop giant fibres in live pigs could 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.

Materials and methods

Pigs of Large White (n=46), Landrace (n=51), Leicoma (n= 44), and Piétrain (n=44) breeds from a German progeny test station were slaughtered at d 191 of age. Samples from *longissimus* muscle (LD) at the level of 13/14th rib were taken 24 h *postmortem*. At the same position meat quality characteristics were measured (Rehfeldt et al., 2008). To simulate giant fibre development *in vitro*, samples from LD muscle were taken from 12 of the Piétrain pigs 5 min before slaughter by biopsy according to Wegner & Schöberlein (1984), placed into pre-warmed tubes and incubated in a water bath at 37°C for 60 min following Hennebach et al. (1980) and Lahucky et al. (1982). All samples were snap-frozen for histological/histochemical analysis. Serial transverse sections of 12 µm were stained for cytoplasm and nuclei, for capillaries, or fibre types (see Rehfeldt et al. 2007; 2008). The abundance, cross sectional area, and type of the giant fibres were determined evaluating 300 to 350 muscle fibres per sample by image analysis. Additional samples from four Piétrain pigs were prepared for electron microscopy and studied in a transmission electron microscope.

For statistical analysis data were subjected to ANOVA using the GLM procedure of SAS including the factors breed and gender and respective interaction. To evaluate the repeatability of the measures of the number of myofibres and of giant fibres/cm² within the animal, intraclass correlation coefficients (ICC) were obtained from one-way ANOVA according to Rasch (1983).

Results and discussion

Appearance of giant fibres in histological muscle cross sections. Giant fibres were found to be located primarily at the edge of, but 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. In addition, giant fibres were not of a uniform type with both oxidative and glycolytic or fast and slow fibres appearing as giant fibres. By qualitative evaluation capillary density was lower around giant fibres as compared with the areas occupied by normal myofibres (not shown). 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 (not shown). This suggests that giant fibres detected in cross sections represent the result of partial myofibre hypercontraction and rupture.

Table 1. Incidence of giant fibres and capillary density in cross sections of *longissimus* muscle samples obtained from pigs of different breeds 24 h *postmortem*

Traits	Large White	Landrace	Leicoma	Piétrain
Hot carcass weight (kg)	86.3 ± 0.36	86.3 ± 0.39	86.1 ± 0.32	87.1 ± 0.22
No. of pigs with giant fibres	25	40	15	41
%age of pigs with giant fibres	54	78	34	93
No. of pigs with giant fibres of type STO/FTO/FTG	0/0/25	4/13/33	1/1/14	9/19/36
Frequency of giant fibres (%)	0.48 ± 0.57^{a}	0.62 ± 0.55^{a}	0.12 ± 0.39^{b}	1.39 ± 1.10^{c}
Giant fibre size (µm²)	9604 ± 2024	9844 ± 2523	8776 ± 3000	11995 ± 3262
Capillaries/mm²	56.4 ± 3.64^{a}	44.9 ± 3.86^{b}	57.6 ± 3.09^{a}	35.2 ± 2.19^{b}
Capillaries/fibre	0.46 ± 0.03	0.44 ± 0.04	0.47 ± 0.03	0.37 ± 0.02

[a,b, c] LSMeans bearing different letters are significantly different (P<0.05).
STO-slow-twitch oxidative, FTO-fast-twitch-oxidative, FTG-fast-twitch glycolytic

Frequency of giant fibres, distribution of capillaries and meat quality in muscle of different pig breeds. Within the Piétrain breed most of the pigs (93%) exhibited giant fibres, followed by Landrace and Large White pigs and finally Leicoma pigs with only 34% abundance (Table 1). The proportion of giant fibres within muscle sections followed the same order with the highest percentage in Piétrain (1.39%) and the lowest percentage in Leicoma (0.12%) pigs. Furthermore, Piétrain pigs exhibited the largest giant fibres. Giant fibres were predominantly of the FTG type. The density of capillaries in muscle cross sections was lower in samples of Piétrain and Landrace pigs compared with Large White and Leicoma pigs. The results on meat quality (not shown) coincided well with the differences in the frequencies of giant fibres. Piétrain pigs exhibited the poorest meat quality in terms of pH_{45}, impedance and conductivity at 24 h *postmortem*, while Leicoma pigs showed the highest quality meat in terms of pH_{45} and impedance with a tendency seen also in drip loss. The results suggest that the abundance of giant fibres is in part genetically determined and confirm the known correlation with meat quality.

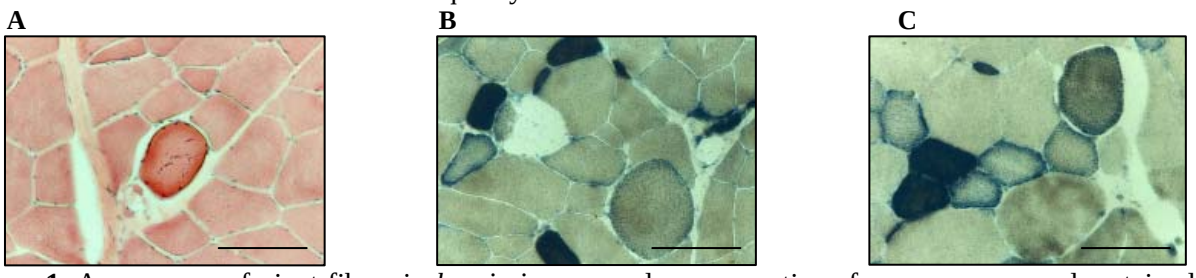

Figure 1. Appearance of giant fibres in *longissimus* muscle cross sections from carcass samples stained for cytoplasm and nuclei (A) or for STO, FTO, and FTG fibres types (B,C) (bar = 100 µm).

In vitro simulation of giant fibre development. Biopsy samples of *longissmus* muscle have been incubated in plastic tubes for 60 min at 37°C in a water bath and analyzed for the occurrence of giant fibres. As shown in Fig. 2, where representative samples from one and the same pig are compared, no giant fibres were detectable in the samples fixed within 2 min after biopsy (Fig. 2A). In another part from the same sample giant fibres have developed during incubation (Fig. 2B). Likewise, giant fibres were detectable in a sample taken 24 h *postmortem* from the same pig (Fig. 2C). These results show that giant fibres not only

develop in the carcass but also in biopsy samples taken from the live pig exposed to specific *in vitro* conditions.

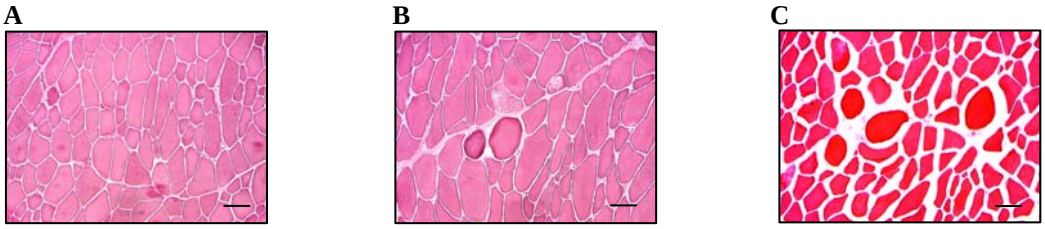

Figure 2. Samples from one and the same pig. (A) 2 min after biopsy (B) incubated 60 min at 37°C after biopsy (C) carcass 24 h *postmortem* (bar = 100 µm).

Repeatability of measurements and comparison of biopsy and carcass samples. In 12 Piétrain pigs the abundance of giant fibres was analyzed in sections of 3 simulated biopsy samples and 3 carcass samples of one and the same pig to assess the repeatability of measurements by estimation of intra-class correlation coefficients. The ICC for the number of giant fibres/cm² was lower for biopsy samples ($\hat{g}$ =0.42) than for carcass samples ($\hat{g}$ =0.69). The repeatability of measurements can be improved by analyzing more than one sample, e.g. 3 as derived from $\hat{g}_3$ =0.69 and 0.87 for biopsy and carcass samples, respectively. Consequently, the analysis of at least 3 muscle samples is necessary to give a reliable estimation for the measurement of giant fibres per unit area in longissimus muscle cross sections. The variability of the average percentage of giant fibres was higher in simulated biopsy samples (0.54±0.59%) than in carcass samples (0.31±0.20%). There was no significant correlation of the giant fibre percentage between biopsy and carcass samples. In conclusion, the environmental conditions represented by the isolation of the sample from the body and subsequent in vitro incubation may cause metabolic changes that are not fully comparable with the *postmortem* conditions in the carcass.

Ultrastructural properties of „simulated" giant fibres. Giant fibres from carcass samples have been characterized previously to exhibit marked changes in ultrastructural properties. In giant fibres of biopsy samples exposed to simulated *postmortem* conditions damages of cell organelles that are essential for the functional and structural integrity of the myofibre were clearly apparent (images not shown). Parts of the sarcolemma of giant fibres showed an irregular, wavelike surface structure, whereas normal myofibres exhibited an even, unruffled membrane. The appearance of mitochondria within giant fibres was changed in that the *cristae mitochondriales* were arranged irregularly instead of parallel to each other or were even disintegrated to different degrees. Myofibrillar structure was partly loosened, which was associated with a loss of the hexagonal array of the myofilaments evident in unaffected parts of giant fibres or in normal fibres. The sarcoplasmatic reticulum consisting of a branched tubular system within the myofibres was changed in that enlarged and swollen tubular channels were apparent. Altogether, giant fibres developed *in vitro* from muscle biopsy samples exhibited ultrastructural properties similar to those detected previously in giant fibres obtained from carcass samples.

Conclusions

The incidence of the giant fibre syndrome in *postmortem* skeletal muscle is a common phenomenon in recent German pig breeds. Giant fibres can be developed from biopsy samples by simulating *postmortem* conditions *in vitro*. Those fibres exhibit morphological and ultrastructural changes, which are typical for giant fibres in carcass samples obtained *postmortem*. The incidence of giant fibres can be measured in *longissimus* muscle with sufficient repeatability using three to four samples per pig. The suitability of the trait giant fibre percentage in 'simulated' biopsy samples to predict pork quality remains to be investigated.

References

Hennebach, H., Albrecht, V., Lengerken, G. V., & Pfeiffer, H. (1980) Investigations into the prediction of meat quality by means of muscle biopsy on the live pig. 3. The pH value of muscle samples 45 minutes after shot biopsy (biopsy-pH_{45} or simulated pH_{45}) and its correlation to postmortem meat quality. *Arch. Anim. Breed. 23*, 183-194.

Lahucky, R., Fischer, K., & Augustini, C. (1982) Predicting meat quality in the live pig by shot biopsy. *Fleischwirtsch. 62*, 1323-1326.

Rasch, D. (1983). *Einführung in die Biostatistik*. Berlin: VEB Deutscher Landwirtschaftsverlag.

Rehfeldt, C., Adamovic, I., & Kuhn, G. (2007) Effects of dietary daidzein supplementation of pregnant sows on carcass and meat quality and skeletal muscle cellularity of the progeny. *Meat Sci. 75*, 103-111.

Rehfeldt, C., Henning, M., & Fiedler, I. (2008) Consequences of domestication on pig skeletal muscle cellularity and growth of myogenic satellite cells. *Livest. Sci. 116*, 30-41.

Wegner, J. & Schöberlein, L. (1984) Suitability of shot bioptate for morphometric and histochemical examination of muscular tissue. *Mh. Vet.-Med 39*, 665-667.