# 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 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 might be responsible for the extent of cell shrinkage and water-holding capacity.

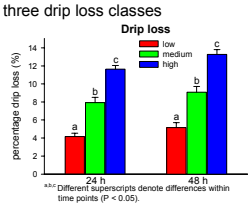
The aim of the present study was to monitor proteolysis of the intermediate filament protein **desmin** and the costameric protein **talin** during storage in selected porcine longissimus muscles (**LM**) known to markedly differ in water holding capacity. Because desmin and talin are substrate of the enzyme μ-calpain relative calpain activity was determined using casein zymography.

## Material and Methods

### *Sample collection*

Based on the 24 h drip loss (48 h postmortem), LM samples collected from 24 Swiss Large White gilts were grouped into three drip loss classes

- **low: ≤ 6%** (n = 9)
- **medium: > 6 and ≤ 10%** (n = 6)
- **high: > 10%** (n = 9).




These samples and LM samples collected 0.5, 2, 4, and 24 h postmortem as well as 48 h drip loss (72 h postmortem) were used to monitor desmin and talin degradation by immunoblotting and μ-calpain activity by casein zymography. Whole-muscle, sarcoplasmic protein extraction, SDS-PAGE, and non-denaturing gel sample preparation were carried out according to Lonergan et al. (2001) and Melody et al. (2004).

### *SDS-PAGE and Immunoblotting*

A 10% polyacrylamide separating gel (acrylamide:N,N`-bis-methylene acrylamide = 37.5:1 [wt/wt], 0.1% [wt/vol] SDS, 0.5% [vol/vol] N`N`N`N`-tetramethylethylenediamine [TEMED], 0.05% [wt/vol] ammonium persulfate [APS], and 500 m*M* Tris-HCl, pH 8.8) was used for determination of desmin. A 8% polyacrylamide gel (acryl-amide:N,N`-bis-methylene acrylamide = 37.5:1 [wt/wt], 0.1% [wt/vol] SDS, 0.5% [vol/vol] TEMED, 0.05% [wt/vol] APS, and 500 m*M* Tris-HCl, pH 8.8) was used for determination of talin.

### *Running conditions*

Gels (10 cm wide x 8 cm tall) for analysis of desmin and talin degradation were run on SE 280 Tall Mighty Small electrophoresis units (Hoefer Scientific Instruments, San Francisco, CA). The running buffer contained 25 m*M* Tris, 192 m*M* glycine, and 0.1% [wt/vol] SDS. Gels were loaded with 30 and 120 mg per lane of total protein for desmin and talin, respectively and run at a constant voltage of 120 V or overnight at 30 V.

### *Transfer conditions*

Gels were transferred to polyvinylidene difluoride (PVDF) membranes at a constant voltage of 90 V for 1.5 h for desmin and at a constant ampere of 1 A for 4 h for talin. The transfer buffer consisted of 25 m*M* Tris, 192 m*M* glycine, 2m*M* EDTA, and 15% (desmin) or 10% (talin) [vol/vol] methanol. The temperature of the transfer buffer was maintained between 0 (desmin) and -10°C (talin) using a refrigerated circulating propylengycole bath.

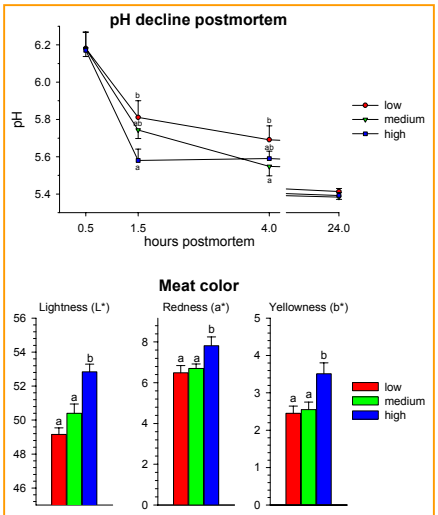
### *Immunoblotting*

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 Chemical Company; 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 degradation 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 postmortem was calculated as the intensity of the immunoreactive protein band in each LM sample at 0.5 h postmortem.

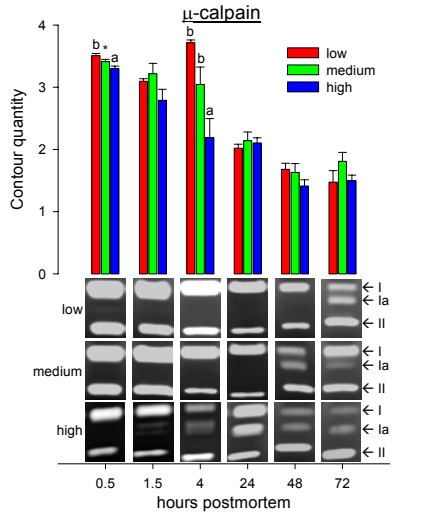
### *Casein zymography*

12.5% non-denaturing acrylamide gels containing casein were used on the Bio-Rad Mini Protean 3 Cell gel system (Bio-Rad Laboratories, Hercules, CA) for analysis. The separating gels were composed of acrylamide (acrylamide: N-N-bis-methylene acrylamide = 37.5:1 [wt/wt]), 0.05% (wt/vol) APS, 0.05% (vol/vol) of TEMED, casein (2.1 mg/ml), and 0.375 *M* Tris-HCl, pH 8.8. The running buffer used was composed of 192 m*M* Glycine, 1 m*M* EDTA, 0.1% (vol/vol) 2-MCE, 25 m*M* Tris-HCl, pH 8.3. All electrophoresis was done at 4°C. All gels were loaded with 120 μg of protein from each sample. Gels were run at 100 V for approximately 24 h. Subsequently, gels were incubated at room temperature in 5 m*M* $CaCl_2$, 0.1% (vol/vol) Mercaptoethanol, 50 m*M* Tris-HCl, pH 7.5, for three 20 minute incubation periods and then fresh buffer was added and gels were incubated overnight. At the end of the incubation, gels were placed into 0.1% (wt/vol) Commassie brilliant blue R-250, 40% (vol/vol) methanol, 7% (vol/vol) glacial acetic acid in water, for 1 h. Gels were then destained in the same solution minus Commassie brilliant blue R-250.

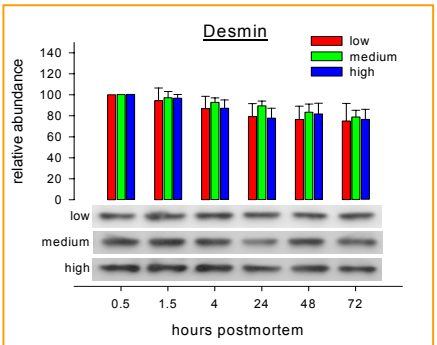
Densitometry scans of the gels were performed using the GS-710 densitometer (Bio-Rad). The contour quantity (pixel intensity x $mm^2$) of the μ-calpain and μ-calpain autolysis product were determined with the Quantity One software (Bio-Rad).



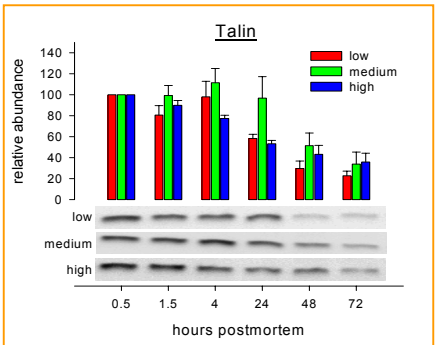

PH values determined 0.5, 1.5, 4, and 24 h postmortem and meat color (L*,a*, b*) measured 24 h postmortem in the LM. Grouping of the LM samples is based on the amount of drip loss determined after 24 h (**low: ≤ 6%**; **medium: > 6 and ≤ 10%**; **high: > 10%**). Different superscripts denote differences within time points and color parameters ($P < 0.05$).



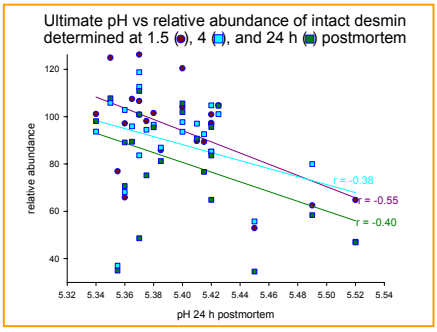

Casein zymography gels depicting μ-calpain (← **I**), autolysis product (← **Ia**) and m-calpain (← **II**) activity in sarcoplasmic extracts of the LM at 0.5, 1.5, 4, 24, 48, and 72 h postmortem. The grouping of the LM samples is based on the amount of drip loss determined after 24 h (**low: ≤ 6%**; **medium: > 6 and ≤ 10%**; **high: > 10%**). Different superscripts denote differences within time points ($P < 0.05$).

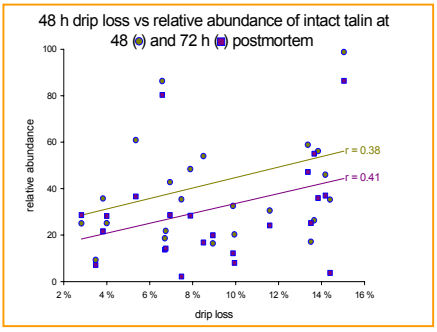



Relative abundance of intact desmin (expressed as percent of the abundance at 0.5 h postmortem of each sample) determined 1.5, 4, 24, 48, and 72 h postmortem in the LM differing in the amount of drip loss after 24 h (**low: ≤ 6%**; **medium: > 6 and ≤ 10%**; **high: > 10%**). Immunoblots showing the intact desmin bands (55 kDa) at the various time points postmortem of representative samples from each drip loss class.




Relative abundance of intact talin *(expressed as percentage of the abundance at 0.5 h postmortem of each sample)* determined 1.5, 4, 24, 48, and 72 h postmortem in the LM differing in the amount of drip loss after 24 h (**low: ≤ 6%**; **medium: > 6 and ≤ 10%**; **high: > 10%**). Immunoblots showing the intact talin bands (225 kDa) at the various time points postmortem of representative samples from each drip loss class.




## Conclusions

- Compared to group **H**, pH values were higher in the **L**-group at 1.5 h postmortem with intermediate values for the **M**-group. These results confirm that a slow pH decline early postmortem positively affect water holding capacity of the LM.
- The activity of μ-calpain early postmortem was lower and autolysis product appeared earlier in the **H**- compared to the **L**-group, which could be caused by the faster pH decline and earlier inactivation of the protease.
- Compared to **desmin** degradation of **talin** occurred at a faster rate**.**
- Degradation of **desmin** and **talin** did not differ among the three drip loss classes. ***However, the significant relationship between the extent of talin proteolysis with 48 h drip loss indicates that talin degradation might play a pivotal role in water holding capacity.***

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