The halothane gene, energy metabolism, adenosine monophosphate-activated protein kinase, and glycolysis in postmortem pig longissimus dorsi muscle[1]

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ABSTRACT: The presence of the halothane gene results in PSE meat. However, the exact mechanisms linking the halothane gene and the incidence of PSE meat remain unclear. We hypothesize that the presence of the halothane gene accelerates energy consumption in postmortem muscle, which activates adenosine monophosphate-activated protein kinase (AMPK), leading to enhanced glycolysis and PSE meat. To test our hypothesis, energy status, AMPK activity, and glycolysis in the postmortem LM of the halothane gene carrier and halothane-negative pigs were compared. The results showed that the presence of the halothane gene accelerated energy depletion in postmortem muscle immediately after exsanguination, leading to rapid and early depletion of ATP, as shown by an increase in the (adenosine monophosphate + inosine monophosphate):ATP ratio in postmortem LM. In addition, an early AMPK activation was observed in LM from halothane carriers. The fructose-2,6-diphosphate concentration in postmortem LM was well correlated with AMPK activation. To be a potent stimulator of phosphofructose kinase, the increase in fructose-2,6-diphosphate is expected to activate phosphofructose kinase, a key enzyme controlling glycolysis, leading to enhanced glycolysis and early accumulation of lactic acid. In summary, this study showed that the presence of the halothane gene induced early energy depletion, which could be a primary reason causing AMPK activation, leading to accelerated glycolysis and an increased incidence of PSE meat. However, AMPK might also be activated by other mechanisms besides energy depletion, which warrants further studies.

Key words: pig, halothane gene, adenosine monophosphate-activated protein kinase, postmortem, glycolysis, pale, soft, and exudative

INTRODUCTION

The halothane gene, referred to as the porcine stress syndrome gene, is so called because it is responsible for porcine stress syndrome, which can be triggered by the anesthetic gas halothane (Eikelenboom and Minkema, 1974). The halothane gene is due to a mutation in the ryanodine receptor, which results in a hypersensitive calcium channel (Fujii et al., 1991). Extensive studies have been carried out on the effect of the halothane gene on animal performance and meat quality (Leach et al., 1996; Hamilton et al., 2000; Pedersen et al., 2001). The most obvious quality defect associated with the halothane gene is the high incidence of PSE pork (Briskey, 1964; Sellier and Monin, 1994). However, the mechanisms linking the halothane gene and PSE pork remain poorly defined.

The AMP-activated protein kinase (**AMPK**), a heterotrimer composed of a catalytic subunit (α) and 2 regulatory subunits (β and γ), is known as the energy status guardian within cells (Woods et al., 1996; Hardie and Carling, 1997; Carling, 2004). The AMPK plays a key role in inducing glycolysis in ischemic myocardium (Marsin et al., 2000; Sambandam and Lopaschuk, 2003; Musi et al., 2005). The AMPK is activated after ATP depletion or, more accurately, a rise in the AMP:ATP ratio within the cells (Corton et al., 1994). Our previous studies showed that AMPK controlled glycolysis in postmortem muscle of mice (Du et al., 2005; Shen and Du, 2005a,b; Shen et al., 2005). Because the presence of the halothane gene leads to hypersensitive calcium gating, it is likely that the halothane gene induces excessive muscle contraction, which causes early elevation of AMP:ATP ratio and early AMPK activation, increasing the incidence of PSE meat.

The object of this study was to evaluate the effects of the halothane gene on the AMP:ATP ratio, AMPK

activation, and glycolysis in postmortem LM and also on meat quality.

MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee.

Samples

Pigs were produced by AI using semen from a Yorkshire, halothane gene carrier boar to impregnate Hampshire sows, which were negative for the halothane gene and the Rendement Napole gene. A total of 16 pigs, 8 halothane negative (**NN**) and 8 halothane positive carriers (**Nn**), were slaughtered. The NN and Nn pigs were identified by the presence of a mutation in the ryanodine receptor as previously described (Otsu et al., 1991). The pigs were 6 mo old with an average BW of 120.9 ± 4.5 and 124.5 ± 5.2 kg for NN and Nn genotypes, respectively. The pigs were slaughtered in the Meat Laboratory at the University of Wyoming. Before slaughter, the pigs were held in lairage overnight to eliminate the effects of preslaughter transport on meat quality.

The pigs were stunned electrically (100 to 110 V, 10 to 15 s). After exsanguination, a sample of LM (about 20 g) between the 10th and 11th ribs on the left side of the carcass was obtained at 0 (immediately after exsanguination), 0.5, 1, 4, and 24 h postmortem. The LM samples were quickly cut into small pieces and mixed. A portion of these samples was used for biological analyses immediately, and the rest of the samples were snap-frozen in liquid nitrogen and stored at $-80°C$ until analyses.

At 24 h postmortem, the pig carcasses were ribbed at the 10th rib and subjected to carcass evaluation, which was conducted according to the guidelines of the Meat Evaluation Handbook (AMSA, 2001). Two loin chops were obtained between the 10th to 12th ribs from the right side of the carcasses. Loin chops were individually packaged (aerobic) and used for the measurements of meat quality.

Meat Quality Measurements

A portable pH meter (HI99161, Hanna Instruments Inc., Woonsocket, RI) with a spear-tipped glass electrode pH probe was used for the measurement of meat pH directly on the carcasses. The pH was measured at the LM between the eighth and ninth ribs at 0, 0.5, 1, 4, and 24 h postmortem. Ultimate pH refers to the pH of the LM at 24 h postmortem.

Surface color [Commission Internationale de l'Eclairage lightness (L*), redness (a*), and yellowness (b*)] of the loin chops was measured in triplicate on a freshly cut surface after a bloom time of at least 60 min at 4°C using a Hunter LabScan Colorimeter (Hunter Laboratory Inc., Reston, VA). The surface color of the chops was measured through the package. To do this, the same package materials were used to cover the white standard plate to eliminate the influence of the packaging materials on meat color. A CIE D–65 10° standard observer and a 2.54-cm viewing port were used (Du et al., 2001).

Boneless loin chops between the 10th to 12th ribs from the right side of carcasses were used for the measurement of drip loss. To do this, boneless loin chops were trimmed free of surrounding connective tissue, individually packaged in a large zip bag, sealed, and hung at 4°C. The chops were weighed before hanging and 24 h after hanging. Drip loss was expressed as the percentage of weight loss over the 24 h of suspension (Gardner et al., 2006).

Cooking yield was obtained by measuring the weights of boneless loin chops before and after cooking to an internal temperature of 71°C (Huff-Lonergan et al., 2002).

The tenderness of the loin chops was measured according to the guidelines of the American Meat Science Association (AMSA, 2001). Briefly, loin chops were pan broiled to an internal temperature of 71°C. Six cores of 1.27 cm in diameter were prepared from each cooked loin chop parallel to the longitudinal orientation of the LM fibers. A Warner-Bratzler shear force machine (G-R Manufacturing Co., Manhattan, KS) was used for the determination of tenderness.

Glycolytic Potential and Lactate Analysis

For lactate determination, a 0.3-g LM sample was homogenized in 900 μL of 0.9 N HClO$_4$ (Sigma, St. Louis, MO; Polytron homogenizer, IKA Works Inc., Wilmington, NC). The homogenates were then centrifuged at $13,000 \times g$ at 4°C for 5 min. The supernatants were removed and neutralized with 2 M KOH (Sigma). After centrifuging again as above to precipitate KClO$_4$, the extracts were used for lactic acid measurement. Lactate acid concentrations were determined by using a commercially available lactate analysis kit (Trinity Biotech, St. Louis, MO).

Glycolytic potential was assayed using 0-h postmortem LM. Glycogen, glucose, and glucose 6-phosphate were determined as previously reported (Shen et al., 2005). The glycolytic potential was calculated using the formula of Monin and Sellier (1985), where glycolytic potential $= 2 \times ([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]$, where the brackets indicate the concentration in micromoles per gram of muscle. The values were presented as micromoles of lactate equivalent per gram of wet LM tissue (μmol/g of muscle).

ATP, ADP, AMP, and Inosine Monophosphate Measurements

The ATP, ADP, AMP, and inosine monophosphate (**IMP**) concentrations in postmortem LM were determined by HPLC, as previously described (Veciana-

Nogues et al., 1997). Briefly, 0.3 g of powdered frozen LM samples were homogenized in 900 µL of ice-cold 0.9 N perchloric acid. After extraction for 30 min in an ice-water bath, the supernatant was obtained by centrifuging at 13,000 × g at 4°C for 10 min. The supernatant was neutralized with 2 M KOH and then centrifuged again to remove $KClO_4$. The neutralized supernatant was then passed through a 0.2-µm filter before being used for the HPLC (Beckman Instruments Inc., Fullerton, CA) analyses.

Ten-microliter aliquots of the final LM extraction were injected into the chromatography 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. Ultraviolet detection was carried out at a wavelength of 254 nm. The peaks were identified and quantified by comparison for retention time and peak area of known external standards, including ATP, ADP, AMP, and IMP (Sigma).

AMPK Activation and Glycogen Phosphorylase Activity

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 ethylene-bis(oxyethylenenitrile) tetraacetic acid; 1 mM dithiothreitol; 50 mM NaF; and 5 mM sodium pyrophosphate). The homogenates were centrifuged at 12,000 × g for 2 min at 4°C. The supernatants were then used for the measurements of AMPK and glycogen phosphorylase activities.

The AMPK and glycogen phosphorylase activities were measured as previously described (Shen and Du, 2005a,b). The AMPK activity was calculated as nanomoles of ATP incorporated into a SAMS (His-Met-Arg-Ser-Ala-Met-Ser-Glu-Leu-His-Leu-Val-Lys-Arg-Arg, Invitrogen, Carlsbad, CA) peptide·g of muscle^{-1}·min^{-1}. Glycogen phosphorylase activity was expressed as milligrams of glucosyl units assimilated into glycogen·g of muscle^{-1}·min^{-1}.

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 1 mL 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 13,000 × g for 5 min. The supernatant was then used for Fru-2,6-P$_2$ determination, as previously described (Dey and Harborne, 1990).

Statistical Analysis

Data were analyzed as a completely randomized design using PROC GLM (SAS Inst. Inc., Cary, NC). 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 the Fisher's Protected LSD test ($P < 0.05$). All data were expressed as means ± SEM.

RESULTS AND DISCUSSION

Energy Metabolism in Postmortem Muscle

In muscle, when ATP is consumed, it produces ADP and 1 phosphate. Two ADP can be rapidly converted to ATP and AMP by myokinase. Then, AMP can be converted to IMP due to the activity of AMP deaminase. To show those changes in postmortem muscle, ATP, ADP, AMP, and IMP concentrations in postmortem LM were determined in NN and Nn pigs (Table 1). At 0 h postmortem, there was no difference ($P < 0.05$) in ATP concentration between NN and Nn genotypes. The difference in ATP between these 2 genotypes became significant ($P < 0.05$) at 0.5 h postmortem, with average values of 5.18 ± 0.24 and 4.51 ± 0.21 µmol/g of muscle for NN and Nn genotypes, respectively. The difference in ATP concentration maintained at 1 h postmortem ($P < 0.05$), indicating faster energy consumption rate in the muscle of Nn genotype compared with NN genotype during early postmortem stage. After 4 h postmortem, ATP in postmortem muscle was largely depleted, and no difference in ATP concentration was detected between the NN and Nn genotypes (Table 1). No difference in ADP and AMP concentrations between postmortem muscle of NN and Nn genotypes was detected. However, a higher IMP concentration ($P < 0.05$) was observed in postmortem muscle of Nn genotype during the very early postmortem stage (Table 1). The IMP is a metabolite of AMP, and the IMP concentration was several times higher than AMP in postmortem muscle (Table 1), which indicates that most of the AMP was rapidly converted into IMP in postmortem muscle.

Based on these data, the halothane gene accelerated ATP depletion in postmortem LM during early postmortem stage, which led to a concomitantly higher (AMP+IMP):ATP ratio (Table 1). This increased energy depletion is linked to the mutation in ryanodine receptor, which results in a hypersensitive Ca^{2+} channel (Fujii et al., 1991). Preslaughter stress might stimulate the gating of this mutant ryanodine receptor, leading to Ca^{2+} influx, inducing muscle contraction and accelerating ATP utilization. It is reported that, even during careful handling, the stress accompanying preslaughter treatment was sufficient to trigger a higher rate of postmortem glycolysis in halothane-positive pigs (Lundström et al., 1989).

AMPK Activation and Glycolysis

The AMPK serves as an energy status gauge within cells. The AMPK is activated by an increase in AMP:ATP ratio (Winder, 2001; Carling, 2004; Hardie,

Table 1. ATP-related compounds in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes[1]

Item	0.0	0.5	1.0	4.0	24.0	SEM	Genotype × time
ATP concentration, μmol/g of muscle							
NN	5.64[a]	5.18[ax]	3.91[b]	0.80[c]	0.08[d]	0.23	$P < 0.0001$
Nn	5.15[a]	4.51[ay]	3.10[b]	0.40[c]	0.12[c]	0.22	
SEM	0.30	0.22	0.27	0.21	0.02		
ADP concentration, μmol/g of muscle							
NN	1.31[a]	1.60[a]	1.49[a]	0.87[b]	0.56[c]	0.10	$P < 0.0001$
Nn	1.29[a]	1.43[a]	1.48[a]	0.79[b]	0.50[b]	0.11	
SEM	0.11	0.09	0.12	0.12	0.07		
AMP concentration, μmol/g of muscle							
NN	0.10[b]	0.17[ab]	0.23[a]	0.20[a]	0.11[b]	0.03	$P < 0.001$
Nn	0.13	0.16	0.26	0.22	0.13	0.03	
SEM	0.02	0.03	0.03	0.04	0.02		
IMP concentration, μmol/g of muscle							
NN	0.97[dy]	1.54[dy]	2.48[c]	6.49[b]	7.71[a]	0.23	$P < 0.0001$
Nn	1.67[cx]	2.18[cx]	3.11[b]	6.96[a]	7.69[a]	0.26	
SEM	0.16	0.16	0.30	0.34	0.23		
AMP:ATP ratio							
NN	0.02[b]	0.03[b]	0.06[b]	0.42[b]	1.87[a]	0.26	$P < 0.0001$
Nn	0.02[c]	0.04[c]	0.09[c]	0.75[b]	1.19[a]	0.10	
SEM	0.00	0.01	0.01	0.11	0.42		
(AMP+IMP):ATP ratio							
NN	0.19[by]	0.34[by]	0.72[by]	18.85[b]	148.00[a]	17.03	$P < 0.0001$
Nn	0.37[bx]	0.54[bx]	1.18[bx]	41.95[b]	105.63[a]	14.31	
SEM	0.04	0.05	0.15	9.58	33.84		

[a–c]Within a row, means lacking a common superscript letter differ, P < 0.05, n = 8.
[x,y]Within a column, means lacking a common superscript letter differ, P < 0.05, n = 8.
[1]AMP = adenosine monophosphate; IMP = inosine monophosphate.

2004). Our previous studies showed that AMPK regulated glycolysis in postmortem mouse LM (Du et al., 2005; Shen and Du, 2005a,b). Thus, it is highly possible that the halothane gene induces high postmortem glycolytic rate and PSE meat through AMPK activation in postmortem muscle.

The AMPK activation and glycolysis in postmortem muscle of NN and Nn genotypes were analyzed. The AMPK in postmortem muscle of halothane carrier pigs was activated earlier and had higher activity immediately after slaughter, with AMPK activity at 1.53 ± 0.10 nmol of ATP·min^{-1}·g of muscle^{-1} for Nn genotype and 0.92 ± 0.08 nmol of ATP·min^{-1}·g of muscle^{-1} ($P < 0.05$) for NN genotype (Figure 1). The difference in AMPK activity between NN and Nn genotypes became larger ($P < 0.01$) at 0.5 h postmortem (Figure 1), increasing from 0.60 in difference to 0.85 nmol of ATP·min^{-1}·g of muscle^{-1} during the first half hour postmortem. The difference in AMPK activity between these 2 genotypes disappeared after 1 h postmortem. In summary, the presence of the halothane gene caused earlier ATP consumption and increased AMP:ATP ratio in postmortem muscle compared with normal pigs. Because AMPK is activated by increased AMP:ATP ratio, the earlier AMPK activation observed in Nn pigs should be due to enhanced ATP consumption in Nn pigs.

No difference in glycogen consumption was observed between NN and Nn pigs (Figure 2). At 24 h postmortem, a significant amount of glycogen remained in mus-

cle, which indicates that glycogen content in muscle might not be the limiting factor in postmortem glycolysis. Instead, reduced enzyme activity might be responsible for the decreased glycolysis during postmortem holding (Copenhafer et al., 2006). The glycolytic potential for NN pigs was 172 ± 15 and 198 ± 18 μmol/g of muscle for Nn pigs, without significant difference. A lower pH and higher lactic acid accumulation ($P < 0.01$) were detected in postmortem muscle of Nn genotype compared with those of NN genotype at 0.5- and 1-h postmortem (Figures 3 and 4), indicating accelerated glycolysis in postmortem muscle of Nn genotype (Fernandez et al., 2002). In fact, pH and lactic concentration were the same for NN and Nn genotypes at beginning (0 h postmortem). These data showed that the presence of the halothane gene may lead to early AMPK activation and accelerated glycolysis in early stage postmortem muscle. The AMPK is activated by an increase in AMP:ATP ratio (Winder, 2001; Carling, 2004; Hardie, 2004). Based on the data obtained in this study and our previous studies in mice (Du et al., 2005; Shen and Du, 2005b), we propose that the mutation in ryanodine receptor accelerates energy consumption in postmortem muscle, which activates AMPK, accelerates glycolysis, and increases the incidence of PSE meat. Besides ATP consumption, reactive oxygen species (**ROS**) produced in postmortem muscle might play a significant role in AMPK activation in postmortem muscle. It has been reported that ROS leads to the activation of AMPK
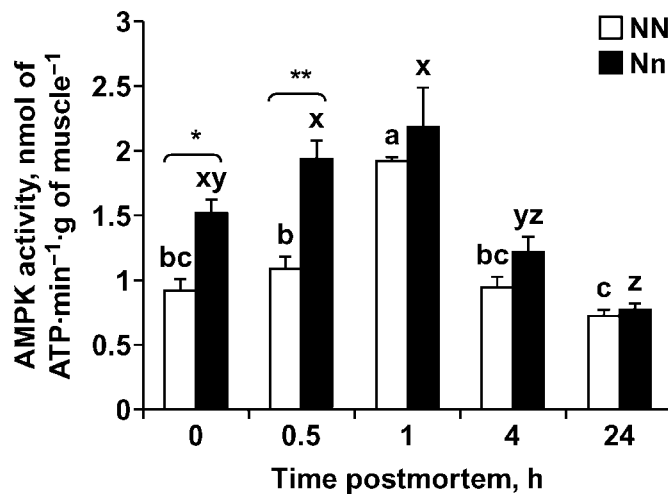
Figure 1. Adenosine monophosphate-activated protein kinase (AMPK) activity in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. *$P < 0.05$; **$P < 0.01$. [a–c]Within NN genotype, means lacking a common superscript letter differ, $P < 0.05$. [x–z]Within Nn genotype, means lacking a common superscript letter differ, $P < 0.05$.

(Hwang et al., 2004; Baron et al., 2005; Hwang et al., 2005). The role of ROS in postmortem glycolysis and PSE incidence is corroborated by the observation that antioxidants can prevent incidence of PSE meat. Using dietary antioxidant, α-lipoic acid supplementation, we observed a slower glycolysis in postmortem muscle and the activation of AMPK in postmortem muscle was inhibited (Shen et al., 2005; Shen and Du, 2005b). In pigs, dietary α-lipoic acid supplementation at 600 mg per pig per d increased the pH of pork at 45 min (Berg et al., 2003), strongly supporting the view that antioxidant slowed down glycolysis in early postmortem stage. Vita-
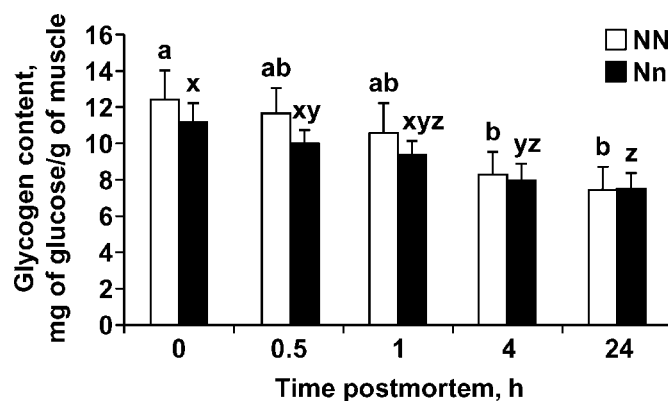

Figure 2. Glycogen glucose and glucose 6-phosphate content in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. [a,b]Within NN genotype, means lacking a common superscript letter differ, $P < 0.05$. [x–z]Within Nn genotype, means lacking a common superscript letter differ, $P < 0.05$.
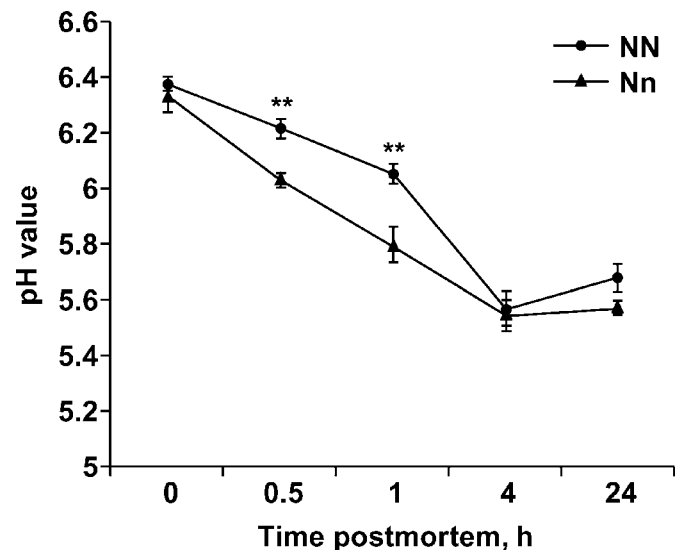

Figure 3. pH values in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. **$P < 0.01$.

min E was frequently used for supplementation in pigs and poultry. Several studies showed that vitamin E supplementation reduced drip loss in pigs (Asghar et al., 1991; Cheah et al., 1995) and prevented incidence of PSE meat in pigs (Cheah et al., 1995; Kerth et al., 2001) and poultry (Olivo et al., 2001).

AMPK Regulation of Glycolysis in Postmortem Muscle

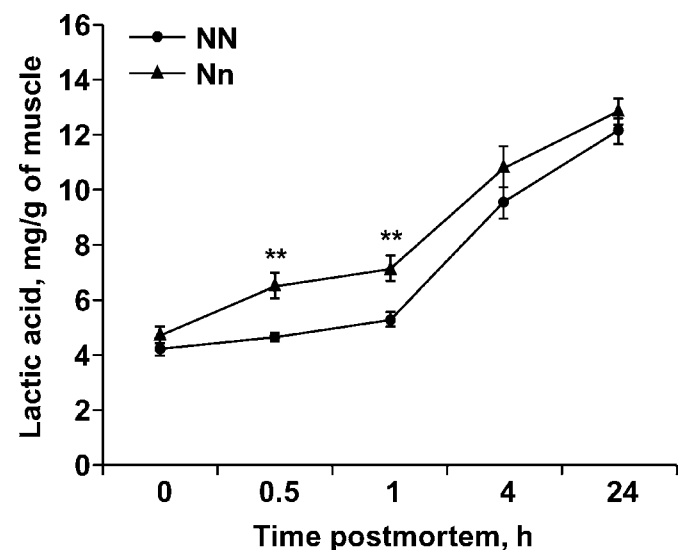The AMPK regulates glycolysis in ischemic cardiac muscle through 2 main pathways. One signaling path-

Figure 4. Lactic acid in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. **$P < 0.01$.
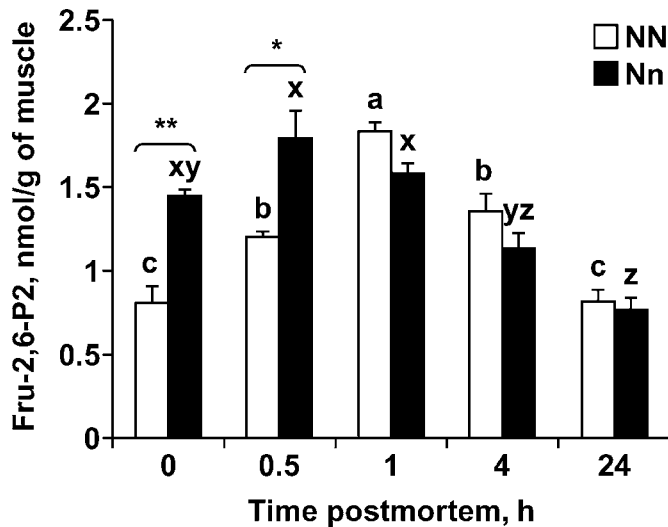
Figure 5. Fructose 2,6-biphosphate (Fru-2,6-P_2) in postmortem LM of halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. *$P < 0.05$; **$P < 0.01$. [a–c]Within NN genotype, means lacking a common superscript letter differ, $P < 0.05$; [x–z]Within Nn genotype, means lacking a common superscript letter differ, $P < 0.05$.

Figure 6. Glycogen phosphorylase activity in postmortem LM for halothane negative (NN) and halothane positive (Nn) genotypes, n = 8. [a,b]Within NN genotype, means lacking a common superscript letter differ, $P < 0.05$.

way is that activated AMPK phosphorylates and activates phosphofructokinase-2 (**PFK-2**; Marsin et al., 2000). The PFK-2 catalyzes the production of Fru-2,6-P_2, which is a potent allosteric activator of phosphofructokinase-1 (**PFK-1**), a most important rate-controlling enzyme in glycolysis. To verify this pathway, we measured the Fru-2,6-P_2 concentration in postmortem muscle. At 0 h postmortem, much higher Fru-2,6-P_2 concentration ($P < 0.01$) was detected in postmortem muscle of Nn genotype than that of NN genotype (Figure 5). This difference did not vanish until 1 h postmortem. Higher Fru-2,6-P_2 concentration in postmortem muscle of halothane carrier pigs indicated higher PFK-1 activity, which should account for the higher glycolysis in Nn muscle during early postmortem stage.

According to report, AMPK also increases glycolysis by phosphorylating and activating phosphorylase kinase, which then phosphorylates and activates glycogen phosphorylase, an enzyme that controls glycogenolysis (Young et al., 1997; Fraser et al., 1999; Russell et al., 1999). In our previous studies on mice, glycogen phosphorylase activity was correlated with AMPK activity (Du et al., 2005; Shen and Du, 2005a,b). In this pig study, glycogen phosphorylase activity did not correlate well with AMPK activity. No difference in glycogen phosphorylase activity was found during the whole postmortem period (Figure 6). Because glycogen phosphorylase is acid sensitive and easy to be denatured in postmortem muscle (Alvarado and Sams, 2002, 2004), this discrepancy between AMPK and glycogen phosphorylase activities may be caused by the denaturation of phosphorylase due to the low pH in combination with high temperature in pork muscle during early postmortem stage.
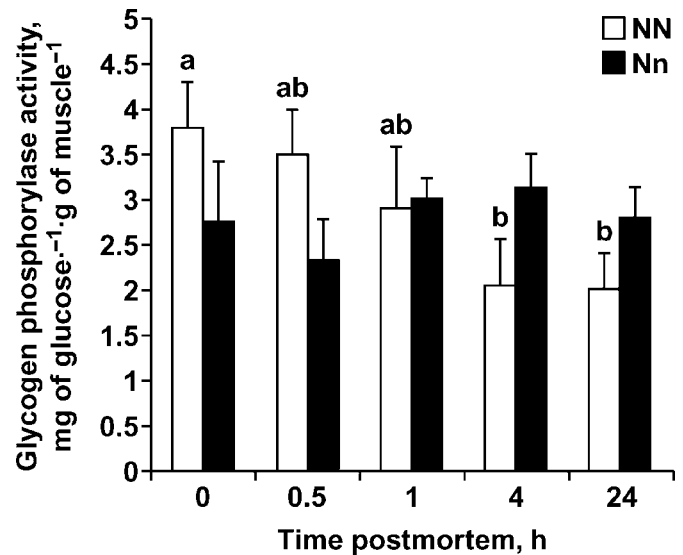
Meat Quality and Carcass Characteristics of Halothane Negative and Carrier Pigs

It is well accepted that the halothane gene elevates glycolysis, which results in low pH during early postmortem stage (Sellier and Monin, 1994; Rosenvold and Andersen, 2003). Just as the name porcine stress syndrome gene indicates, carrier pigs are highly sensitive to stress (Mittchel and Heffron, 1982; Lundström et al., 1989). Our results confirmed this notion. Although pigs were rested overnight before slaughter and careful handling was employed to reduce preslaughter stress, the presence of the halothane gene negatively affected meat quality. Meat from the Nn genotype pigs had higher drip loss and lightness value ($P < 0.05$) when compared with the NN genotype (Table 2), indicating higher tendency to develop PSE meat.

Some researchers observed no noticeable difference in meat quality between NN and Nn pigs (Sellier and Monin, 1994). Most researchers tend to agree that Nn pigs have intermediate meat quality between NN and

Table 2. Meat quality of pork of halothane negative (NN) and halothane positive (Nn) genotypes

Item	NN	Nn	SEM
Drip loss, %	3.18[b]	5.78[a]	0.49
Cooking yield, %	77.56	74.80	1.24
Shear force, kg	4.49	5.19	0.34
Lightness, L*	58.54[b]	62.09[a]	0.89
Redness, a*	10.68	10.34	0.60
Yellowness, b*	16.76	17.63	0.41

[a,b]Within a column, means lacking a common superscript letter differ, $P < 0.05$, n = 8.

Table 3. Carcass characteristics of pigs of halothane negative (NN) and halothane positive (Nn) genotypes[1]

Item	NN	Nn	SEM
Live weight, kg	120.88	124.47	4.91
Carcass weight, kg	93.33	95.20	4.13
Dressing percent	77.13	76.41	0.56
Length, cm	83.42	86.60	1.64
Last rib fat, cm	1.84	1.65	0.19
Loin eye area, cm^2	45.16	48.71	2.34
Marbling	1.38	1.25	0.17

[1]n = 8.

nn pigs, which have the most noticeable quality problems (Rosenvold and Andersen, 2003). It has been suggested that the halothane gene changes from being a recessive gene in lightweight pigs (80 kg) to being additive at normal market weight (100 to 105 kg), and then become a dominant gene in heavyweight pigs (130 kg; Sather et al., 1991). The pigs used in this study weighed 120.88 kg for NN and 124.47 kg for Nn pigs (Table 3). Thus, the presence of the halothane gene is expected to become dominant and negatively affects meat quality.

Live weight, carcass weight, dressing percent, carcass length, last rib fat thickness, loin eye area, and marbling did not differ between NN and Nn pigs (Table 3), though the length and loin eye area tended to be higher and the last rib fat thickness and marbling tended to be lower in Nn pigs (Table 3). Lack of difference could be due to the relatively low number of pigs used in this study.

CONCLUSION

The halothane gene, a mutation in ryanodine receptor, accelerates energy depletion in postmortem muscle, which should be responsible for the rapid and early AMPK activation in postmortem muscle. To be a main kinase regulating glycolysis, activated AMPK is expected to accelerate glycolysis in postmortem muscle, resulting PSE meat. The AMPK regulates glycolysis in postmortem muscle through enhancing Fru-2,6-P_2 production.

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