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

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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

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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),