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Deactivation of AMPKα/GSK-3β Leads to High-Level Glycogen Synthase in Poor Pork Meat Quality

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Abstract

We investigated glycogen synthase and upstream regulatory proteins determining meat quality in porcine *longissimus dorsi* at 24 h *post-mortem*. The general meat quality traits of 300 muscle samples were estimated. Muscle samples were classified into two groups based on ultimate pH of meat 24 h *post-mortem* (pH_{24h}). Muscle glycogen synthase belonging to the low pH_{24h} group showed remarkably higher expression than that in the high pH_{24h} group. AMP activated protein kinase (AMPK) and glycogen synthase kinase 3 (GSK-3) as negative regulators of glycogen synthase deactivated the suppression of glycogen synthase by phosphorylating Ser485 of AMPK and Ser9 of GSK-3β. These inhibitory kinases lead to high glycogen synthase expression. These results suggest that the accumulation of glycogen by up-regulating glycogen synthase and inhibiting AMPKα and GSK-3β was rapidly converted to lactate resulting in acidic meat. This molecular clue representing acidic meat based on *post-mortem* muscular pH can be used to estimate meat quality via Akt-AMPKα/GSK-3β-mediated up-regulation of glycogen synthase.

Keywords: glycogen synthase, AMPK, GSK-3β, porcine meat quality, *post-mortem* pH

1. Introduction

Porcine meat quality is determined by various intrinsic factors such as meat color, water holding capacity (WHC), hardness, and fat content of muscle tissue (Rosenvold et al., 2001; Hocquette et al., 2005). Pale, soft, and exudative (PSE), which are poor meat qualities, are very problematic in the pork industry (Barbut et al., 2008). Severe PSE quality occurs during the early *post-mortem* stage and is triggered by a rapid pH fall and high temperature in the muscle. The pale color and WHC of meat are influenced by a number of factors including low ultimate pH and protein denaturation (Briskey & Wismer-Pedersen, 1961; Warner 1997; Bowker et al., 2000; Wang et al., 2009). Thus, *post-mortem* muscular pH is a critical factor determining meat quality. In general, the final pH of meat is dependent on muscular glycogen content after slaughter.

Muscular glycogen is an important energy source for muscle contraction. However, fast glycogen degradation plays a major role in changes occurring in muscle *post-mortem* and induces the accumulation of lactic acid in

muscle tissue (Bowker et al., 2000; Pösö & Puolanne, 2005). The accumulation of lactic acid leads to a rapid decline in muscular pH known as acidic meat (referred to as poor meat quality) and *vice versa* during the *post-mortem* period, particularly during the early phase (Ryu et al., 2005). Thus, *post-mortem* pH value has a considerable effect on meat quality. In particular, the ultimate pH of pig muscle at 24 h post-slaughter (pH_{24h}) is a critical factor determining meat quality traits. In addition, pH_{24h} is closely related to WHC (Warner et al., 1997; Kang et al., 2010; Nam et al., 2012).

The relationship between glycogen and meat quality has been widely studied. For example, pork muscle with low glycogen and high lactate levels has a low muscular pH at 45 minute *post-mortem* (Choe et al., 2008). A rapid pH fall caused by accelerated glycogenolysis and ATP breakdown produces the PSE defect (Pryzbylski et al., 2006). Nevertheless, why glycogen synthases (GSs) are differentially expressed in low and high pH muscle, which refer to low and good quality meat, respectively, has not been addressed.

In this study, we evaluated pork meat quality by elucidating the molecular mechanism occurring during *post-mortem* metabolism. We examined the expression levels of GS and upstream regulatory proteins involved in *post-mortem* muscular pH affecting glycogen. In several studies, AMP-activated protein kinase (AMPK) has been shown to regulate muscular glycogen synthesis (Jørgensen et al., 2004). By quantitative immunoblot analysis, the expression levels of GS and regulatory proteins such as phosphorylated AMPK at Ser485 (inhibitor of GS) and Thr172 (activator of GS) and the AMPK-upstream regulator Akt were compared between low and high pH_{24h} groups. Taken together, this molecular study of *post-mortem* muscular pH provides possible indicators of pork meat quality traits.

2. Materials and Methods

2.1 Animals and Meat Samples

A total of 300 Berkshire pigs were bred under the same conditions (Da-San-Genetics Co. Ltd., Namwon, Korea) and then slaughtered in 10 batches according to standard slaughtering procedures, when their body weight reached 80-90 kg as described previously (Kang et al., 2010). Subsequently, the samples were used for meat quality trait evaluation analyses.

2.2 Meat Quality Evaluation

Pork meat quality such as carcass weight, backfat thickness, pH_{24h}, meat color, WHC, chemical composition (collagen, fat, and protein), drip loss, cooking loss, and Warner–Bratzler shear force were evaluated. Samples of all Berkshire pigs (n = 300) including 133 castrations, 165 boars, and 2 sows, were divided into two groups such as the low pH value group (LpH_{24}; the lower 5% pH_{24h} of the 300 samples, n = 15) and the high pH value group (HpH_{24h}; the upper 5% pH_{24h}, n = 15).

2.3 Glycogen and Lactate Contents Measurement

Muscular glycogen content was measured according to Choe et al. (2008). Ten samples were randomly chosen from the LpH_{24} and HpH_{24} groups. In brief, approximately 2 g of muscle tissue was minced, suspended in 10 mL of 9% (v/v) cold perchloric acid, and thoroughly homogenized. After centrifugation (15,000 × g at 4°C for 20 min), the supernatant was used for glycogen determination. Iodine color reagent was added to a glycogen standard. Linear regression equations of the glycogen standard for each set of samples were applied to determine glycogen concentration in the corresponding samples. Lactate content was determined spectrophotometrically (absorbance at 340 nm) using a commercial kit (Boeringer, Mannheim, Germany) as described previously (Choe et al., 2008) with minor modifications. Briefly, approximately 5 g of muscle sample was homogenized in 20 mL perchloric acid (1 M), and potassium hydroxide was added to neutralize the solution. The final volume was brought up to 10 mL with distilled water. Lactate concentration was measured after 20 min of refrigeration and centrifugation.

2.4 Muscle Protein Extraction and Quantification

Muscle tissues (30 mg) were homogenized in lysis buffer purchased from iNtRON (Daejeon, Korea) according to the manufacturer's protocol. Protein concentration was quantified in triplicate using the Coomassie Dye Binding Assay (Bio-Rad, Hercules, CA, USA) employing BSA as the standard (Bradford, 1976).

2.5 Western Blot Analysis

Protein expression was examined by Western blot analysis according to Lee et al. (2007). We selected eight samples randomly from each pH_{24h} group and subjected them to Western blot analysis. An equal amount of total protein (30 μg) was separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The blotted membranes were blocked for 1 h in TTBS (50 mM Tris-HCl, pH 8.2,

0.1% v/v Tween 20, and 150 mM NaCl) containing 5% (w/v) nonfat dry milk. Subsequently, the membranes were incubated overnight at 4°C with primary antibodies to glycogen synthase (1:4000, #3886, Cell Signaling Technology, MA, USA), AMPKα (1:4000, #2532, Cell Signaling Technology, MA, USA), p-AMPKα (Ser485) (1:1000, #4185, Cell Signaling Technology, MA, USA), p-AMPKα (Thr172) (1:1000, #2531, Cell Signaling Technology, MA, USA), Akt (1:4000, #1085-1, EPIT-MICS, CA, USA), p-Akt (Ser473) (1:1000, #4058, Cell Signaling Technology, MA, USA), GSK-3β (1:1000, #sc-8257, Santa Cruz, CA, USA) p-GSK-3β (Ser9) (1:1000, #9336, Cell Signaling Technology, MA, USA), and GAPDH (1:6000, #sc-25778, Santa Cruz, CA, USA). Blots were washed with TTBS and incubated with horse radish peroxidase-conjugated monkey anti-rabbit IgG secondary antibody at 1:4000 dilutions for GS, AMPKα, Akt, GAPDH, whereas 1:2000 dilution was used for both p-AMPKα and p-AKt. Secondary monkey anti-goat IgG antibody (1:2000, #sc-2020, Santa Cruz, CA, USA) was used for GSK-3β and p-GSK-3β. After blotting, the membranes were washed with TTBS, the immunoblot signals were detected using an enhanced chemiluminescence and visualized with a LAS-4000 (GE Healthcare, Uppsala, Sweden). GAPDH was used as an internal control. All data were analyzed using a paired Student's *t*-test, and difference with a *P* value.

3. Results and Discussion

3.1 Meat quality Evaluation in Berkshire Muscle

Post-mortem pH values were measured from 300 Berkshire *longissimus dorsi* 24 hours after slaughter. The lowest 5% pH values (LpH_{24}) and highest 5% pH values (HpH_{24}) were selected based on the 24 hour *post-mortem* values. The mean values for the meat quality traits of the 300 Berkshires are shown in Table 1. Berkshire meat generally exhibits a high *post-mortem* pH value and WHC compared with those of other breeds, whereas drip and cooking losses are lower than those of other breeds (Suzuki et al., 2003; Lee et al., 2011). Our previous study indicated that porcine serum metabolites are clearly different between low and high pH groups based on the pH_{24h} value of the *post-mortem* samples by ^1H nuclear magnetic resonance and high performance liquid chromatography analyses (Kim et al., 2011). Taken together with these reports, the present results indicate that pH_{24h} is an important factor for evaluating meat quality.

Table 1. Characters of meat quality traits in Berkshire longissimus dorsi (n = 300)

Trait		Average ± SD
Carcass weight (kg)		85.51 ± 4.70
Backfat thickness (mm)		24.95 ± 5.23
pH_{24h}*		5.76 ± 0.15
	CIE *L***	48.42 ± 2.48
Meat color	CIE *a***	6.28 ± 0.81
	CIE *b***	3.91 ± 1.07
Water-holding capacity (%)		61.31 ± 2.10
	Collagen	0.88 ± 0.14
Chemical composition (%)	Fat	2.84 ± 1.06
	Protein	24.18 ± 0.78
Drip loss (%)		3.67 ± 1.27
Cooking loss (%)		23.85 ± 4.51
Warner-Bratzler shear force (kg)		2.59 ± 0.58

* The pH value 24 h after slaughter.

** CIE L, a, and b represent meat color lightness, redness, and yellowness, respectively.

We chose individual meat samples separately based on the pH_{24h} corresponding to the lower 5% (n = 15, LpH_{24h}) and the higher 5% group (n = 15, HpH_{24h}). The average pH_{24h} values for the low and high pH groups were 5.52 ± 0.02 and 6.16 ± 0.09, respectively ($P < 0.01$) (Figure 1 and Table 2). The pH_{24h} value of LpH_{24h} ranged from 5.48-5.54, whereas that for the HpH_{24h} group was 6.07-6.31. Besides, middle pH_{24h} group (n = 15) shown 5.73 ± 0.0 which are ranged from 5.73-5.74.

Table 2. Characters of meat quality traits in Berkshire *longissimus dorsi* groups based on pH value at 24 hour *post-mortem* corresponding to low pH_{24h} (n = 15) and high pH_{24h} (n = 15) groups

Traits		Low pH_{24h} group	High pH_{24h} group
		Average ± SD	Average ± SD
Sample number		15	15
Carcass weight (kg)		84.93 ± 7.24	86.67 ± 3.77
Backfat thickness (mm)		26.73 ± 4.50	27.07 ± 5.56
pH_{24h}*		5.52 ± 0.02	6.16 ± 0.09[b]
Meat color	CIE *L***	50.21 ± 1.54	45.17 ± 1.71[b]
	CIE *a***	6.68 ± 0.74	6.17 ± 0.71
	CIE *b***	5.03 ± 0.83	2.60 ± 0.59[b]
Water-holding capacity (%)		60.86 ± 2.68	63.28 ± 2.38[a]
Chemical composition (%)	Collagen	0.68 ± 0.12	0.90 ± 0.20
	Fat	3.49 ± 1.52	3.01 ± 0.90
	Protein	24.76 ± 0.71	23.48 ± 0.66[b]
Drip loss (%)		4.66 ± 1.33	2.83 ± 1.23[b]
Cooking loss (%)		24.56 ± 6.26	19.74 ± 4.30[a]
Warner-Bratzler shear force (kg)		2.71 ± 0.67	2.30 ± 0.48

* The pH value 24 h after slaughter.

** CIE *L*, *a,* and *b* represent meat color lightness, redness, and yellowness, respectively.

a, $P < 0.05$; b, $P < 0.01$.

Lightness (*L*) and yellowness (*b*) of the meat samples were significantly lower in HpH_{24h} group than those in the LpH_{24h} group. But, redness (*a*) was not significantly different between the two groups. WHC of meat is one of the most important properties as it affects the quality of the end meat product (Huff-Lonergan and Lonergan, 2005). WHC was higher as a percentage in the HpH_{24h} group than that in the LpH_{24h} group ($P < 0.05$), whereas drip loss (%) and cooking loss (%) were *vice versa* with a significant difference ($P < 0.01$) between the two groups.

3.2 Quantitative Protein Comparison

Berkshire breeds have excellent meat quality (Suzuki et al., 2003; Lee et al., 2011). The differences in the genetic effects between individuals such as single nucleotide polymorphisms have broad effects on meat quality traits (Dalvit et al., 2007; Gao et al., 2007; Williams, 2008). Proteome strategies have been attempted to understand the relationship between protein profiles and meat quality traits (Lametsch et al., 2003; Hwang et al., 2005; Kwaskborski et al., 2008; Te Pas et al., 2009). However, potential meat quality protein markers are not as well considered for evaluating as those of other meat quality traits such as genetic variations. Berkshire *longissimus dorsi* muscle has been used to quantitatively compare proteins between low and high pH_{24h}, in which the dominant expressions of Igc, Prep, Ldhb, Aco2 were identified in the LpH_{24h} group by shotgun proteome analysis (Nam et al., 2012).

3.2.1 Effect of GS on Meat Quality in Berkshire Muscle

Post-mortem glycogenolysis has a significant effect on meat quality (Przybylisk et al., 2006). Muscle glycogen is one of the most important energy sources for muscle contraction. Glycogen degradation during the *post-mortem* period has a considerable effect on meat quality. The levels of glycogen and ATP decrease when oxygen is no longer available such as at slaughter and then muscle glycogen degradation during *post-mortem* produces lactic acid that accumulates and lowers muscle pH (Kang et al., 2010; Przybylisk et al., 2006). The analysis of enzymes related to glycogen synthesis and degradation during the *post-mortem* period can be a useful way to discover protein biomarkers for evaluating meat quality.

GS (glycogen synthase, UDP-glucose-glycogen glucosyltransferase), a key enzyme in glycogen synthesis, converts glucose to glycogen (Bouskila et al., 2010). We analyzed the protein expression levels of GS between the LpH_{24h} and HpH_{24h} groups in 24 hour *post-mortem* muscle (Figure 1). GS was expressed significantly higher in LpH_{24h} than in HpH_{24h}. The accumulated glycogen levels in LpH_{24h} would be expected to be higher than that of HpH_{24h} due to highly expressed GS. Consequently, LpH_{24h} muscle may have produced more lactic acid than that of HpH_{24h} muscle, which leads to acidic meat (know as poor meat quality) and accelerates the fall in meat pH. Accumulation of lactic acid causes a rapid decline in muscle pH during the early *post-mortem* period (Ryu et al., 2005). These results suggest that GS can be used as a critical trait to evaluate meat quality.

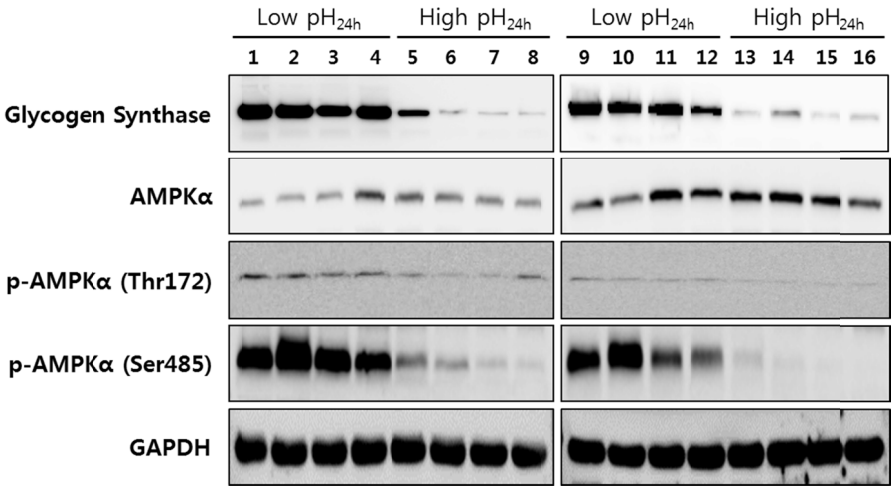

Figure 1. Western blot analysis of glycogen synthase, AMPKα and phospho-AMPKα in low and high pH value at 24 hour post-mortem (pH_{24h}) of Berkshire *longissimus dorsi*. Randomly selected samples from the low and high pH_{24h} groups were subjected to Western blotting to compare the expression levels of glycogen synthase, AMPKα, and phospho-AMPK. GAPDH was used as the control

3.2.2 Down-Regulation of GS by p-AMPKα at Ser485

AMPK is a serine-threonine kinase and a highly conserved enzyme from yeasts to animals that plays a key role in cellular energy homeostasis (Carling, 2004). AMPK is a heterotrimeric protein complex consisting of three subunits with a catalytic α and regulatory β and γ domains that together make a functional enzyme. This kinase is activated by cellular or environmental stressors such as low glucose, hypoxia, and heat shock in which AMPK can sense the decreasing ATP level in cells while increasing AMP/ATP ratios (Williams & Brenman, 2008). Shen and coworks (2007) reported that early AMPK activation was observed in early *post-mortem* stage of porcine muscle in the presence of halothane gene, leading to accelerated glycolysis and increasing the incidence of PSE meat. We analyzed the protein expression level of AMPK between the LpH_{24h} and HpH_{24h} groups (Figure 1). The AMPK expression level was not significantly different between the two groups. AMPK inhibits GS at a low energy level (know as decreasing ATP level) under hypoxia such as the *post-mortem* condition.

The α subunit of AMPK contains several residues that can be phosphorylated. One of these residues is threonine-172, and phosphorylation at this site is essential for AMPK activity (Carling, 2004). The expression levels of AMPKα phosphorylated at Thr172 were not significantly different between the low and high pH_{24h} groups (Figure 1). These results do not explain why GS was differentially expressed between LpH_{24h} and HpH_{24h}. Next, we focused on phosphorylation at serine-485 of AMPKα. AMPKα phosphorylated at Ser485 was remarkably expressed in the LpH_{24h} group compared to that in the HpH_{24h} group (Figure 1). The role of phosphorylation at Ser485 is poorly understood (Horman et al., 2006). Kayampilly and Menon (2008) reported that phosphorylation of AMPK at serine residues (Ser485/491) inhibits hormonally stimulated AMPK activity. Carling (2004) suggested that phosphorylation of AMPK at Ser485 inhibits phosphorylation at Thr172 by AMP-kinase. Furthermore, AMPK phosphorylated at Ser485 has been proposed to have an inhibitory effect on AMPK activation in primary porcine aortic endothelial cells (Zu et al., 2010). Together with these reports, the current results provide a clear indication that p-AMPKα (Ser485) plays an important role diminishing the effects of AMPK activity and GS under *post-mortem* conditions in porcine *longissimus dorsi*.

Ten samples were randomly selected from each group and their contents were measured to evaluate the changes in glycogen and lactate (surrogate to lactic acid) contents between the low and high pH_{24h} groups. No significant difference was observed in glycogen content between the two groups at 24 h *post-mortem* (Figure 2). However, the lactate content of the low pH_{24h} group was significantly higher than that of the high pH_{24h} group (Figure 2). Ryu et al. (2005) reported that glycogen content is not different in metabolic rate or meat quality classes at 24 h *post-mortem*. Moreover, lactate content was not different between the meat quality classes at 24 h *post-mortem* even though it is not significantly related to metabolic rate.

Accumulation of glycogen contents by highly expressed GS in the low pH_{24h} group may have resulted in active degradation of glycogen, in turn, producing lactic acid, which leads to acidic meat and poor meat quality (Figures 1 and 2). Taken together with these reports, the current results provide a clear indication that GS expression could be a useful meat quality protein marker.

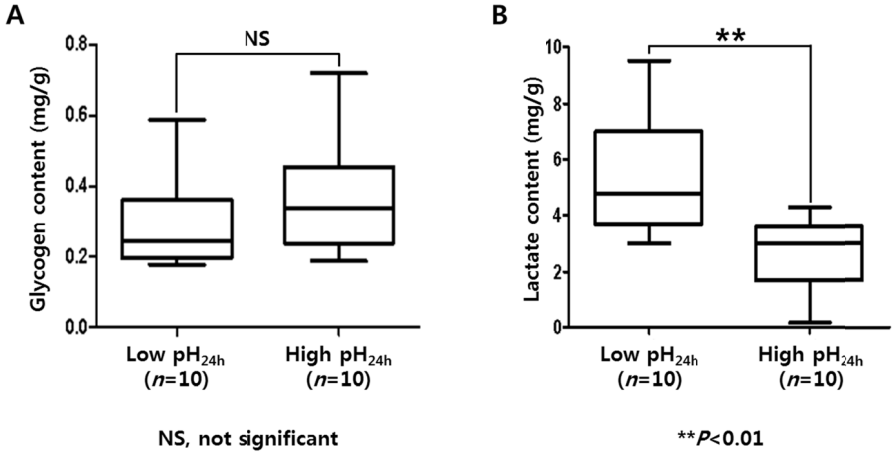

Figure 2. Glycogen and lactate contents of Berkshire *longissimus dorsi* in low and high pH value groups at 24 hour post-mortem (pH_{24h})

3.2.3 Down-Regulation of AMPKα and Glycogen Synthase Kinase 3β (GSK-3β) by Akt

Akt is a serine/threonine protein kinase that plays a critical role in multiple cellular processes such as glucose metabolism, apoptosis, and transcription (Manning & Cantley, 2007). Akt can be phosphorylated at Thr308 and Ser473 of the Akt protein itself. Three-phosphoinositide-dependent kinase 1 (PDK1) phosphorylates Thr-308 on the activation loop of the Akt kinase domain, when PDK1 binds to phosphotadylinositol-3,4,5-triphosphatate on the plasma membrane. Additionally, Akt can be phosphorylated at Ser473 on the hydrophobic motif by mammalian target of rapamycin complex 2 (Manning & Cantley, 2007). GSK-3 is a ubiquitous serine/threonine protein kinase that negatively regulates glycogen synthesis in response to insulin (Patel et al., 2008). GSK-3 is rapidly phosphorylated in the *N*-terminal domain serine residue (Ser21 of GSK-3α and Ser9 of GSK-3β) on insulin or other growth factors in mammalian cells (Fang et al., 2000; Patel et al., 2008). Porcine GSK-3β isoforms identified from various tissues, and the effect of different isoform on GS activity were analyzed (Wang et al., 2012a). Molecular characterization of porcine GS was reported (Wang et al., 2012b). Glycogen synthase 1 (GS1) was highly expressed in the porcine skeletal muscle, and the transcription was not significantly affected by insulin treatment. However, the information on porcine GSK-3β and GS are still very limited. GS is well known as a direct target of GSK-3 and a regulator of cellular glucose homeostasis. Active GSK-3 phosphorylates the serine residues of GS, resulting in the inactivation of GS and subsequent reduction of glycogen synthesis.

We analyzed the protein expression levels of GSK-3β and phospho-GSK-3β at Ser9 between the LpH_{24h} and HpH_{24h} groups (Figure 3). GSK-3β was dominantly expressed at LpH_{24h}. Phospho-GSK-3β at Ser9 expression was quite different between the two groups. Similarly, Akt and phospho-Akt at Ser473 were highly expressed in LpH_{24h} compared to those at HpH_{24h} (Figure 3). Phospho-GSK-3β at Ser9 by insulin inhibited its own GSK-3 kinase activity leading to activation of GS. Serine residues of GSK-3 isoforms have been identified as targets of activated Akt, resulting in inhibition of GSK-3 activity (Sutherland et al., 1993; Halse et al., 1999). The isoforms of GSK-3 are phosphorylated and inactivated by cAMP-dependent protein kinase A (Fang et al., 2000).

Epidermal growth factor also inhibits GSK-3 in human muscle cells (Halse et al., 1999). The lack of GSK-3β expression in skeletal muscle of mice results in improved glucose tolerance (Patel et al., 2008). In addition, overexpression of active Akt in transgenic mice reduces heart phospho-AMPKα at Thr172 (Kovacic et al., 2003). Taken together with these reports, the current results provide strong evidence that phospho-Akt (Ser473) phosphorylates both Ser485 of AMPKα and Ser9 of GSK-3β, resulting in the inhibition of AMPKα and GSK-3β kinase activity followed by activation of GS to produce more glycogen under *post-mortem* conditions in porcine muscle (Figure 4).

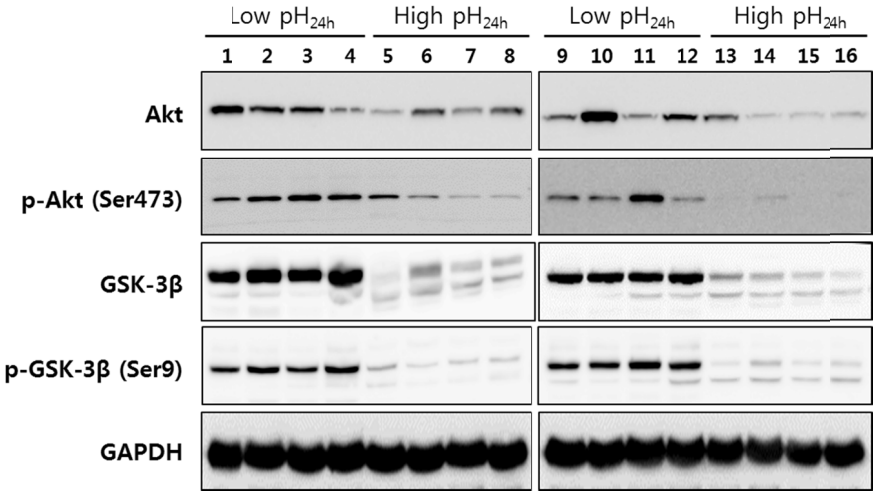

Figure 3. Western blot analysis of Akt and phospho-Akt in low and high pH value at 24 hour post-mortem (pH_{24h}) of Berkshire *longissimus dorsi*. Randomly selected samples from low and high pH_{24h} groups were subjected to Western blotting to compare the expression levels of Akt, glycogen synthase kinase 3β (GSK-3β), and phosphorylated forms. GAPDH was used as the control

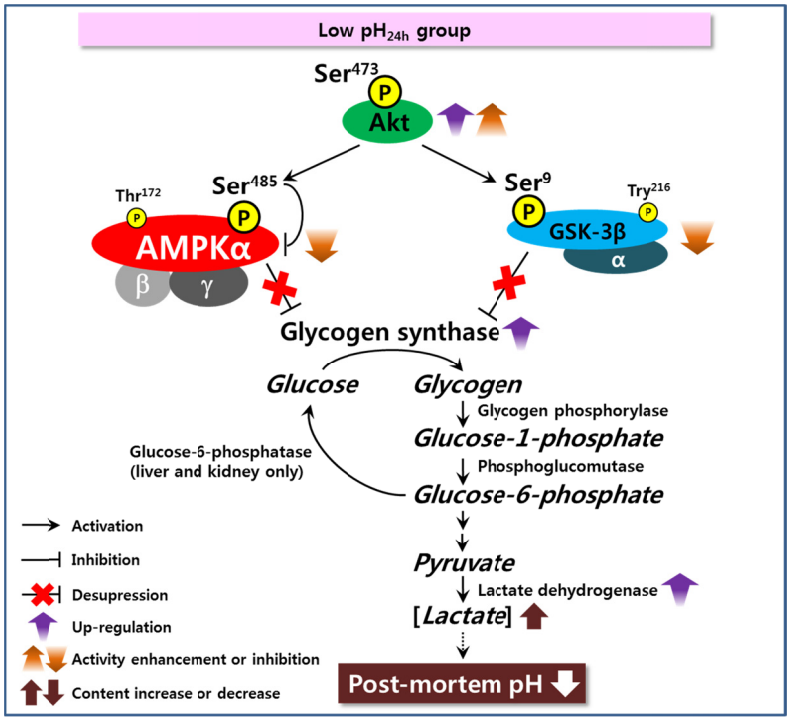

Figure 4. Molecular working model for low pH values at 24 hour post-mortem (pH_{24h}) of post-mortem porcine muscle

Glycogenolysis, the breakdown of glycogen to glucose-1-phosphate and glucose, is initiated by glycogen phosphorylase, and then the phosphate group is shifted to glucose-6-phosphate by phosphoglucomutase. The rate of glycogenolysis, phosphorylase activity, and lactate production rapidly increases in the perfused rat heart under anoxia, which is a surrogate to the *post-mortem* condition (Cornblath et al., 1963). In our previous shotgun proteomics report, lactate dehydrogenase B chain, which catalyzes the interconversion of pyruvate to lactate, was highly expressed in the low pH meat quality group compared to that in the high pH group (Nam et al., 2012). In summary, we suggest that muscle glucose was converted to glycogen by GS, followed by increased lactate concentration through glycogenolysis and glycolysis, which leads to a drop in pH at 24 h *post-mortem* (Figure 4).

4. Conclusions

Although several pork meat quality traits analyses have been conducted to analyze the metabolic rate and general proteome profile under different meat conditions, no study has investigated how they regulate GS expression under different meat conditions related to good and poor meat quality. Thus, understanding the biochemical perspective is an important issue to facilitate the signal transduction cascade analysis in different meat conditions to evaluate meat quality traits. Highly expressed GS in the low pH_{24h} group suggests that accumulated glycogen produced more lactic acid leading to acidic meat. AMPK and GSK-3 are negative regulators of GS. However, phospho-Akt (Ser473) phosphorylated GSK-3β (Ser9) which acts as an Akt substrate and a regulatory site. This phosphorylation inactivated the kinase leading to decreased phosphorylation and activation of GS. Furthermore, phosphorylation of AMPKα at Ser485 and inhibition of AMPKα by activated Akt deactivated the suppression of GS and increased GS expression. Thus, our results suggest that glycogen synthesis occurs in a complex manner after slaughter, where the Akt-AMPKα/GSK-3β complex plays a pivotal role under different meat conditions. Although this study is an initial biochemical investigation into meat quality during the *post-mortem* period, we believe that this kind of study provides new insight into the biochemical mechanism and provides biochemical protein markers for meat quality evaluations, particularly for the porcine industry.

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