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Early Post-mortem AMP-Activated Protein Kinase (AMPK) Activation Leads to Phosphofructokinase-2 and -1 (PFK-2 and PFK-1) Phosphorylation and the Development of Pale, Soft, and Exudative (PSE) Conditions in Porcine Longissimus Muscle

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Pale, soft, and exudative (PSE) meat has been recognized for decades. Fast glycolysis during early post-mortem stage while the muscle temperature is still high is the cause of PSE meat. To elucidate the molecular mechanism underlying this fast glycolysis in muscle to become PSE meat, post-mortem ATP metabolism, fructose-2,6-diphosphate content, and the activities of AMPK, glycogen phosphorylase, and pyruvate kinase were examined in post-mortem muscle. Earlier and faster post-mortem AMPK activation was responsible for the significantly lower pH and higher lactic acid accumulation ($p < 0.05$) seen in PSE muscle, which resulted in the occurrence of PSE meat. In muscle that became PSE meat, AMPK was activated at 0 h post-mortem and reached maximal activation at 0.5 h post-mortem, whereas AMPK reached maximal activation at 1 h post-mortem in the normal pork loin. Higher fructose-2,6-diphosphate content ($p < 0.05$) was detected in PSE muscle compared to normal muscle at early post-mortem stage. However, no difference in the activities of glycogen phosphorylase and pyruvate kinase, rate-controlling enzymes in glycogenolysis and glycolysis, respectively, was detected between PSE and normal pork loins. Because fructose-2,6-diphosphate is a product of phosphofructokinase-2 (PFK-2), these data suggest that AMPK regulates post-mortem glycolysis through its phosphorylation and activation of PFK-2, which then up-regulates the activity of phosphofructokinase-1 (PFK-1), a key rate-controlling enzyme in glycolysis. Early AMPK activation in PSE muscle is associated with early consumption of ATP, because higher AMP and IMP contents and lower ATP content were detected in PSE meat compared to normal meat. Other mechanisms causing early AMPK activation in PSE meat may exist, which warrants further investigation.

KEYWORDS: AMP-activated protein kinase; pork; PSE; glycolysis; lactic acid; meat

INTRODUCTION

Pale, soft, and exudative (PSE) meat has been recognized for decades (*1*) and causes a huge economic loss to the meat industry due to its inferior quality, such as low water-holding capacity, pale and watery appearance, and tough texture after cooking. Cannon et al. (*2*) estimated that the PSE and reddish pink, soft, and exudative (RSE) pork resulted in approximately $100 million annual loss in the United States alone. Fast and excessive glycolysis in post-mortem muscle, especially at early post-mortem stage, in combination with high muscle temperature is the cause of PSE meat (*3*−*7*). However, molecular mechanisms leading to fast glycolysis in post-mortem muscle are not fully understood.

AMP-activated protein kinase (AMPK) comprises a catalytic subunit (α) and two regulatory subunits (β and γ) (*8*−*10*). AMPK regulates energy status within cells (*8*, *9*, *11*−*13*), which is activated following ATP depletion or, more accurately, a rise in the AMP/ATP ratio within the cell (*14*). Once activated, AMPK phosphorylates downstream substrates, which leads to the switching off of ATP-consuming pathways and the switching on of catabolic pathways that generate ATP, such as glycolysis, in an attempt to restore the cellular energy balance (*9*, *12*, *14*−*16*).

AMPK plays a crucial role in the initiation of glycolysis in the ischemic heart (*13*, *17*). Our previous studies showed that AMPK regulated glycolysis in post-mortem muscle (*18*−*21*). In those studies, preslaughter swim and/or intraperitoneal injection of 5-aminoimidazole-4-carboxamide-1−4-ribofuranoside (AICAR), a specific activator of AMPK, increased AMPK activation and led to lower pH in post-mortem mouse longis-

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simus muscle (LM) when compared to control (*22*, *23*). On the other hand, glycolysis and pH decline in post-mortem LM of AMPK knockout mice, which express a cardiac and skeletal muscle-specific dominant negative AMPK-α2 subunit and, consequently, have very low AMPK activity (*24*, *25*), were very slow, showing the essential role of AMPK in post-mortem glycolysis (*18*, *26*). In another study, dietary α-lipoic acid supplementation prevented AMPK activation and decreased glycolysis in post-mortem muscle (*20*, *21*). These studies showed that AMPK was crucial for post-mortem glycolysis. Because fast glycolysis at early post-mortem stage leads to PSE meat, AMPK is hypothesized to play a key role in the incidence of PSE meat. The objective of the current study was to define the role of AMPK in the occurrence of PSE pork and to explore the underlying mechanisms.

EXPERIMENTAL PROCEDURES

Samples. Crossbred (Yorkshire × Hampshire) pigs with an average weight of ≈110 kg were slaughtered at the Meat Laboratory, University of Wyoming. All pigs used in this study were halothane gene and Rendement Napole (RN^-) gene negative. A slice of LM muscle (≈20 g) between the 10th and 11th ribs in the left side of carcasses was obtained at 0 (immediately following exsanguination), 0.5, 1, 4, and 24 h post-mortem. After sampling, samples were quickly cut into small pieces and mixed. A portion of the samples was used for biological analyses directly, and the rest of the samples were snap-frozen in liquid nitrogen and stored at −80 °C until analyses.

Samples from eight pigs with PSE meat and eight pigs with normal meat were selected for further analyses. Criteria for the selection of PSE and normal meat (*26*) were as follows: PSE meat, $L^* > 60$, pH ≤ 6.0 at 0.5 h post-mortem, ultimate pH < 5.5. drip loss > 5%; normal meat, $L^* < 60$, pH > 6.0 at 0.5 h post-mortem, ultimate pH > 5.5, drip loss < 5%.

At 24 h post-mortem, two loin chops were obtained between 10th and 12th ribs from the right side of the carcass. Chops were individually packaged (aerobic) and used for the measurements of color and water-holding capacity.

pH Measurement. A portable pH-meter (HI99161, Hanna Instruments Inc.) with a spear-tipped glass electrode pH probe was used for the measurement of pH change in carcasses. The pH was measured at loin muscle between the eighth and ninth ribs at 0, 0.5, 1, 4, and 24 h post-mortem. Ultimate pH refers to the pH of muscle at 24 h post-mortem.

Lactic Acid. For lactate determination, 0.3 g of muscle sample was homogenized in 900 μL of 0.9 N $HClO_4$ (Polytron homogenizer; IKA Works, Inc., Wilmington, NC). The homogenate was centrifuged at 13000*g* and 4 °C for 5 min. The supernatant was removed and neutralized with 2 M KOH and centrifuged again as above to precipitate potassium perchlorate. The extracts were used for lactic acid measurement using a commercially available lactate analysis kit (Trinity Biotech, St. Louis, MO).

Color Measurement. Surface color (CIE $L^*a^*b^*$) of loin chops was measured after a 30 min bloom time at 4 °C using a Hunter LabScan Colorimeter (Hunter Laboratory, Inc., Reston, VA). Color was measured through the plastic packaging. To do this, the same package materials were used to cover a standard white plate to eliminate the influence of packaging materials on meat color. A CIE D-65 10° standard observer and a 2.54-cm viewing port were used (*27*).

Drip Loss. Loin chops were trimmed of bone, fat, and connective tissue to obtain LM chops, which were then individually packaged in a large zip bag and hung at 4 °C. Chops were weighed before and after hanging for 24 h, and drip loss was expressed as the percentage of weight loss over 24 h of suspension (*28*).

Cooking Yield. After color measurement, LM chops were obtained by trimming off the bone and surrounding fat and connective tissue. Then, LM chops were vacuum packaged and submerged in boiling water for cooking. Cooking yield was obtained by measuring the weight of LM chops before and after cooking to an internal temperature of 71 °C (*29*). The internal temperature was measured by inserting a thermoprobe into the geometrical center of the chops.

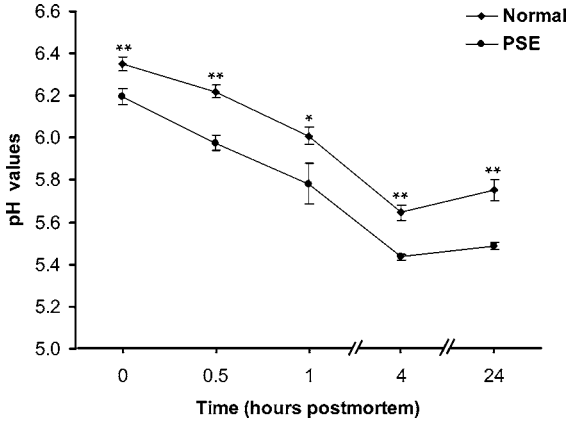

Figure 1. pH values of post-mortem muscle of normal and PSE pork. $n = 8$. * indicates significant difference at $p < 0.05$; ** indicates significant difference at $p < 0.01$.

ATP, AMP, and IMP Content Measurements. ATP and AMP contents in post-mortem muscle were determined by high-performance liquid chromatograph (HPLC, Beckman Instruments, Inc., Fullerton, CA) as previously described (*30*). Briefly, powdered frozen LM samples were homogenized in 3 volumes of ice-cold 0.9 N perchloric acid. After extraction for 30 min in ice, supernatant was obtained by centrifuging at 13000*g* and 4 °C for 10 min. The supernatant was neutralized with 2 M KOH and centrifuged again under the same condition to remove $KClO_4$. The neutralized supernatant was passed through a 0.2 μm filter. Ten micrometer aliquots of the final muscle extraction were injected into the chromatograph column (Phenomenex C_{18}-MC1, 250 × 4.60 mm, 5 μm). Mobile phase A was phosphate buffer (0.04 M potassium dihydrogen orthophosphate and 0.06 M dipotassium hydrogen orthophosphate, pH 7.0). Mobile phase B was acetonitrile. UV detection was carried out at a wavelength of 254 nm. Peaks were identified and quantified by comparison for retention time and peak area with known external standards.

AMPK, Glycogen Phosphorylase, and Pyruvate Kinase Activities. Frozen LM samples (0.1 g) were powdered in liquid nitrogen and homogenized in 500 μL of ice-cold homogenization buffer (0.25 M mannitol, 0.05 M Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM NaF, and 5 mM sodium pyrophosphate). Homogenates were centrifuged at 12000*g* for 2 min at 4 °C. The supernatants were used for AMPK and glycogen phosphorylase activity determination.

AMPK, glycogen phosphorylase, and pyruvate kinase activities were measured as previously described (*20*, *22*). AMPK activity was calculated as nanomoles of ATP incorporated into a SAMS peptide per gram of muscle per minute. Glycogen phosphorylase activity was expressed as milligrams of glucosyl units assimilated into glycogen per minute per gram of muscle. Pyruvate kinase activity was calculated as millimoles of NADH oxidized per minute per gram of muscle.

Fructose 2,6-Biphosphate Measurement. For measurement of fructose 2,6-biphosphate (fru-2,6-P_2), 0.1 g of LM powder was homogenized in 10 volumes of 50 mM NaOH and kept for 10 min at 90 °C. After neutralization with 0.25 M sodium acetate (pH 4.0), the homogenate was centrifuged at 13000*g* for 5 min. The supernatant was used for fru-2,6-P_2 determination as previously described (*31*).

Statistical Analysis. Data were analyzed as a completely randomized design using PROC GLM (General Linear Model Procedure of Statistical Analysis System, SAS, 2000). For all data analyses, each animal was considered as an experimental unit. Time course data were analyzed as a split-plot in time. Differences in the mean values within the same treatments were compared by Fisher's protected least significant difference (LSD) test ($p < 0.05$). All data were expressed as mean ± SEM.

RESULTS

Meat Quality Measurements. Longissimus muscle pH, drip losw, and color L^* values were used to select normal or PSE

Table 1. Quality Measurement of Normal and PSE Pork[a]

quality class	drip loss (%)	cooking yield (%)	lightness (L^*)	redness (a^*)	yellowness (b^*)
normal	2.26 ± 0.48 x	77.98 ± 1.17 x	56.04 ± 0.89 x	9.76 ± 0.64 x	15.69 ± 0.62 x
PSE	7.59 ± 0.62 y	76.60 ± 1.38 x	63.75 ± 1.10 y	10.12 ± 0.52 x	18.15 ± 0.45 y

[a] Within a column, least-squares means lacking a common letter (x, y) differ, $p < 0.05$. $n = 8$ for both normal and PSE quality classes.

Table 2. ATP, AMP, and IMP Contents in Normal and PSE Pork and Calculated AMP/ATP and (AMP + IMP)/ATP Ratios[a]

	time				
	0.0 h	0.5 h	1.0 h	4.0 h	24.0 h
ATP contents (μmol/g of muscle)					
normal	5.53 ± 0.43 ax	5.01 ± 0.35 ax	3.12 ± 0.49 bx	0.51 ± 0.19 cx	0.14 ± 0.02 cx
PSE	4.08 ± 0.34 ay	3.18 ± 0.41 by	1.49 ± 0.30 cy	0.20 ± 0.09 dx	0.10 ± 0.03 dx
AMP contents (μmol/g of muscle)					
normal	0.12 ± 0.02 cx	0.18 ± 0.03 abcx	0.26 ± 0.03 ax	0.21 ± 0.03 abx	0.15 ± 0.02 bcx
PSE	0.18 ± 0.05 bx	0.30 ± 0.07 ax	0.33 ± 0.03 ax	0.14 ± 0.03 bx	0.11 ± 0.02 bx
IMP contents (μmol/g of muscle)					
normal	1.35 ± 0.39 cx	1.64 ± 0.27 cx	3.29 ± 0.58 bx	6.68 ± 0.29 ax	7.72 ± 0.10 ax
PSE	3.25 ± 0.62 cy	3.44 ± 0.61 cy	5.43 ± 0.59 by	7.61 ± 0.51 ax	7.42 ± 0.28 ax
AMP/ATP ratio					
normal	0.02 ± 0.00 cx	0.04 ± 0.01 cx	0.14 ± 0.06 cx	0.67 ± 0.14 bx	1.12 ± 0.08 ax
PSE	0.05 ± 0.02 bx	0.12 ± 0.04 bx	0.38 ± 0.13 bx	1.14 ± 0.16 ay	1.31 ± 0.23 ax
(AMP + IMP)/ATP ratio					
normal	0.31 ± 0.10 cx	0.40 ± 0.09 cx	2.05 ± 0.97 bcx	26.57 ± 6.33 bx	76.64 ± 18.52 ax
PSE	0.98 ± 0.28 by	1.55 ± 0.43 by	7.50 ± 3.01 bx	110.50 ± 34.80 ay	117.73 ± 23.65 ax

[a] Within a row, least-squares means lacking a common letter(a–d) differ, $p < 0.05$. Within a column, least-squares means lacking a common letter (x, y) differ, $p < 0.05$. $n = 8$ for both normal and PSE quality classes.

pork. Due to the experimental design, the means of all three traits were significantly different between normal and PSE quality classes ($p < 0.05$) (**Figure 1** and **Table 1**). The PSE group had an average drip loss of 7.59 ± 0.62% versus 2.26 ± 0.48% for the normal group ($p < 0.01$). The lightness (L^*) for normal meat group was 56.04 ± 0.89, whereas that for PSE meat was 63.75 ± 1.10 ($p < 0.01$). In **Table 1**, the values of redness (a^*), yellowness (b^*), and pork loin cooking yield are also presented. There was no difference in a^* values and cooking yield between normal and PSE meat. However, PSE meat was more yellow than normal meat ($p < 0.01$).

As expected from the experimental design, the pH of the PSE group was constantly lower than that of the normal group ($p < 0.05$). Furthermore, the pH of the PSE group declined much more rapidly during the early post-mortem stage ($p < 0.01$) as compared to the normal group. For both normal and PSE groups, the pH declined to the ultimate value of 5.65 and 5.43, respectively, by 4 h post-mortem (**Figure 1**).

Correspondingly, at 0, 0.5, 1, and 4 h post-mortem, PSE pork had higher lactic acid contents than normal pork ($p < 0.05$) (**Figure 2**). At 24 h post-mortem, no difference was observed in lactic acid contents between these two groups.

ATP-Related Compounds. ATP, AMP, and IMP contents in post-mortem LM and calculated AMP/ATP and (AMP + IMP)/ATP ratios are listed in **Table 2**. The ATP content was consistently higher in normal pork compared to PSE pork before 4 h post-mortem ($p < 0.05$), showing higher energy level in the post-mortem muscle of normal meat. After 4 h post-mortem, ATP was depleted to the same level in both normal and PSE groups. In the PSE group, ATP decreased from 4.08 ± 0.34 at 0 h post-mortem to 3.18 ± 0.41 mmol/g of muscle at 0.5 h post-mortem ($p < 0.05$). In normal pork, however, no difference in ATP content was found between these two post-mortem stages ($p < 0.05$), indicating a faster ATP utilization rate in the PSE group (**Table 2**).

Fast consumption of ATP did not result in a corresponding increase in AMP content in the PSE quality group (**Table 2**).

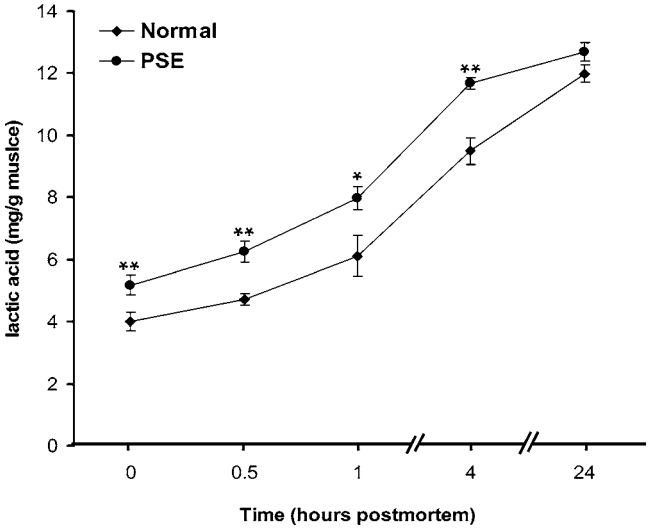

Figure 2. Lactic acid content in post-mortem muscle of normal and PSE pork. $n = 8$. * indicates significant difference at $p < 0.05$; ** indicates significant difference at $p < 0.01$.

No difference in AMP content was found between normal and PSE pork during the whole post-mortem period ($p < 0.05$). Instead, much higher IMP ($p < 0.05$), a metabolite from AMP, was detected in PSE pork before 4 h post-mortem. As shown in **Table 2**, the calculated AMP/ATP and (AMP + IMP)/ATP ratios increased throughout the entire post-mortem period in both normal and PSE pork. From 0 to 24 h post-mortem, the AMP/ATP ratio increased 41- and 26-fold in the normal and PSE groups, respectively (**Table 2**). The (AMP + IMP)/ATP ratio was higher in the PSE group compared to the normal group before 1 h post-mortem (**Table 2**).

Post-mortem AMPK and Glycolytic Enzyme Activities and Fruc 2,6-P_2 Content. Although no difference in AMP/ATP ratio existed between normal and PSE groups before 4 h post-mortem, differences in AMPK activity were observed

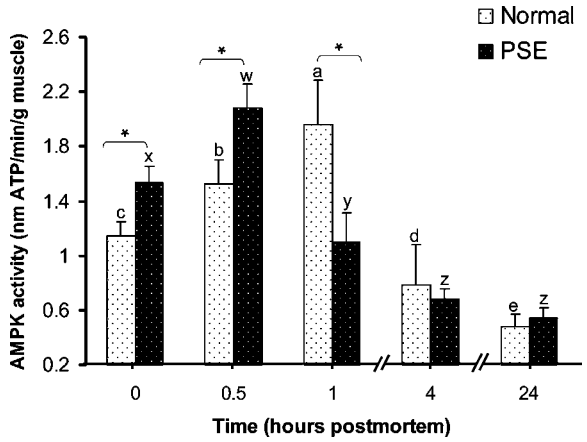

Figure 3. AMPK activity in post-mortem muscle of normal and PSE pork. $n = 8$. * indicates significant difference at $p < 0$ 0.05. Within normal pork, means lacking a common letter (a–e) differ, $p < 0.05$. Within PSE pork, means lacking a common letter (w–z) differ, $p < 0.05$.

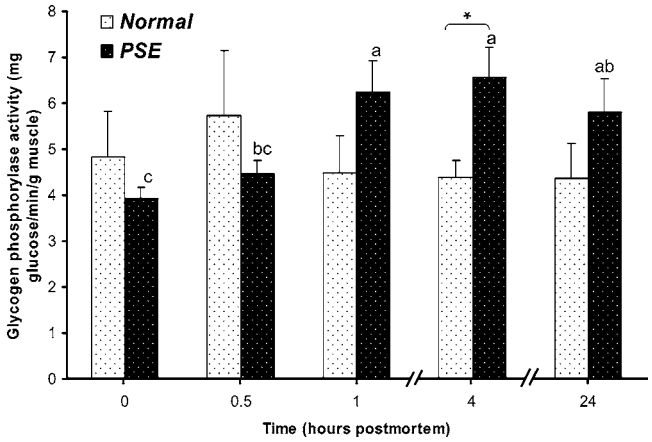

Figure 4. Glycogen phosphorylase activity in post-mortem muscle of normal and PSE pork. $n = 8$. * indicates significant difference at $p < 0.05$. Within PSE pork, means lacking a common letter (a–c) differ, $p < 0.05$.

between these two groups (**Figure 3**). In the PSE group, AMPK was activated earlier, starting at 0 h post-mortem, and reached maximal activation at 0.5 h post-mortem (**Figure 3**). For the normal group, however, AMPK activity did not reach maximum until 1 h post-mortem. The AMPK activity in normal pork was lower than that of PSE pork at 0 and 0.5 h post-mortem ($p < 0.05$) but higher at 1 h post-mortem (**Figure 3**). After 1 h post-mortem, the AMPK activity attenuated, with the lowest activity at 24 h post-mortem.

Glycogen phosphorylase activity is shown in **Figure 4**. No difference was observed between normal and PSE pork. In the PSE group, glycogen phosphorylase was activated at 1 h post-mortem, with the highest activity at 1 and 4 h post-mortem ($p < 0.05$). In normal pork, however, glycogen phosphorylase activity remained unchanged ($p < 0.05$) throughout the post-mortem period.

Pyruvate kinase, another rate-eontrolling enzyme in glycolysis, was activated in post-mortem LM (**Figure 5**), with the highest activity at 0.5 and 1 h post-mortem in both normal and PSE pork. After 1 h post-mortem, pyruvate kinase activity started to decrease in both groups and reached the lowest values at 24 h post-mortem. No difference in pyruvate kinase activity was observed between PSE and normal pork.

Fru-2,6-P_2 is the allosteric activator of phosphofructokinase-1 (PFK-1), the most important rate-controlling enzyme in glycolysis. Fru-2,6-P_2 content increased during the early post-mortem stage (**Figure 6**). In PSE pork, fru-2,6-P_2 increased to its highest value of 2.15 ± 0.27 nmol/g of muscle at 0.5 h post-mortem. After this point, fru-2,6-P_2 decreased. In contrast, fru-2,6-P_2 in normal pork did not achieve its highest value until 1 h post-mortem. Furthermore, fru-2,6-P_2 content was higher ($p < 0.05$) in the PSE group than in the normal group at both 0 and 0.5 h post-mortem. In short, fru-2,6-P_2 content increased earlier in PSE pork than in normal pork.

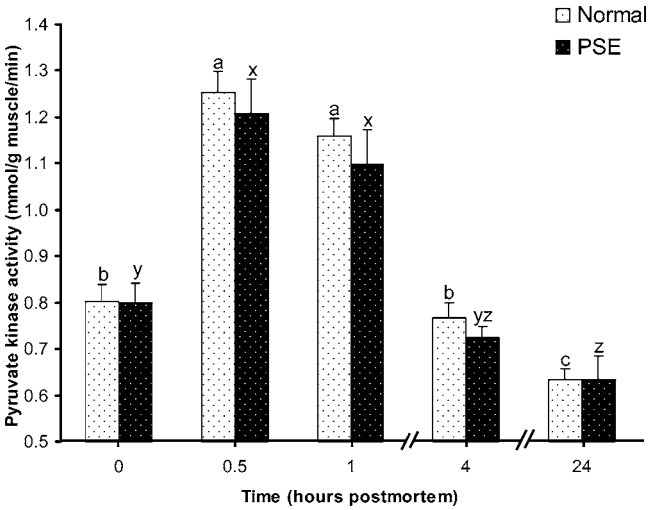

Figure 5. Pyruvate kinase activity in post-mortem muscle of normal and PSE pork. $n = 8$. Within normal pork, means lacking a common letter (a–c) differ, $p < 0.05$. Within PSE pork, means lacking a common letter (x–z) differ, $p < 0.05$.

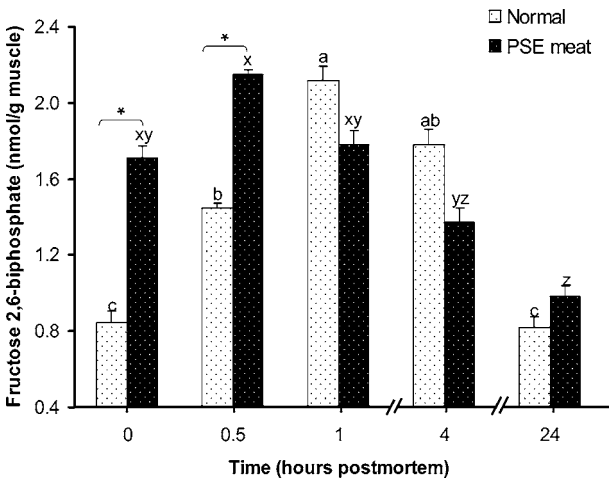

Figure 6. Fru-2,6-P_2 content in post-mortem muscle of normal and PSE pork. $n = 8$. * indicates significant difference at $p < 0.05$. Within normal pork, means lacking a common letter (a–c) differ, $p < 0.05$. Within PSE pork, means lacking a common letter (x–z) differ, $p < 0.05$.

DISCUSSION

AMPK is a cellular energy sensor, regulating food intake (*32*, *33*), glucose and fatty acid metabolism (*17*, *34*), and protein synthesis (*35*, *38*). Thus, AMPK is potentially a molecular target to improve farm animal growth performance, body composition, and meat quality to benefit both producers and consumers. The importance of AMPK in animal industry is first evidenced by the identification of RN^- mutation in pigs. The RN^- gene is associated with "acid meat" in Hampshire pigs (*39*). RN^- carriers have a high lean ratio but inferior meat quality

characterized by a low ultimate pH, a reduced water-holding capacity, and a decreased yield after processing (*40*−*42*). It was later identified that the RN^- mutation in Hampshire pigs is an amino acid substitution (R200Q) in the muscle-specific isoform of AMPK γ3 subunit (*43*). This mutation results in enhanced glycogen storage in muscle (*44*), resulting in acid meat (*45*−*47*). Interestingly, another mutation in the AMP γ3 subunit reduces the glycogen content in muscle and improved meat quality (*43*, *48*).

Consistent with our previous studies (*18*−*21*), in this study we showed that AMPK was crucial in post-mortem glycolysis and the incidence of PSE meat. AMPK was activated earlier in the muscle that became PSE meat. This earlier AMPK activation led to faster pH decline at initial post-mortem stage, resulting in PSE pork.

AMPK increases glycolysis through two main pathways (*17*, *49*). Activated AMPK up-regulates glycolysis by phosphorylating and activating phosphorylase kinase, which then phosphorylates and activates glycogen phosphorylase, an enzyme that controls glycogenolysis and catalyzes production of substrate for glycolysis (*50*−*52*). In our previous studies on mice, higher glycogen phosphorylase activity was found in treatments with higher AMPK activity, indicating that AMPK activated glycogen phosphorylase in post-mortem muscle of mice (*18*, *20*, *22*). However, in this study, glycogen phosphorylase activity in pig muscle did not correlate well with AMPK activity. This discrepancy may be caused by denaturation of phosphorylase due to low pH in combination with high temperature in pork during the early post-mortem stage (*53*, *54*). A much lower pH was detected in pork loin compared to that in mouse muscle, and the bulk quantity of pork limited the heat dissipation, which might cause significant denaturation of phosphorylase.

Another important mechanism by which AMPK increases glycolysis is implemented through phosphorylation and activation of phosphofructokinase-2 (PFK-2) (*55*). PFK-2 catalyzes the production of fru-2,6-P_2 within cells. Fru-2,6-P_2 is a potent allosteric activator of PFK-1, a committed and key rate-controlling enzyme in glycolysis. Within 0.5 h after exsanguination, higher fru-2,6-P_2 content was detected in the post-mortem muscle becoming PSE pork. The high fru-2,6-P_2 content activated PFK-1 and promoted glycolysis, as shown by lower pH and higher lactic acid accumulation in post-mortem muscle eventually becoming PSE meat. Because no difference in glycogen phosphoylase and pyruvate activity was found between normal and PSE meat before 4 h post-mortem, high PFK-2 and PFK-1 activities should play a major role in post-mortem glycolysis and the development of PSE pork.

AMPK is activated by an increase in the AMP/ATP ratio (*8*, *9*, *12*, *13*). However, changes in the AMP/ATP ratio cannot explain the early AMPK activation in PSE group and the difference of AMPK activity between normal and PSE pork before 4 h post-mortem (**Figure 3**). In our study, no difference in AMP/ATP ratio was found between normal and PSE pork before 4 h post-mortem, despite a significant earlier AMPK activation in PSE group (**Table 2**). Because AMP is converted to IMP in post-mortem muscle, the combination of AMP and IMP reflects the total amount of AMP formed in post-mortem muscle. Therefore, the (AMP + IMP)/ATP ratio was used to replace the AMP/ATP ratio. A significantly higher (AMP + IMP)/ATP ratio was observed in the early-stage post-mortem muscle of the PSE group, which could explain the observed differences in AMPK activation between normal and PSE pork. These data suggest that the (AMP + IMP)/ATP ratio rather than the AMP/ATP ratio works as a better predictor for the activation of AMPK. These data show that a lower energy level in PSE pork at early post-mortem stage activates AMPK. This is in line with a previous study (*56*).

Nevertheless, the lack of difference in AMP/ATP and (AMP + IMP)/ATP ratio in post-mortem muscle at 0 and 0.5 h, despite a dramatic AMPK activation during this period, suggests that other mechanisms leading to AMPK early activation might exist. In ischemic heart and cell culture, an activation of AMPK was observed in the absence of change in the AMP/ATP ratio (*57*−*60*). Recently, Ca^{2+}/calmodulin-dependent protein kinase kinase-β was identified as an upstream activator of AMPK which leads to AMPK activation without a change in AMP/ATP ratio (*61*, *62*). Thus, further studies to explore whether other mechanisms exist and their roles in post-mortem glycolysis and the incidence of PSE meat are of great interest.

In conclusion, data presented in this paper lead to three major conclusions. First, early AMPK activation in pork loin results in PSE meat. Second, activated AMPK up-regulates glycolysis through its phosphorylation and activation of PFK-2, leading to the accumulation of fru-2,6-P_2. Third, early AMPK activation in post-mortem PSE muscle is linked to accelerated ATP utilization, although other mechanisms leading to AMPK activation may exist.

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