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Effect of muscle-fiber type on glycogenin-1 gene expression and its relationship with the glycolytic potential and pH of pork

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ABSTRACT. This study analyzed the effect of muscle-fiber type composition on glycogenin-1 (GYG) gene expression and its impact on pH. The longissimus dorsi (LD) muscle contains more type IIB fibers (75.10%) than does the psoas major (PM) muscle (41.58%), while the PM has more type I (3.65 *vs* 0.94%), type IIA (34.15 *vs* 10.63%), and type IIX (20.62 *vs* 13.33%) fibers. Compared with PM, glycolytic potential (GP), $pH_{45\ min}$, and ΔpH from 45 min to 24 h post-mortem were all relatively higher in LD. Glycogen metabolites (lactate and GP) were negatively correlated with $pH_{24\ h}$ and positively correlated with ΔpH. Expression of GYG was generally higher in LD. GYG expression was positively correlated with glycogen metabolite (lactate and GP) content and ΔpH, and was negatively correlated with $pH_{24\ h}$. These data confirm that the muscle-fiber type and GP have significant effects on ultimate pH and pH decline, and suggest that expression of GYG in muscles is related to the metabolism of glycogen and may impact GP, ΔpH, and ultimate pH. High expression of GYG was associated with a high

glycogen content, large pH decline, and low ultimate pH in muscles post-mortem.

Key words: Pig; Glycogenin-1; Muscle-fiber type; Glycolytic potential; Myosin heavy chains

INTRODUCTION

Muscle is the most important part of the livestock carcass, and determines its economic value. Previous studies have indicated that muscle fiber characteristics may affect post-mortem changes that occur during the conversion of muscle to meat, which can ultimately affect many traits related to meat quality (Henckel et al., 1997; Choe et al., 2008; Lee et al., 2012). In mammals, most skeletal muscles consist of a mixture of muscle fiber types. Generally, based on the differential expression of isoforms of myosin heavy chains (MyHCs), the pork skeletal muscles consist of 4 types of muscle fibers: type I, type IIA, type IIB, and type IIX (Tanabe et al., 1999). Type I is a slow aerobic metabolism muscle fiber, which contains relatively more mitochondria and cytochromes and has low glycogen content. Type I muscle is also known as slow muscle fiber or red muscle fiber. Type IIA is a fast aerobic metabolism muscle fiber, which contains relatively more glycogen and some myosin. Type IIB is a fast glycolytic muscle fiber, which contains relatively less mitochondria and cytochromes and has high glycogen content. Type IIB muscle is also known as white muscle fiber or fast muscle fiber (Estrade et al., 1993; Tanabe et al., 1999). Type IIX is pink in appearance and its features are intermediate between types IIA and IIB.

Post-mortem pH is a large determinant of overall pork quality impacting its water-holding capacity, color, shelf life, texture, and functional properties of the meat (Fernandez and Tornberg, 1991; Boler et al., 2010). The decline in post-mortem pH from physiological levels to the ultimate pH, reached 8-24 h post-mortem, is a reflection of biochemical changes occurring in the muscle, and is largely dependent on muscle glycogen content at the time of slaughter (Lawrie, 1966). As glycogen content increases, often estimated post-mortem by glycolytic potential, ultimate pH declines. However, there is a threshold at which further increases in glycolytic potential do not result in lowering of the ultimate pH (Bendall, 1973; Fernandez et al., 1992). Furthermore, several studies have shown a relationship between increased glycolytic potential and poorer meat quality, including paler, softer pork with poor water-holding capacity (Enfält et al., 1997; Hamilton et al., 2003).

Glycogenin-1 (GYG) is the protein core of glycogen (Smythe et al., 1990) and serves as an autocatalytic protein substrate for glycogen synthase (Alonso et al., 1995). Therefore, the number of GYG molecules available within the skeletal muscle dictates the number of glycogen particles available and hence the amount of glycogen that can be stored (Alonso et al., 1995). The expression of GYG may be the rate-limiting step of glycogen synthesis and may be more important than the activity of glycogen synthase (Alonso et al., 1995).

As glycogen content at the time of slaughter is an important determinant of post-mortem pH, which in turn has a large impact on meat quality, and as glycogen content may depend on the presence and activity of GYG, the impact of muscle-fiber type on GYG expression and the effect of the GYG content of post-mortem muscles on glycolytic potential and pH decline were investigated in this study.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Care and Ethics Committee of Sichuan Agricultural University, China.

Animals and treatments

Seventy-five market weight commercial barrows (103.5 ± 8.4 kg) were slaughtered for this study. These pigs were standard halothane-free Duroc x (Landrace x Yorkshire) breeds and raised under uniform conditions to slaughter weight at the research pig farm of Sichuan Agricultural University. From 24 h prior to harvest, pigs were only allowed to have free access to water. On the day of harvest, all pigs were harvested according to humane, standard commercial procedures (Xiao et al., 1999).

Muscle sampling and pH measurement

Samples for RNA extraction

Two 70-mg muscle samples were collected as soon as possible post-mortem from the core of the longissimus dorsi muscle (LD, between the tenth and the last rib) and the psoas major muscle (PM), on the left side of the carcass. Muscle samples were immediately frozen in liquid nitrogen and subsequently stored at -80°C until use in MyHC and GYG expression analyses.

Samples for glycolytic potential (GP) analysis

Each pig was sampled once at 45 min and 24 h after exsanguination. At each time point, a 6-g muscle sample was collected from the core of the LD and from the PM, on the left side of the carcass. Muscle samples were divided into two 3-g samples for chemical analysis at both sampling times. All samples were obtained from a previously unexposed area of tissue (>1 cm from any cut surface) and were handled so as to minimize exposure to air and purge loss. Samples were immediately frozen in liquid nitrogen and subsequently stored at -80°C.

The carcasses of slaughtered pigs were kept in 4°C coolers for 24 h. The pH and temperature of the intact muscle of the carcass were measured using a pH Star Probe with a glass electrode (SFK Technologies Inc., Cedar Rapids, IA, USA) on the cut surface of each section at 45 min ($pH_{45\ min}$) and 24 h ($pH_{24\ h}$) post-mortem. The difference in the pH value between these 2 time points was defined as ΔpH ($\Delta pH = pH_{45\ min} - pH_{24\ h}$).

GP

Muscle samples were assayed for GP according to methods described in Hartschuh et al. (2002), except that concentrations were determined in comparison to standard curves of lactate and glucose. Glucose assays were performed with a coupled enzymatic assay kit (hexokinase and glucose-6-phosphate dehydrogenase; Sigma-Aldrich, St. Louis, MO, USA). Lactate content was measured using an enzyme assay, including lactate dehydrogenase (Sig-

ma-Aldrich). Both assays measured the conversion of NAD^+ to NADH using a plate reader (Bio-Stack Ready, BIO-TEK Instruments Inc., Winooski, VT, USA) at 340 nm. All results are reported as micromole per gram (μmol/g) wet muscle tissue. The concentrations of glucose-6-phophate and glucose were not independently determined but were included in the glycogen determination, and the GP was calculated using the equation: GP = 2 x (glycogen + glucose + glucose-6-phosphate) + lactate (Monin and Sellier, 1985; Hambrecht et al., 2005).

Gene expression

Total RNA was prepared by disrupting tissues in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) with a TissueLyzer (Qiagen, Valencia, CA, USA), following manufacturer instructions. One microgram of RNA was treated with DNAase before reverse transcription into cDNA (Quantitect Reverse Transcription kit, Qiagen). Expression of type I, IIA, IIB, and IIX MyHC isoforms, and expressions of GYG and β-actin were measured by Taqman real-time reverse transcriptase-polymerase chain reaction (RT-PCR) normalized to glyceraldehyde-3-phophate dehydrogenase. The Taqman primer/probe sets for all genes examined were obtained from Applied Biosystems (Foster City, CA, USA). The sequences of primers for type I, IIA, IIB, IIX, TOP2B, and TBP, were designed by Tanabe et al. (1999). The sequences of primers for glycogenin and β-actin were designed by Ylä-Ajos et al. (2007). Data are reported as the change in the cycle threshold (ΔCt) values (Ct of GYG minus Ct of β-actin). Increased ΔCt is reflective of reduced expression.

Statistical analysis

All data were analyzed with SAS (SAS 9.2, SAS Inst. Inc., Cary, NC, USA). The MIXED procedure was used when analyzing the effect of muscle type and sampling time on GP, lactate, and pH. The model included the fixed effects of muscle type, sampling time, and the interaction between muscle type and sampling time, and the identity of the individual animal was used as a random term. The model for analysis of the types I, IIA, IIB, and IIX MyHC isoforms on GYG expression included only muscle type as a fixed factor. The REG procedure was used to analyze the relationship among GP, lactate, pH, and GYG expression. Least square means were evaluated using the PDIFF and STDERR options in SAS. Differences were considered to be significant when $P < 0.05$.

RESULTS

Composition of muscle fiber types

According to the expression ratio of MyHCs mRNA, significant differences were observed in the composition of muscle fiber types between the 2 muscles (Table 1). Fast-type MyHC isoforms were dominant in the porcine LD and more slow-type MyHC isoforms were present in the PM muscle. The LD muscle contains a significantly higher proportion of type IIB muscle fibers than does the PM (75.10 *vs* 41.58%), whereas the PM has a higher proportion of type I (3.65 *vs* 0.94%), type IIA (34.15 *vs* 10.63%), and type IIX (20.62 *vs* 13.33%) muscle fibers than does the LD.

Table 1. Expression ratio of myosin heavy chains mRNA of longissimus dorsi and *psoas major* (N = 75; least squares means ± SE).

Muscle	I	IIA	IIX	IIB
Longissimus dorsi	0.94%^{B}	10.63%^{B}	13.33%^{b}	75.10%^{A}
Psoas major	3.65%^{A}	34.15%^{A}	20.62%^{a}	41.58%^{B}

Within a column, least squares means with different lowercase superscript letters differ significantly at $P < 0.05$, those bearing different capital superscript letters differ significantly at $P < 0.01$.

Effect of muscle and sampling time on glycogen metabolites and pH

The LD had higher GP and lactate contents 24 h post-mortem ($P < 0.05$) compared to the PM (Table 2). In addition, the LD had more energy reserves (GP, lactate) at both sampling times.

Table 2. Glycolytic potential, lactate concentration (μmol/g wet muscle), and pH in longissimus dorsi and psoas major muscles.

Muscle	Sampling time	Lactate	GP	pH
LD	45 min	86.79 ± 17.14^{bc}	104.60 ± 12.71^{bc}	6.27 ± 0.30^{a}
	24 h	99.76 ± 17.91^{a}	117.00 ± 13.75^{a}	5.67 ± 0.11^{c}
PM	45 min	80.46 ± 12.14^{c}	95.86 ± 11.63^{c}	5.88 ± 0.18^{b}
	24 h	92.61 ± 13.13^{b}	107.08 ± 13.98^{b}	5.72 ± 0.34^{bc}

Means within columns with different superscript letters differ significantly ($P < 0.05$). GP = glycolytic potential (GP = 2 x [glycogen + glucose + glucose-6-phosphate) + lactate]. LD = longissimus dorsi; PM = psoas major.

With respect to pH, there was a significant interaction effect between muscles and sampling time (Table 2). Specifically, the LD had higher pH at 45 min post-mortem and lower pH at 24 h post-mortem, and the difference was pronounced ($P < 0.05$). The pH of the PM declined quickly in the first 45 min post-mortem, and the pH value at 45 min post-mortem was significantly lower than that of the LD ($P < 0.05$). The pH value, however, did not change significantly from 45 min to 24 h post-mortem in the PM ($P > 0.05$).

Correlations between glycogen metabolites and pH

In both the LD and PM, lactate and GP showed significantly negative correlation coefficients with the ultimate pH value, and significantly positive correlation coefficients with ΔpH (Table 3). The GP at 45 min post-mortem was negatively correlated with $pH_{24\,h}$. As a deduced estimation of energy reserves derived from the combination of glucose and lactate concentrations, GP at 45 min post-mortem showed the highest negative correlation with $pH_{24\,h}$ ($r = -0.77$, $P < 0.001$) and a significant positive correlation with ΔpH ($r = 0.72$, $P < 0.001$) in the LD. These results indicate that the low $pH_{24\,h}$ and pH decline post-mortem were correlated with the glycogenolysis process. High GP values in the muscle resulted in low $pH_{24\,h}$ and a greater decrease in pH.

GYG expression and correlations with glycogen metabolites and pH

At 45 min post-mortem, the transcription level of the housekeeping gene β-actin was similar in the 2 muscles ($P > 0.05$), but the GYG ΔCt of the LD was lower than that of the PM

(-0.2 *vs* 1.1, $P < 0.05$), which indicated that transcription of the GYG gene was significantly higher in the LD than in the PM ($P < 0.05$). The correlation coefficients between GYG expression and the glycogen metabolites (lactate and GP), pH, and ΔpH are listed in Table 4. It should be noted that because increased ΔCt is related to decreased gene expression, negative correlations with ΔCt indicate positive correlations with gene expression. GYG expression at 45 min post-mortem was significantly positively correlated with glycogen metabolite concentrations (lactate and GP) and ΔpH, and negatively correlated with pH at 24 h post-mortem, but was not correlated with pH at 45 min post-mortem in either muscle. In the LD, GYG expression showed its highest positive correlation with GP at 24 h post-mortem ($r = 0.59$, $P < 0.01$). These results indicate that increased expression of GYG was associated with increased glycogen metabolite concentrations and decreased ultimate pH values.

Table 3. Correlation coefficients between metabolites and pH.

Muscle	Traits	45 min post-mortem		24 h post-mortem	
		Lactate	GP	Lactate	GP
LD	$pH_{24\,h}$	-0.73**	-0.77**	-0.48*	-0.61**
	ΔpH	0.68**	0.72**	0.45*	0.56*
PM	$pH_{24\,h}$	-0.39*	-0.45*	-0.39*	-0.50**
	ΔpH	0.70**	0.72**	-0.68**	-0.74**

*Significant at $P < 0.05$. **Significant at $P < 0.01$. $pH_{24\,h}$ = pH at 24 h post-mortem. For abbreviations, see legend to Table 2.

Table 4. Correlation coefficients between glycogenin-1 gene expression (ΔCt), pH change, and glycogen metabolites.

Muscle	45 min post-mortem			24 h post-mortem			ΔpH
	Lactate	GP	pH	Lactate	GP	pH	
PM	-0.46*	-0.57**	0.19^{ns}	-0.49*	-0.54*	0.49*	-0.45*
LD	-0.51*	-0.58**	-0.24^{ns}	-0.46*	-0.59^{ns}	0.53*	-0.49^{ns}

Increased ΔCt is reflective of reduced expression. ΔCt = cycles to threshold of glycogenin-1 minus Ct of β-actin. *Significant at the 5% level. **Significant at the 1% level. ns = not significantly different ($P > 0.05$). For abbreviations, see legend to Table 2.

DISCUSSION

The 2 muscles in this study, LD and PM, were chosen due to their difference in fiber-type composition (Henckel et al., 1997; Gil et al., 2008). The LD is representative of a mixed fiber-type muscle, which consists of a higher proportion of type IIB fibers, while the PM has a higher proportion of type I, type IIA, and type IIX fibers. These results were in good agreement with the results of Tanabe et al. (1999). Previous studies have indicated that glycogen particles mainly accumulate in muscles containing type IIB fibers (Estrade et al., 1993; Lee et al., 2012), and compared with glycolytic muscles, oxidative muscles have a lower glycogen content and lower glycolytic potential (Hambrecht et al., 2005). Ferguson et al. (2008) suggested that a high proportion of type IIB fibers would cause a greater pH decline; however, the rate of pH decline is actually faster in muscles with more type I fibers than in muscles with more type IIB fibers. This was confirmed in the current study as pH at 45 min post-mortem in the LD was higher than that in the PM, but the overall change in pH was greater in the LD. In

fact, pH did not change significantly in the PM between 45 min and 24 h, which was similar to the results of Melody et al. (2004). In the present study, the lower GP of the PM relative to the LD matched results of previous studies comparing oxidative- and glycolytic-type muscles (Hambrecht et al., 2005).

In this study, the ultimate pH value was strongly correlated with muscle metabolites, when measured at both 45 min and 24 h post-mortem. Muscle GP is an indicator of glycogen storage and potential lactate production, and is often negatively correlated with ultimate pH (Hartschuh et al., 2002; Bertol et al., 2006). It has previously been shown that the metabolite content and the pH of post-mortem muscle influence its quality measures, including color, water-holding capacity, and tenderness loss (Huff-Lonergan et al., 2002; Gil et al., 2008). Meat with increased glycolytic potential often has poorer quality, resulting in paler, softer pork with poor water-holding capacity (Enfält et al., 1997; Hamilton et al., 2003).

The GYG protein forms the core of glycogen and its physiological function is to initiate the formation of a new glycogen granule in muscle (Whelan, 2007). Therefore, the positive correlation between GYG expression and available glycogen was to be expected. However, GYG expression was also positively correlated to the lactate content at 45 min and 24 h post-mortem, as well as to GP, suggesting an overall relationship between GYG expression and glycolysis. The expression of GYG was significantly higher in the LD than in the PM post-mortem. This difference in GYG expression may reflect an expression difference in living muscle. It is possible, however, although beyond the scope of this study, that the increase in GYG expression in the LD muscle post-mortem was concomitant with glycogenolysis. As both the PM and LD muscles experienced glycogenolysis post-mortem, it is also possible that differences in GYG expression are related to the difference in muscle fiber types between the 2 muscles. Further research relating muscle fiber type, physiological glycogen stores, and expression of GYG in living muscle is needed.

CONCLUSION

Glycogen metabolites (lactate and GP) were significantly negatively correlated with ultimate pH and significantly positively correlated with the ΔpH from 45 min to 24 h post-mortem. Glycogen metabolism, pH decline, and ultimate pH post-mortem may be affected by muscle fiber composition. Furthermore, the expression level of the GYG gene was related to the glycogenolysis process, and may be associated with the muscle fiber composition. Finally, the process of anaerobic metabolism of muscle glycogen had a significant impact on GP and on the ultimate pH of pork.

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