Extent of cytoskeletal protein degradation is related to pH decline and water holding capacity in porcine longissimus muscle.

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Introduction

Accelerated pH decline and low ultimate pH are related to the development of low water-holding capacity and unacceptable high purge loss. The source of drip from pork is intracellular water which is lost from muscle fibre postmortem driven by a pH and calcium-induced shrinkage of myofibrils during rigor development. A prerequisite to translate shrinkage of myofibrils into shrinkage of the whole cell is an intact cytoskeleton (Offer and Cousins 1992) which is a highly complex network consisting of a large numbers of connections between myofibrils and between peripheral myofibrils and the sarcolemma. The rate and degree of cytoskeletal protein degradation postmortem (**PM**) might be responsible for the extent of cell shrinkage and water-holding capacity (Kristensen and Purslow 2001; Rowe et al. 2001a; Bee et al. 2004). The aim of the present study was to monitor proteolysis of the intermediate filament protein desmin and the costameric protein talin during storage in selected porcine longissimus muscles (**LM**) known to markedly differ in water holding capacity. Because desmin and talin are substrate of the enzyme μ-calpain relative calpain activity was determined using casein zymography.

Materials and Methods

Twenty-four Swiss Large White gilts of similar age and slaughter weight were used in this study. At slaughter the pH and temperature of LM were monitored 30 min, 1.5, 4, and 24 h PM, using a WTW pH meter (WTW pH196-S, Weilheim, Germany) equipped with a WTW electrode (WTW Eb4, Weilheim, Germany) and a temperature probe. Sets of measurements were obtained at different locations at the 13^{th} (pH) and 12^{th} rib (temperature), by insertion of the pH and temperature probe between the ribs from the inside of the left carcass side. One day after harvest, four chops (1 cm each) of the LM at the 13^{th} rib level were removed from the left side of each carcass and drip loss and color were determined. Drip loss was measured as the amount of purge resulting during the storage of the chop for 24 and 48 h at 2°C (Honikel 1998). Following a 10-min bloom period, L* (higher value indicates a lighter color), a* (higher value indicates a redder color), and b* (higher

value indicates a more yellow color) values of the LM were measured using a Minolta Chroma Meter (CR-300; Minolta, Dietikon, Switzerland) and illuminant D_{65}. Three replicate measures were performed on each muscle sample, resulting in six measurements per muscle.

Whole muscle protein extracts were prepared from LM samples collected 30 min, 1.5, 4, and 24 h PM and from the 24 (48 h PM) and 48 h (72 h PM) drip loss samples for Western blotting of desmin and talin as described by Melody et al. (2004). Immunoblotting and chemiluminescent detection were done as described earlier (Huff-Lonergan et al. 1996). Primary antibodies included polyclonal rabbit anti-human desmin (No. V2022; Biomeda, Foster City; diluted 1:20,000) and monoclonal mouse anti-talin (clone 8D4; Sigma; diluted 1:1,000). Secondary antibodies included goat anti-rabbit peroxidase conjugate (No. A 9169; Sigma; diluted 1:20,000 for desmin) and anti-mouse peroxidase conjugate (No. A 2554, Sigma; diluted 1:10,000 for talin). Densities of immunoreactive bands were quantified by densitometry using GeneGnome (Syngene, Cambridge, UK) and Gene Tools Analysis Software (Version 3.02; Syngene). Desmin and talin proteolysis were indicated by a decrease in intensity of the 55 and 225 kDa bands, respectively. Intact protein degradation ratio at 1.5, 4, 24, 48, and 72 h PM was calculated as the intensity of the immunoreactive protein band of the respective 30 min PM LM sample. Differences in μ-calpain activity (determined as [average optical density]$^{-1}$) and autolysis were determined in the sarcoplasmic fraction of the LM samples at each time point using casein zymography. Sarcoplasmic protein extraction and the casein zymography procedure was conducted according to the procedures described by Melody et al. (2004).

Results and Discussion

Based on the results obtained from the 24 h drip loss, LM samples were divided into the following three drip loss classes: low (**L** = ≤ 6%; n = 9), medium (**M** = > 6 and ≤ 10%; n = 6), and high (**H** = > 10%; n = 9). Twenty-four and 48 h drip loss was on average 3.5 and 4.4%, 7.1 and 8.3%, and 11.6 and 13.3% in the L-, M-, and H-group, respectively. Compared to group H, pH values were higher ($P < 0.05$) in the L-group at 1.5 h PM (L: 5.9 and H: 5.6) but did not differ ($P > 0.05$) at 4 h PM (L: 5.8 and H: 5.6). For the M-group intermediate pH values were observed at 1.5 h PM (5.7) whereas at 4 h PM the pH values (5.6) were similar ($P > 0.05$) to the pH values of the H- but lower ($P < 0.05$) than the pH values of the L-group. Neither at 30 min (6.2 for all) nor at 24 h PM (5.4 for all) pH values differed among the drip loss classes. The LM of the L- (L*: 48.7 and b*: 2.4) and M-group (L*: 50.3 and b*: 2.6) was darker and less yellow ($P < 0.05$) than the LM of the H-group (L*: 52.8 and b*: 3.5). The LM of the H-group (a*: 7.8) was redder ($P < 0.05$) than the LM of the M-group (a*: 6.5) with intermediate values for the L-group (a*: 6.7). Twenty-four and 48 h drip loss was correlated ($P < 0.01$) with lightness (0.88 and 0.90), redness (0.52 and 0.43), yellowness

(0.69 and 0.63), and with 1.5 h pH (-0.60 and –0.62). These results suggest that a fast pH decline early PM results in distinctly higher drip loss and have detrimental effects also on meat colour. Because lower pH values early PM are related to faster lactic acid build-up the differences in pH decline between the drip loss classes could be caused by an increased glycolysis and/or glycogenolysis rate combined with higher at death glycogen concentration (Bee 2002).

Regardless of the drip loss classes the extent of proteolysis PM between desmin (Figure 1) and talin (Figure 2) markedly differed. Compared to 30 min PM, at 72 h intact desmin was less ($P < 0.05$) degraded than intact talin. Neither desmin nor talin proteolysis differed between drip loss classes. However, the extent of desmin proteolysis at 1.5 (r = -0.55; $P < 0.01$), 4 (r = -0.38; $P = 0.08$), and 24 h (r = -0.40; $P = 0.06$) was negatively correlated with pH values measured at 24 h PM. These results might indicate that LM with higher ultimate pH tended to have greater desmin degradation which coincide with results reported by Rowe et al. (2001a). In agreement with previous observations (Bee et al. 2004) drip loss at 48 h was positively correlated with talin proteolysis at 48 (r = 0.38; $P = 0.08$) and 72 h PM (r = 0.41; $P = 0.05$). The LM samples of the L-group had greater ($P < 0.05$) μ-calpain activity at 30 min and at 24 h PM than the LM of the H-group (Figure 3). Furthermore, in six out of nine samples of the H-group autolysis product appeared as early as 2 h PM, which concurs with the observed earlier loss of μ-calpain activity. The present findings are in agreement with results pre-

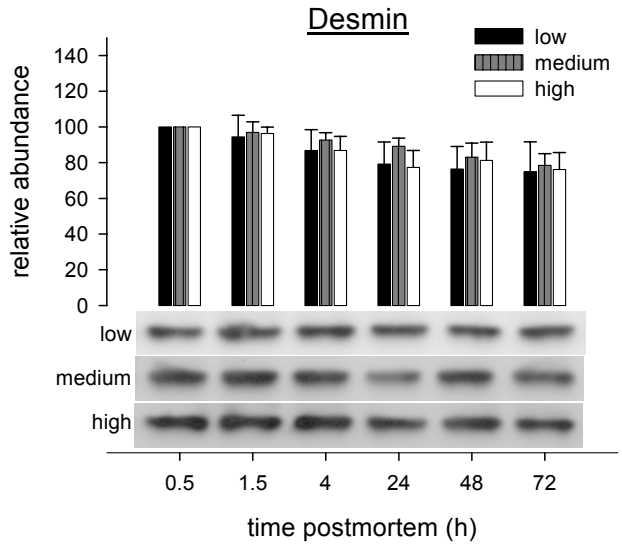

Figure 1 Relative abundance of intact desmin determined 1.5, 4, 24, 48, and 72 h postmortem in the LM differing in the amount of drip loss after 24 h (L: ≤ 6%; M: > 6 and ≤ 10%; H: > 10%). Immunoblots showing the intact desmin bands (55 kDa) at the various time points PM of representative samples from each drip loss class.

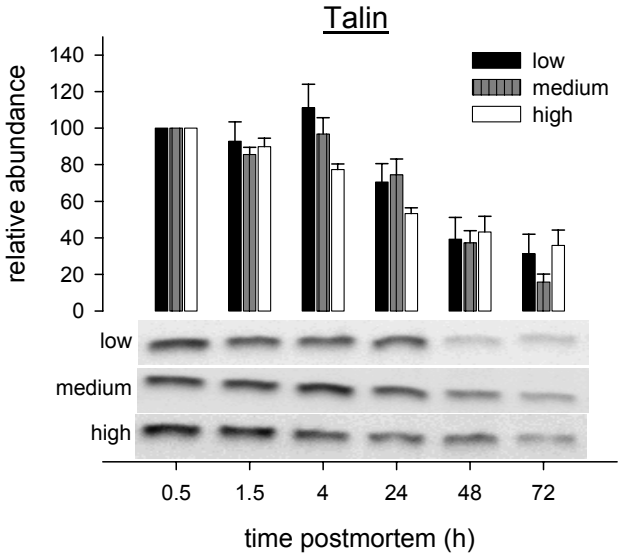

Figure 2 Relative abundance of intact talin determined 1.5, 4, 24, 48, and 72 h postmortem in the LM differing in the amount of drip loss after 24 h (L: ≤ 6%; M: > 6 and ≤ 10%; H: > 10%). Immunoblots showing the intact talin bands (225 kDa) at the various time points PM of representative samples from each drip loss class.

sented by O'Halloran et al. (1997) and Rowe et al. (2001b) who reported greater loss of μ-calpain activity and earlier appearance of autolysis in LM samples with rapid glycolysis and accelerated pH decline. In the present study proteolysis of desmin and talin did not differ among the three drip loss classes. However, the significant relationship between the extents of talin proteolysis with 48 h drip loss indicates that talin degradation might play a pivotal role in water holding capacity.

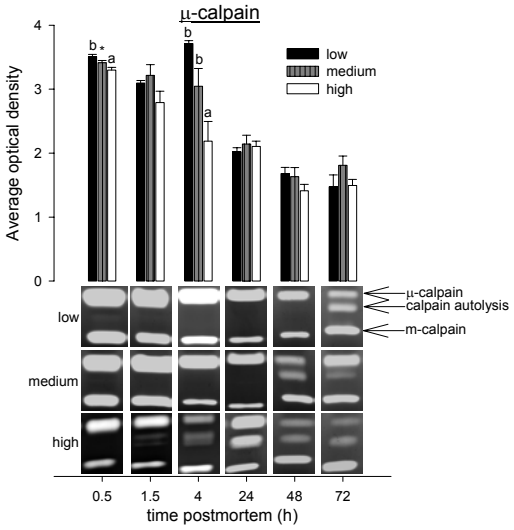

Figure 3 Casein zymography gels depicting μ-calpain, autolysis products and m-calpain activity in sarcoplasmic extracts of the LM at 0.5, 1.5, 4, 24, 48, and 72 h postmortem. Different superscripts denote differences within time point ($P < 0.05$).

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