Available online at www.sciencedirect.com
ScienceDirect
Meat Science 76 (2007) 359–365
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Rate and extent of pH decline affect proteolysis of cytoskeletal proteins and water-holding capacity in pork

Giuseppe Bee [b], Abbey L. Anderson [a], Steven M. Lonergan [a], Elisabeth Huff-Lonergan [a,*]

[a] Department of Animal Science, Iowa State University, 2278 Kildee Hall, Ames, IA 50011, United States
[b] Agroscope Liebefeld-Posieux, Swiss Federal Research Station for Animal Production and Dairy Products (ALP), Posieux 1725, Switzerland

Received 31 July 2006; received in revised form 5 December 2006; accepted 7 December 2006

Abstract

The objective of this study was to determine the extent to which early postmortem (PM) pH decline influences proteolysis of the intermediate filament protein desmin, the costameric proteins vinculin and talin and autolysis of μ-calpain in the longissimus muscle (LM) of pigs from two genetic lines. Based on the LM 3 h pH (H = 3 h pH of LM > 6.0; L = 3 h pH of LM pH < 5.7) PM, 10 carcasses per line and pH group were selected. The average 3 h pH within pH group was 6.23 (H) and 5.44 (L). The LM samples were collected 24, 48, 72, and 120 h PM and percent drip loss was measured after 1, 2, and 4 d of storage. Samples collected at 24, 48, 72, and 120 h PM were used to monitor desmin, vinculin, and talin degradation and samples collected at 24 h PM were used to determine the extent of μ-calpain autolysis by immunoblotting. Higher ($P < 0.01$) pH values at 45 min, 6 h, and 24 h PM and lower ($P < 0.01$) drip losses after 1, 2, and 4 d of storage were recorded in the H-compared to the L-group. Abundance of the 76 kDa μ-calpain autolysis product was greater ($P < 0.01$), proteolysis of talin at all measured time points and proteolysis of desmin after 24 and 48 h PM was greater ($P \leqslant 0.03$) in the H-group than in the L-group. The current findings indicate activation rate of μ-calpain may be associated with proteolysis of desmin and talin and could play a role in the development of drip loss. The rate of early PM pH decline can partly explain the variation of desmin and talin degradation by affecting the activation of μ-calpain.

Keywords: μ-Calpain; Desmin; pH; Talin; Vinculin; Water-holding capacity

1. Introduction

Variation in pork quality attributes such as water-holding capacity and tenderness continues to reduce value of fresh pork. While it is clear that pH and temperature decline influence fresh pork quality, identification of undefined sources of variation contributing to poor quality is required to provide opportunities to improve pork quality. Postmortem changes in muscle proteins contribute to fresh meat quality. Myofibrillar fragmentation has long been associated with improved tenderness of meat. A current hypothesis proposes that proteolysis of key muscle proteins (including desmin, vinculin and talin) minimizes the loss of water-holding capacity (Huff-Lonergan & Lonergan, 2005; Melody et al., 2004; Morrison, Mielche, & Purslow, 1998) caused by lateral shrinkage of myofibrils in postmortem muscle (Diesbourg, Swatland, & Millman, 1988). In contrast, degradation of membrane proteins such as integrin has been associated with larger drip channel size (Lawson, 2004) and increased drip loss (Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006). The proteolysis of muscle proteins that contribute to these fresh pork characteristics (including desmin, talin, and vinculin) is most frequently attributed to the calcium dependent proteinase, μ-calpain (Koohmaraie, 1992). It is therefore proposed that activation of μ-calpain is expected to predict variation in fresh pork water-holding capacity and tenderness. In addition

* Corresponding author. Address: Department of Animal Science, Iowa State University, 2278 Kildee Hall, Ames, IA 50011, United States. Tel.: +1 515 294 9125; fax: +1 515 294 9143.
E-mail address: elonerga@iastate.edu (E. Huff-Lonergan).

0309-1740/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2006.12.004

to calcium concentration, μ-calpain activation is regulated by pH (Huff-Lonergan et al., 1996), calpastatin (Maddock, Huff-Lonergan, Rowe, & Lonergan, 2005), and oxidation (Carlin, Huff-Lonergan, Rowe, & Lonergan, 2006). Because pH can clearly influence the rate and extent of activation, autolysis, and autolytic inactivation of μ-calpain, the objective of these experiments was to determine the extent to which early postmortem (PM) pH decline influences proteolysis of the intermediate filament protein desmin, the costameric proteins vinculin and talin, and autolysis of μ-calpain in the longissimus muscle (LM) of pigs from two genetic lines.

2. Materials and methods

2.1. Animals and collection of tissue samples

In this study, 309, commercial pigs from two genetic lines (A and B) were slaughtered at a commercial slaughter facility on the same day. These pigs did not carry the mutations in the PRKAG3 and ryanodine receptor loci that are associated with poor pork quality. The pH of the longissimus muscle (LM) from the right side of each carcass was monitored 45 min, 3, 6, and 24 h PM using a pH-Star S meter (SFK Technology Inc., Herlev, Denmark) equipped with a glass tipped probe (Mettler-Toledo Process Analytical Inc., Wilmington, MA). The pH electrode was calibrated at the appropriate temperature fro each time point and its calibration was checked periodically. Based on the 3 h pH in the LM, 10 carcasses with a high (H = pH > 6.0) and 10 carcasses with a low pH (L = pH < 5.7) were selected within line for continued analysis. Loin pH was measured at 6 h PM and 24 h PM. Loins were vacuum-packaged at 24 h PM and immediately transported to the Iowa State University Meat Science Laboratory.

2.2. Drip loss analysis

After transport, nine 2.54-cm thick boneless chops were removed from each loin at 1 d PM and the initial weight was recorded for determination of drip loss. Chops were stored in a sealed plastic bag under atmospheric pressure at 4 °C. After 1 (48 h PM), 2 (72 h PM), and 4 d (120 h PM) of storage, three chops for each time point were blotted, reweighed (final weight), and drip loss percentage was calculated (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001). Muscle samples were then vacuum-packaged and stored at −20 °C until further analysis.

2.3. Whole-muscle sample preparation and SDS–PAGE gel sample preparation

Vacuum-packaged muscle samples stored at −20 °C from the four time points (24, 48, 72 and 120 h PM) were used for Western blotting of desmin, vinculin, and talin and 24 h PM samples were also used for Western blotting of μ-calpain. Whole-muscle protein extraction and SDS-PAGE gel sample preparation was conducted according to Lonergan et al. (2001). The solubilized protein content of the supernatant was determined according to Lowry, Rosebrough, Farr, and Randall (1951) using premixed reagents (Bio-Rad Laboratories, Hercules CA). Gel samples were frozen at −80 °C until analysis.

2.4. SDS-PAGE and Western blotting

Gel samples were thawed and run on polyacrylamide separating gels (acrylamide: *N*,*N*′-bis-methylene acrylamide = 100:1 [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 mM Tris–HCl, pH 8.8) for determination of desmin (10% acrylamide), vinculin (10% acrylamide), talin (6.5% acrylamide) degradation, and μ-calpain autolysis (9% acrylamide), respectively. A 5% polyacrylamide gel (acrylamide: *N*,*N*′-bis-methylene acrylamide = 100:1 [wt/wt], 0.1% [wt/vol] SDS, 0.125% [vol/vol] TEMED, 0.075% [wt/vol] APS, and 125 mM Tris–HCl, pH 6.8) was used for the stacking gel.

2.4.1. Running conditions

Gels (10 cm wide × 12 cm tall × 1.5 mm thick) for analysis of desmin and talin degradation were run on SE 280 Tall Mighty Small electrophoresis units (Hoefer Scientific Instruments, San Francisco, CA) and for analysis of vinculin degradation and μ-calpain autolysis were run on SE 260 Tall Mighty Small electrophoresis units (Hoefer Scientific Instruments; 10 cm wide × 10 cm tall × 1.5 mm thick). The running buffer contained 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 0.1% [wt/vol] SDS. Gels were loaded with 30, 80, 120, and 120 μg per lane of total protein for desmin, vinculin, talin, and μ-calpain, respectively, and run at a constant voltage of 120 V.

2.4.2. Transfer conditions

Gels were transferred to polyvinylidene difluoride membranes (PVDF; NEN Life Science Products Inc., Boston, MA) at a constant voltage setting of 90 V for 1.5 h for desmin, vinculin, μ-calpain and at a constant amperage setting of 1A for 4 h for talin using a TE22 Mighty Small Transphor or a TE62 Transphor electrophoresis unit (Hoefer Scientific Instruments), respectively. The transfer buffer consisted of 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 15% (desmin, vinculin, and μ-calpain) or 10% (talin) [vol/vol] methanol. The temperature of the transfer buffer was maintained at 0 °C for desmin, vinculin, and μ-calpain and at −10 °C for talin using a refrigerated circulating ethylene glycol (50% [vol/vol]) bath (Ecoline RE106; Lauda Brinkmann, Westbury, NY).

2.4.3. Immunoblotting

Immunoblotting and chemiluminescent detection were done as described earlier (Melody et al., 2004). Primary antibodies included polyclonal rabbit anti-desmin (No. V2022; Biomeda, Foster City, CA; diluted 1:10,000),

monoclonal anti-vinculin (No. V9131; Sigma–Aldrich Inc., St. Louis, MO; diluted 1:10,000), monoclonal anti-μ-calpain (No. MA3-940; Affinity BioReagents, Golden, CO), and monoclonal anti-talin (clone 8D4; Sigma–Aldrich; diluted 1:1,000). Secondary antibodies included goat anti-rabbit horseradish peroxidase (HRP) (No. A 9169; Sigma–Aldrich; diluted 1:10,000 for desmin), sheep anti-mouse HRP (No. Na931; Amersham Pharmacia Biotech, Piscataway, NJ) diluted 1:5,000 for vinculin, talin, and μ-calpain. Desmin, vinculin, and talin degradation were indicated by a decrease in intensity of the 53, 120, and 225 kDa bands, respectively. Intact desmin (53 kDa), vinculin (120 kDa), and talin (225 kDa) degradation ratio was calculated as the intensity of each immunoreactive desmin, vinculin, and talin band over the intensity of the respective protein bands in a reference sample (porcine LM sampled 45 min PM) that was loaded on each gel. For μ-calpain autolysis within sample, percentages of the unautolyzed 80 kDa, the autolyzed 78, and 76-kDa band intensity were calculated as a percentage of the total peak area of calpain bands in each lane.

2.5. Statistical analysis

Data were analyzed with the MIXED procedure of SAS (Version 8.02, SAS Institute, Cary, NC, USA). The model used for the analyses of drip loss percentage, intact desmin, vinculin, and talin degradation at each time point PM as well as μ-calpain autolysis at 24 h PM included genetic line (line), pH group (pH), and line × pH interactions as fixed effects. The course of the pH decline in the LM was analyzed as repeated measurements with line, pH, time, and the two- and three-ways interactions as fixed effects using the MIXED procedure (Littell, Henry, & Ammerman, 1998). Least squares means were calculated and considered statistically significant at $P \leqslant 0.05$.

3. Results

The pH-values 3 h PM of the selected loins were on average 0.7 to 0.8 units lower in the L-group (3 h longissimus dorsi pH <5.7) compared to the H-group (3 h longissimus dorsi pH >6.0) (Table 1). Across pH groups, the values 45 min, 3, and 6 h PM did not differ ($P = 0.09$) between genetic lines, whereas ultimate pH was lower (line × time interaction; $P < 0.01$) in line A (5.60) compared to line B (5.76). Regardless of the genetic line, muscle pH was lower ($P < 0.01$) at all time points in the carcasses of the L- than the H-group. The course of pH decline did differ between the pH groups (pH × time interaction; $P > 0.05$). From 45 min to 3 h PM, pH markedly decreased by 0.52 units, remained unchanged from 3 to 6 h, and then slightly increased by 0.11 units from 6 to 24 h PM in the L-group. By contrast, in the H pH group, 3 h pH did not differ from that determined at 45 min, however, it decreased by 0.41 units from 3 to 6 h PM and remained unchanged at 24 h PM. Since all pigs were slaughtered on the same day it is unlikely that this difference in pH decline is systematic, rather, it may point to some biological variation that needs to be explored more deeply.

Line A had higher ($P < 0.01$) drip loss after storage for 2 and 4 d, but not after 1 d, compared to line B (Fig. 1). At all three time points, chops from the loins selected based on the low 3 h pH had 1.49–1.73% higher drip loss ($P < 0.01$) compared to loins of the H-group.

Abundance of intact desmin (53 kDa) in the LM was lower ($P \leqslant 0.03$) at 24 and 48 h and tended to be lower ($P = 0.06$) at 120 h PM in the H- compared to the L-group, indicating a higher degradation of desmin in the H-group at those time points (Table 2). Compared to line A, more desmin was degraded at 72 ($P < 0.09$) and 120 h PM ($P < 0.01$) in line B, but no line differences ($P \geqslant 0.44$) were observed at 24 and 48 h PM. The degradation of intact vinculin was not affected by genetic line or pH group. Detection of intact talin was lower ($P \leqslant 0.03$) at all time points (24, 48, 72, and 120 h PM) in the H-group compared to the L-group, indicating greater proteolysis in the H-group. At 48 and 72 h PM talin was degraded to a greater ($P \leqslant 0.07$) extent in line A compared to line B whereas no line differences ($P \geqslant 0.42$) were found at 24 and 120 h PM.

Abundance of intact desmin and talin at 48, 72, and 120 h PM was positively ($P < 0.05$) correlated with drip loss after 1, 2, and 4 d of storage, indicating that a high abundance of the intact cytoskeletal proteins was associated with higher drip losses (Table 3) compared to samples that had a low abundance of intact cytoskeletal proteins. By contrast, intact vinculin intensity at 48 h PM was nega-

Table 1
Decline of pH in the longissimus muscle from pigs of two genetic lines and selected for high and low 3 h pH postmortem (PM)

Time PM	pH group[a]				*P*-value[b]			
	Line A		Line B					
	H	L	H	L	SEM	Line	pH	Time
45 min	6.27	6.02	6.21	5.91				
3 h	6.19	5.41	6.27	5.47	0.042	0.09	<0.01	<0.01
6 h	5.78	5.47	5.86	5.49				
24 h	5.66	5.54	5.86	5.65				

[a] pH classification H = pH > 6.0 at 3 h PM and L = pH < 5.7 at 3 h PM.
[b] Probability values for genetic line (Line), 3 h pH PM classification (pH), and time PM (Time).

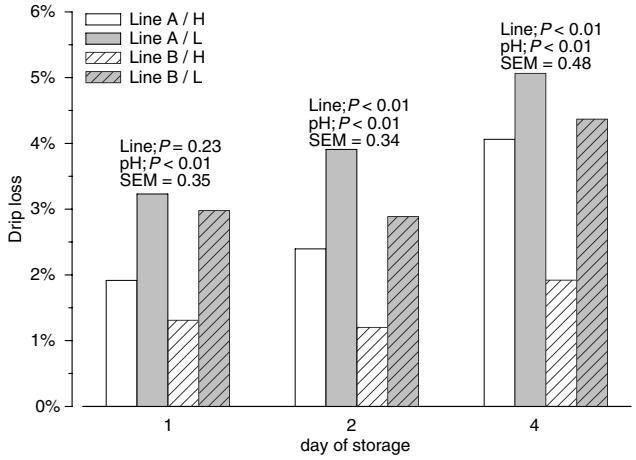

Fig. 1. Drip loss from longissimus dorsi muscle chops from carcasses from two genetic lines (lines A and B) and selected based on 3 h pH postmortem (high [H] >6.0 and low [L] <5.7). Probability (P) values for the main effects of genetic line (line) and pH group (pH) and SEM are presented for each time point. Drip loss determined as described by Lonergan et al. (2001).

Table 2
Effect of the genetic line and 3 h pH on degradation of intact desmin, vinculin, and talin determined in the longissimus dorsi muscle 24, 48, 72, and 120 h postmortem (PM)[a]

	pH group[b]				P-value[c]		
	Line A		Line B				
	H	L	H	L	SEM	Line	pH
Desmin							
24 h PM	92.3	108.4	80.1	110.7	6.35	0.44	<0.01
48 h PM	80.5	98.5	76.1	93.0	7.87	0.54	0.03
72 h PM	70.2	84.8	59.8	68.3	7.74	0.09	0.14
120 h PM	56.4	74.7	26.6	45.6	9.83	<0.01	0.06
Vinculin							
24 h PM	100.5	104.7	95.0	98.1	8.93	0.50	0.69
48 h PM	92.2	86.8	98.5	80.6	7.52	0.99	0.13
72 h PM	67.7	80.6	79.6	71.8	9.28	0.87	0.78
120 h PM	89.1	98.8	77.8	65.3	17.27	0.20	0.94
Talin							
24 h PM	56.9	109.8	46.1	152.1	19.42	0.42	<0.01
48 h PM	24.2	63.9	39.5	122.4	16.80	0.03	<0.01
72 h PM	12.8	64.0	13.1	19.5	14.39	0.07	0.03
120 h PM	1.4	39.2	0.6	20.8	12.09	0.44	0.02

[a] Intact desmin (53 kDa), vinculin (120 kDa), and talin (225 kDa) degradation ratios were calculated as the intensity of each immunoreactive desmin, vinculin, and talin band over the intensity of the respective immunoreactive desmin, vinculin and talin bands in a reference sample (whole muscle protein extract of a porcine longissimus dorsi muscle sample collected 45 min PM) that was loaded on each gel.

[b] pH classification H = pH > 6.0 at 3 h PM and L = pH < 5.7 at 3 h PM.

[c] Probability values for genetic line (Line) and 3 h pH PM classification (pH).

tively correlated with drip loss after 1 d of storage whereas no ($P > 0.05$) correlations were found at 2 and 4 d of storage. The positive correlation between intact desmin and intact talin abundance from 24 to 120 h PM suggests that during aging, degradation of desmin parallels degradation of talin. Intact desmin and talin were positively ($P < 0.05$) correlated with intact vinculin abundance at 120 h PM but not between 24 and 72 h PM.

Table 3
Pearson correlation coefficients between relative intact desmin, vinculin, and talin immunoreactive band abundance at 48, 72, and 120 h postmortem (PM) and drip loss percentage determined after 1, 2 and 4 d of storage[a,b]

	Drip loss (d of storage)[c]		
	1	2	4
Desmin	0.41**	0.45**	0.42**
Vinculin	−0.36*	−0.04	0.07
Talin	0.35*	0.60**	0.51**

[a] Significant correlations are noted.

[b] Intact desmin (53 kDa), vinculin (120 kDa), and talin (225 kDa) degradation ratios were calculated as the intensity of each immunoreactive desmin, vinculin, and talin band over the intensity of the respective immunoreactive desmin, vinculin, and talin bands in a reference sample (whole muscle protein extract of a porcine longissimus dorsi muscle sample collected 45 min PM) that was loaded on each gel.

[c] Drip loss was determined on longissimus dorsi chops after 1 d of storage at 4 °C.

* $P < 0.05$.

** $P < 0.01$.

Marked effects of pH group but not of the genetic line were observed for the relative abundance of the unautolyzed (80 kDa) μ-calpain subunit and its autolysis products (78 and 76 kDa) at 24 h PM (Table 4). In the H-group the 80 and 78 kDa bands were more abundant ($P < 0.01$) and the 76 kDa band was less ($P < 0.01$) abundant compared to the L-group. Abundance of the 80 and 78 kDa bands were positively ($P < 0.01$) correlated with the band intensity of intact desmin and talin (24 h PM) and 1 d drip loss (Table 5). Correlation coefficients determined for the abundance of the proteins and drip loss at later time points PM were of similar magnitude (data not shown). By contrast, abundance of the autolyzed 76 kDa band was negatively correlated with intact desmin, vinculin, talin (24 h PM) and drip loss (1 d after storage).

Table 4
Effect of the genetic line and the 3 h pH postmortem (PM) on relative abundance of the unautolyzed 80 kDa μ-calpain subunit and its large subunit autolysis products (78 and 76 kDa) determined 24 h PM

	pH group[a]				P-value[b]		
	Line A		Line B				
	H	L	H	L	SEM	Line	pH
80 kDa	1.0	10.0	0.7	14.0	3.63	0.61	<0.01
78 kDa	10.9	18.3	6.3	26.4	4.45	0.69	<0.01
76 kDa	88.2	71.7	92.9	59.7	7.42	0.63	<0.01

[a] pH classification H = pH > 6.0 at 3 h PM and L = pH < 5.7 at 3 h PM.

[b] Probability values for genetic line (Line) and 3 h pH PM classification (pH).

Table 5
Pearson correlation coefficients between unautolyzed 80 kDa μ-calpain subunit and its large subunit autolysis products (78 and 76 kDa) determined 24 h postmortem (PM) and intact desmin, vinculin, talin abundance at 24 h PM as well as drip loss after 1 d of storage[a]

μ-Calpain[b]	Desmin[c]	Vinculin[c]	Talin[c]	Drip loss[d]
80 kDa	0.45**	0.21*	0.48**	0.59**
78 kDa	0.59**	0.13	0.73**	0.58**
76 kDa	−0.57**	−0.18*	−0.66**	−0.63**

[a] Significant correlations are noted.
[b] 80 kDa, relative abundance of unautolyzed large μ-calpain subunit; 78 kDa, relative abundance of 78 kDa calpain large subunit autolysis product; 76 kDa, relative abundance of 76 kDa calpain large subunit autolysis product. Relative abundance of each calpain large subunit determined at 24 h PM.
[c] Intact desmin (53 kDa), vinculin (120 kDa), and talin (225 kDa) degradation ratios were calculated as the intensity of each immunoreactive desmin, vinculin, and talin band over the intensity of the respective immunoreactive desmin, vinculin, and talin bands in a reference sample (whole muscle protein extract of a porcine longissimus dorsi muscle sample collected 45 min PM) that was loaded on each gel.
[d] Drip loss was determined on longissimus dorsi chops after 1 d of storage at 4 °C.
* $P < 0.05$.
** $P < 0.01$.

4. Discussion

Assignment of loins in groups based on 3 h pH resulted in striking differences in the pH determined at earlier (45 min PM) and at later time points (6 and 24 h PM). In the L-group the lowest pH values were measured at 3 h PM whereas in the H-group the lowest pH values were determined at 6 h PM. One factor affecting initial and ultimate pH is the muscle glycogen concentration at the time of slaughter, which determines the potential formation of lactic acid during PM conversion of muscle to meat (Bendall & Swatland, 1988). Resting muscle glycogen level can be affected by many exogenous factors like feeding strategies (Bee et al., 2006), pre-slaughter handling (Warriss, Bevis, & Ekins, 1989) as well as endogenous factors like breed (Enfält, Lundström, Karlsson, & Hansson, 1997), genetic selection strategies (Lonergan et al., 2001) as well as animal to animal variations (Bee, 2002). Furthermore, the muscle's ability to buffer the increase in lactic acid formation plays an additional role (Kylä-Puhju, Ruusunen, Kivikari, & Puolanne, 2004) because it has been shown that muscles with equal lactic acid concentration can display different ultimate pH values (Van Laack & Kauffman, 1999). Thus, the higher pH values at all measured time points in the H- compared to the L-group and in line B compared to A could be caused either by lower resting glycogen levels at slaughter and consequently a slower lactic acid accumulation PM and/or by a higher buffer capacity of the muscle.

As expected, pH group affected drip losses after 1, 2, and 4 d of storage. Although the highest correlations were found between ultimate pH (24 h PM) and 1 and 4 d drip loss ranging from −0.62 to −0.75, negative correlations were also observed for pH determined at earlier time points (45 min pH vs. 1 and 4 d drip loss: −0.49 and −0.51; 6 h pH vs. 1 and 4 d drip loss: −0.56 to −0.60). These relationships are in agreement with various previous reports (e.g. Huff-Lonergan et al., 2002; Kauffman et al., 1998; Schäfer, Rosenvold, Purslow, Andersen, & Henckel, 2002) and highlight the impact of high early pH, and minimal pH decline on water holding capacity. The genetic background of the pigs influenced drip loss at 2 and 4 d of storage with drip loss being lower in line B than in line A. This coincides with the fact that line B tended ($P = 0.09$) to have higher pH values at all time points compared to line A. These results agree with a previous study of Lonergan et al. (2001) who reported higher drip losses combined with more rapid pH decline PM in the LM of pigs selected for growth efficiency compared to control pigs.

Recently, it has been recognized that PM degradation of muscle proteins not only determines meat tenderness (Huff-Lonergan, Parrish, & Robson, 1995; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995) but affects the extent of drip formation (Kristensen & Purslow, 2001; Morrison et al., 1998; Rowe, Huff-Lonergan, & Lonergan, 2001; reviewed by Huff-Lonergan & Lonergan, 2005). Offer & Knight (1988) postulated that in order to translate shrinkage of myofibrils into shrinkage of the whole muscle fiber during rigor development inter-myofibrillar and costameric connections must be intact. Thus, the extent of rigor-shrinkage and muscle protein denaturation affects the amount of intracellular water, the main source of drip from pork, lost during conversion of muscle to meat. The present results demonstrate that early PM pH is related to degradation of both the intermediate filament protein desmin and the costameric protein talin, but not of vinculin. Disappearance of both cytoskeletal proteins occurred faster in the H- compared to the L-group. In agreement with the present results, Melody et al. (2004) reported greater desmin degradation in the LM compared to the psoas major which at the same time PM displayed a more rapid pH decline compared to the LM. In the current study, relative abundance of talin (compared to a reference porcine LM sample collected 45 min PM) was already diminished by 50% at 24 h PM and decreased gradually to 1% at 120 h PM in the H-group, whereas in the L-group intact talin intensity at 120 h PM was still 30% of the reference standard. By contrast, disappearance of intact desmin occurred at a slower rate in both pH groups. Kristensen & Purslow (2001) showed a similar degradation pattern for talin and desmin during 10 d of aging. In the current study, vinculin was degraded over time, which was evidenced by a decreasing abundance of intact vinculin and simultaneously appearance of two degradation products, but in contrast to results of Ertbjerg, Henckel, Karlsson, Larsen, & Moller (1999), the extent of vinculin proteolysis was not affected by the course of the pH decline. Nevertheless, the present results indicate that pH decline in early postmortem muscle may account for a portion of the extent of proteolytic disruption of intermediate filament and costamere,

which may dictate the degree of muscle fiber shrinkage that is caused by myofibrillar shrinkage. This would have an impact on the amount of intracellular water shifted toward extracellular water during rigor development, thus affecting water loss during aging (Kristensen & Purslow, 2001; reviewed in Huff-Lonergan & Lonergan, 2005).

The calpain protease system plays a key role in PM proteolysis through degradation of key myofibrillar and myofibril-associated proteins (Huff-Lonergan et al., 1996; Taylor et al., 1995). A well documented characteristic of calpains is that they are susceptible to calcium-induced autolysis which in case of μ-calpain reduces the Ca^{2+} requirement for calpain activity from approximately 1–50 μM to 0.5–2.0 μM (Goll, Thompson, Li, Wei, & Cong, 2003) thereby reaching levels observed in muscles entering rigor (Jeacocke, 1993). Therefore, proteolytic activity of μ-calpain is highly related to autolysis of the 80 kDa subunit which under PM conditions depends on the pH and temperature (Koohmaraie, 1992). The results of the present study revealed significantly greater autolysis of the 80 kDa subunit of μ-calpain, evidenced by a higher abundance of the 76 kDa band, in the H- compared to the L-group at 24 h PM. The negative correlation between the abundance of the autolyzed 76 kDa μ-calpain subunit and pH values at all measured time points ranging from −0.59 (45 min PM) to −0.34 (24 h PM) confirms the close relationship between the extent of autolysis and muscle pH. The present findings suggest that μ-calpain autolysis occurs earlier with faster pH decline and eventually results in earlier loss of proteolytic activity which could explain the lower degradation of desmin and talin in the L- compared to H-group. In accordance, Rowe, Lonergan, Rothschild, & Huff-Lonergan (2001) showed in a preliminary study that the activity of μ-calpain revealed by casein zymography decreased faster in porcine LM with low pH at 2 h PM than in LM samples that had slower and limited pH decline. In accordance with the present findings they reported earlier degradation of troponin-T and desmin in samples that had more extensive autolysis of μ-calpain at 24 h. This observation was supported by Carlin et al. (2006) who showed that μ-calpain had greater activity at pH 6.5 than at pH 7.5. Thus, it is possible that slightly lower pH values may encourage more rapid activation of μ-calpain. This phenomenon deserves more attention.

5. Conclusions

Disruption of the inter-myofibrillar and costameric connections by degradation of desmin and talin early in the postmortem period has the potential to affect water-holding capacity. This is hypothesized to occur by limiting the extent of lateral shrinkage of the myofibrils and overall shrinkage of the entire muscle fiber caused by rigor shortening and denaturation of myofibrillar proteins. Under such circumstances, intracellular space is independent of myofibrillar volume or shrinkage. The current work extends this hypothesis by providing evidence that the rate of activation of μ-calpain is linked to the extent of degradation of intact desmin and talin. Furthermore the data presented here strongly suggest that the rate of early pH decline can explain a portion of the variation of degradation of desmin and talin by dictating the rate of activation of μ-calpain.

References

Bee, G. (2002). Effect of available dietary carbohydrate on glycolytic potential and meat quality of swine muscles. *Canadian Journal of Animal Science, 82*, 311–320.

Bee, G., Biolley, C., Guex, G., Herzog, H., Lonergan, S. M., & Huff-Lonergan, E. (2006). Effects of available dietary carbohydrate and preslaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. *Journal of Animal Science, 84*, 191–203.

Bendall, J. R., & Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. *Meat Science, 24*, 85–126.

Carlin, K. R. M., Huff-Lonergan, E., Rowe, L. J., & Lonergan, S. M. (2006). Effect of oxidation, pH and ionic strength on calpastatin inhibition of μ- and m-calpain. *Journal of Animal Science, 84*, 925–937.

Diesbourg, L., Swatland, H. J., & Millman, B. M. (1988). X-ray-diffraction measurements of postmortem changes in the myofilament lattice of pork. *Journal of Animal Science, 66*, 1048–1054.

Enfält, A. C., Lundström, K., Karlsson, A., & Hansson, I. (1997). Estimated frequency of the RN-allele in Swedish Hampshire pigs and comparison of glycolytic potential, carcass composition, and technological meat quality among Swedish Hampshire, Landrace, and Yorkshire pigs. *Journal of Animal Science, 75*, 2924–2935.

Ertbjerg, P., Henckel, P., Karlsson, A., Larsen, L. M., & Moller, A. J. (1999). Combined effect of epinephrine and exercise on calpain/calpastatin and cathepsin B and L activity in porcine longissimus muscle. *Journal of Animal Science, 77*, 2428–2436.

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiological Reviews, 83*, 731–801.

Huff-Lonergan, E., Baas, T. J., Malek, M., Dekkers, J. C. M., Prusa, K., & Rothschild, M. F. (2002). Correlations among selected pork quality traits. *Journal of Animal Science, 80*, 617–627.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity in meat: The role of postmortem biochemical and structural changes. *Meat Science, 71*, 194–204.

Huff-Lonergan, E., Mitsuhashi, T., Beekman, D. D., Parrish, F. C., Jr., Olson, D. G., & Robson, R. M. (1996). Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. *Journal of Animal Science, 74*, 993–1008.

Huff-Lonergan, E., Parrish, F. C., Jr., & Robson, R. M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science, 73*, 1064–1073.

Jeacocke, R. E. (1993). The concentration of free magnesium and calcium ions both increase in muscle fibres entering rigor mortis. *Meat Science, 35*, 27–45.

Kauffman, R. G., van, L. R., Russell, R. L., Pospiech, E., Cornelius, C. A., Suckow, C. E., et al. (1998). Can pale, soft, exudative pork be prevented by postmortem sodium bicarbonate injection? *Journal of Animal Science, 76*, 3010–3015.

Koohmaraie, M. (1992). The role of Ca(2+)-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. *Biochimie, 74*, 239–245.

Kristensen, L., & Purslow, P. P. (2001). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. *Meat Science, 58*, 17–23.

Kylä-Puhju, M., Ruusunen, M., Kivikari, R., & Puolanne, E. (2004). The buffering capacity of porcine muscles. *Meat Science, 67*, 587–593.

Lawson, M. A. (2004). The role of integrin degradation in post-mortem drip loss in pork. *Meat Science, 68*, 559–566.

Littell, R. C., Henry, P. R., & Ammerman, C. B. (1998). Statistical analysis of repeated measures data using SAS procedures. *Journal of Animal Science, 76*, 1216–1231.

Lonergan, S. M., Huff-Lonergan, E., Rowe, L. J., Kuhlers, D. L., & Jungst, S. B. (2001). Selection for lean growth efficiency in Duroc pigs influences pork quality. *Journal of Animal Science, 79*, 2075–2085.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry, 193*, 265–275.

Maddock, K. R., Huff-Lonergan, E., Rowe, L. J., & Lonergan, S. M. (2005). Effect of pH and ionic strength on inhibition of μ- and m-calpain by calpastatin. *Journal of Animal Science, 83*, 1370–1376.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science*, 1195–1205.

Morrison, E. H., Mielche, M. M., & Purslow, P. P. (1998). Immunolocalisation of intermediate filament proteins in porcine meat. Fibre type and muscle-specific variations during conditioning. *Meat Science, 50*, 91–104.

Offer, G., & Knight, P. (1988). The structural basis of water-holding in meat. Part 2. Drip losses. In R. A. Lawrie (Ed.), *Developments in meat science* (pp. 173–243). London, UK: Elsevier Applied Science.

Rowe, L. J., Huff-Lonergan, E. J., & Lonergan, S. M. (2001). Desmin degradation influences water-holding capacity and tenerness of fresh pork. *Journal of Animal Science, 79*(Suppl. 1), 443.

Rowe, L. J., Lonergan, S. M., Rothschild, M. F., & Huff-Lonergan, E. (2001). Relationship between porcine longissimus dorsi pH decline and μ-calpain activity/autolysis and protein degradation. *Journal of Animal Science, 79*(Suppl. 1), 20.

Schäfer, A., Rosenvold, K., Purslow, P. P., Andersen, H. J., & Henckel, P. (2002). Physiological and structural events postmortem of importance for drip loss in pork. *Meat Science, 61*, 355–366.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science, 73*, 1351–1367.

Van Laack, R. L. J. M., & Kauffman, R. G. (1999). Glycolytic potential of red, soft, exudative pork longissimus muscle. *Journal of Animal Science, 77*, 2971–2973.

Warriss, P. D., Bevis, E. A., & Ekins, P. J. (1989). The relationships between glycogen stores and muscle ultimate pH in commercially slaughtered pigs. *British Veterinary Journal, 145*(4), 378–383.

Zhang, W. G., Lonergan, S. M., Gardner, M. A., & Huff-Lonergan, E. (2006). The role of integrin and desmin degradation in water holding capacity in pork. *Meat Science*. doi:10.1016/jmeatsci.2006.05.008.