DECREASING MUSCLE PHOSPHOCREATINE SLOWS POSTMORTEM GLYCOLYSIS AND IMPROVES PORK QUALITY

Tracy L. Scheffler, Jason M. Scheffler, Steven C. Kasten, Eric M. England, Sungkwon Park, and David E. Gerrard*

Department of Animal & Poultry Sciences, Virginia Tech, Blacksburg, Virginia, USA

*Corresponding author (phone: 1+1-540-231-9157; fax: +1-540-231-3010; e-mail: dgerrard@vt.edu)

Abstract— **Rate and extent of postmortem glycolysis are the main factors controlling pork quality development. The objective of this study was to evaluate the role of phosphocreatine (PCr) on postmortem glycolysis and resulting pork quality attributes. Phosphocreatine and creatine content of muscle were manipulated by feeding pigs the creatine analogue, β-guanidinopropionic acid (β-GPA). Animals received a standard (control) diet or β-GPA supplemented (2%) diet (1 wk or 2 wk). Animals were harvested and muscle samples were taken from the longissimus at 0, 20, and 45 min and 24h postmortem. As expected, supplementation with β-GPA resulted in reduced muscle PCr at 0 min (P=0.05). Other indicators of muscle energy status at harvest, including ATP, pH, and lactate, were similar among control and treatment animals. However, by 20 min postmortem, G6P was lower (P<0.05) in muscle from β-GPA fed animals. G6P remained lower at 45 min (P=0.06) and was accompanied by lower muscle glucose (P=0.03) and lactate (P=0.08), and increased muscle pH (P=0.08). The decreased rate of glycolysis resulted in improved pork quality, as demonstrated by decreased reflectance (P=0.003), higher subjective color scores (P=0.004), and decreased drip loss (P=0.1). In total, this supports that decreased muscle phosphocreatine contributes to reduced glycolytic flux and promotes development of more desirable pork quality.**

Index Terms—β-guanidinopropionic acid, glycolysis, phosphocreatine, pork quality.

I. INTRODUCTION

Pale, soft, and exudative (PSE) is a major industry concern due to its undesirable appearance and reduced functionality. Pork quality development is largely governed by the rate and extent of postmortem glycolysis (Briskey, 1964, Monin and Sellier, 1985). Hastened postmortem glycolysis generates heat and causes a rapid accumulation of lactate and hydrogen ions. This combination of high carcass temperature at relatively low muscle pH results in the denaturation of sarcoplasmic and myofibrillar proteins. Conversely, 'acid meat' or meat with a low ultimate pH (pH_u), is generally considered to result from increased muscle glycogen stores; this is suggested to allow greater glycolysis and a low ultimate pH. There is certainly a relationship between glycogen and ultimate pH at extreme pH values; for example, dark, firm and dry meat is the result of limiting substrate resulting in minimal postmortem glycolysis. However, glycogen alone does not fully explain differences in pH_u.

An improved ability to buffer ATP levels in postmortem muscle may contribute to increased glycolytic capacity. After exsanguination, muscle attempts to maintain homeostasis by generating ATP. The most immediate means of buffering ATP is via the creatine kinase catalyzed transfer of phosphate from phosphocreatine (PCr) to ADP, yielding ATP and creatine. Continued ATP hydrolysis may result in depletion of PCr and accumulation of ADP. Additional ATP is generated by the myokinase reaction ($2ADP \rightarrow AMP + ATP$). Subsequently, AMP is deaminated to IMP by AMP deaminase. The combined efforts of myokinase and AMP deaminase are thought to maintain ATP/ADP ratio during high energy demands and prevent muscle fatigue. Increases in AMP and ADP stimulate anaerobic degradation of glycogen, resulting in lactate and H^+. Hydrolysis of ATP ($ATP + H_20 \leftrightarrow ADP + Pi + H^+$) also generates H^+. Thus, the capacity to generate ATP may influence H^+ accumulation and pH decline, thereby impacting meat quality development.

Our previous work (Copenhafer, Richert, Schinckel, Grant & Gerrard, 2006) showed that, in addition to elevated glycogen, Rendement Napole pigs possess elevated PCr levels. This elevated PCr could contribute to an enhanced capacity to buffer ATP, thereby promoting extended glycolysis. Along these same lines, reduced PCr should result in a reduced capacity to buffer ATP. Therefore, the objective of our study was to reduce PCr content of muscle and determine its effect on postmortem metabolism and meat quality development. Dietary supplementation of the creatine analogue, β-guanidinopropionic acid (β-GPA), is well documented to deplete PCr and Cr levels in skeletal muscle of rodents in a time-dependent manner. β-GPA is a much poorer substrate for creatine kinase and cannot substitute for PCr (Chevli and Fitch, 1979). We hypothesized that the reduced PCr pool in muscle from β-GPA fed animals would result in a reduced capacity to buffer ATP, quicker initiation of glycogenolysis in order to maintain ATP levels, which in turn would yield reduced

hydrogen ion production and ultimately a higher muscle pH.

II. MATERIALS AND METHODS

Animals

All procedures with animals were conducted in accordance and with prior approval of the Virginia Tech Animal Care and Use Committee. Twelve castrated male pigs (Yorkshire/Large White × Duroc) were obtained from Murphy Brown, LLC (Waverly, VA). Pigs were individually housed in 1.44 m^2 pens at the Litton Reaves Animal Facility at Virginia Tech. Animals were blocked by initial weight and assigned to control or treatment diet (1 wk or 2 wk). Treatment diet consisted of a standard (control) swine diet supplemented with 2% β-GPA. At initiation of the experiment, pigs in the 2 wk group (n=4) were provided with treatment diet, while remaining pigs (n=8) were given the control diet. After 1 wk, 4 pigs in the control group were switched to the treatment diet. Feed and water were provided ad libitum. At the end of the 2 wk period, pigs were transported to the Virginia Tech Meats Laboratory and harvested. Pigs were electrically stunned and exsanguinated. Exsanguination was considered time 0.

Muscle sampling

Muscle samples (~5 g) were collected from the lumbar region of the *longissimus* muscle at 0, 20, and 45 min and 24h (1440 min) postmortem. Samples were immediately frozen in liquid nitrogen and stored at –80 °C until analysis.

Meat quality characteristics

At 24h postmortem, carcasses were ribbed between the 10th and 11th costae. Two 2.54-cm thick chops were removed from the carcass anterior to the cut surface at the 10th rib and trimmed to remove excess backfat and connective tissue. Subjective color, marbling (NPPC, 2000) and firmness (NPPC, 1991) were evaluated by five individuals at the cut surface of the *longissimus* at the 10^{th} rib. On the same surface, objective color measurements were determined using a portable Minolta CR300 chromameter (Ramsey, NJ, USA). Mean L* (lightness), a* (redness), and b* (yellowness) values were collected from three separate locations on the surface of the chop. Water holding capacity was determined on the adjacent chop using the drip loss method (Rasmussen and Andersson, 1996). Briefly, muscle samples were collected from one chop using a coring device and placed in drip loss tubes. After 24h at 4°C, the drip loss containers plus samples were weighed. Muscle samples were removed and containers were reweighed with exudates. Percentage drip loss was calculated and recorded.

Muscle metabolite analysis and pH measurements

Muscle glucose, glucose 6-phosphate (G6P), glycogen, and lactate concentrations were determined using enzyme analytical methods (Bergmeyer, 1974) modified to a 96-well configuration. Metabolite concentrations were used to calculate glycolytic potential (GP) using the formula described by Monin and Sellier (1985): GP (μmoles/g wet tissue) = 2 (glucose + glucose 6-phosphate + glycogen) + lactate. ATP and PCr were determined using neutralized muscle extracts analyzed by a standard sequential enzyme assay (Passonneau and Lowry, 1993). For pH determination, ~2 g of frozen muscle was homogenized in 20 ml of 5mM iodoacetate. The pH of the homogenate was determined using a ROSS electrode attached to an Orion pH meter (Thermo Fisher Scientific, Beverly, MA, USA).

Statistical analysis

Relationships between GPA administration and meat quality development were analyzed using SAS JMP Version 8. The model included the effect of treatment (control, 1 wk, or 2 wk). Differences between least square means were evaluated using Tukey's adjustment for multiple comparisons. Due to relatively small number of animals, we had insufficient power to detect differences for some variables. When relevant, contrasts were used to compare control (-1) relative to 1 wk (0.5) and 2 wk (0.5) β-GPA treatment combined.

III. RESULTS AND DISCUSSION

Inclusion of β-GPA in the diet led to an expected decline in PCr in *longissimus* muscle (P=0.05, Figure 1). In support, β-GPA was detected using high performance liquid chromatography analyses of muscle extracts from animals fed the treatment diet (data not shown). Interestingly, treatment was associated with striking improvements in meat quality attributes. Two wk treatment with β-GPA decreased reflectance (lower L* values; P=0.003; Figure 2) and resulted in more reddish-pink meat color, evidenced by higher subjective color scores (P=0.004). Moreover, β-GPA treatment decreased drip loss (P=0.10; contrast for control vs. β-GPA, P=0.05), indicating increased water-holding capacity of muscle from treatment animals. To understand potential mechanisms by which β-GPA feeding and reduced muscle PCr augment meat quality, we investigated several

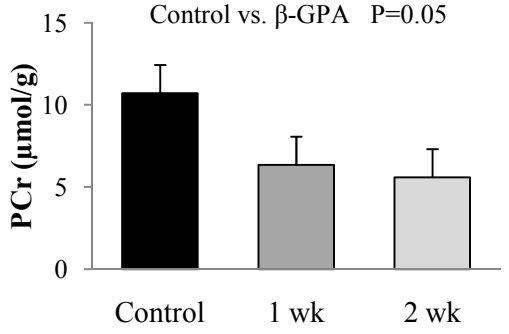

Figure 1. Phosphocreatine content (μmol/g) of *longissimus* muscle of pigs fed control or β-GPA (1 wk or 2 wk) diet.

key metabolites in postmortem metabolism. ATP (data not shown) and lactate (Table 1) were not different between treatment groups at 0 min, suggesting muscle was at a similar energetic status at the time of death. However, at 20 min postmortem, G6P was increased in muscle from control compared to 2 wk β-GPA treatment (P=0.02), and G6P remained elevated at 45 min (contrast, control vs. β-GPA, P=0.04). β-GPA treatment also influenced glucose (P=0.03) at 45 min. During anaerobic glycolysis, glycogen phosphorylase cleaves the outer chains of glycogen, generating glucose 1-phosphate, which is isomerized to glucose 6-phosphate. Glycogen debranching enzyme breaks the α-1,6 linkages, releasing free glucose, which accumulates in postmortem muscle. Therefore, increased G6P and glucose suggest that glycogen breakdown and glycolytic flux are enhanced in control muscle compared to β-GPA muscle. In agreement, the final product of anaerobic glycolysis, lactate, was increased at 45 min (P=0.06). More rapid postmortem glycolysis is also consistent with an effect of treatment on pH (P=0.03); at 45 min, control animals exhibited lower muscle pH than 2 wk β-GPA muscle. At 24h, there were no differences in residual glycogen, glucose, G6P, or lactate (P>0.3).

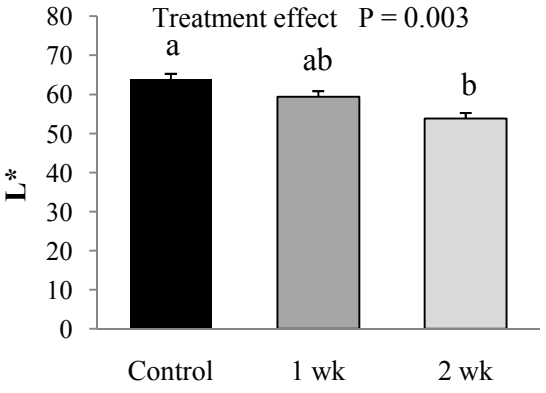

Figure 2. L* (lightness) values of *longissimus* muscle of pigs fed control or β-GPA diet. Means with different letters differ (P<0.05).

Altogether, these data imply that faster postmortem metabolism occurs more readily in muscle from control compared to β-GPA treated animals. Certainly, in β-GPA muscle, glycogen is available (~45 μmol/g) and thus is not limiting glycolysis; in fact, virtually all glycogen appears to be broken down in the 24h postmortem period. Curiously, the increase in glucose and G6P and greater decrease in glycogen in the first 45 min postmortem suggest that metabolism occurs more rapidly in control than in β-GPA muscle. Intuitively, increased PCr would allow for a greater capacity to buffer ATP, which should delay/slow glycolysis. However, our results show control animals with greater PCr exhibit more rapid postmortem metabolism. Yet, it must be noted that stress-susceptible (i.e., Halothane carrier) pigs also exhibit rapid metabolism but have decreased PCr (Essen-Gustavsson, Karlstrom & Lundstrom, 1992). In stress-susceptible animals, aberrant calcium regulation contributes to rapid glycolysis, and muscle exhibits reduced ATP at 'time 0'. Our data, therefore, suggest another biochemical mechanism is responsible for altering metabolism in β-GPA muscle.

Table 1. Postmortem levels of metabolites (μmol/g) and pH of *longissimus* muscle.

	Time (min)	Control	1 wk	2 wk	SEM	P-value
Glycogen	0	55.83	41.96	45.17	4.80	0.15
	20	45.27	35.73	37.95	4.09	0.27
	45	26.75	32.04	33.94	5.17	0.58
	1440	3.59	1.30	4.14	1.62	0.45
Glucose	0	0.65	0.85	0.61	0.31	0.83
	20	2.71	2.49	2.26	0.31	0.61
	45	4.42	3.27	2.65	0.41	0.03
	1440	7.84	7.69	7.03	0.90	0.80
G6P	0	7.32	8.55	7.51	0.80	0.52
	20	7.60	6.46	5.84	0.36	0.02
	45	6.18	4.87	3.72	0.66	0.06
	1440	10.50	8.82	7.27	1.55	0.37
Lactate	0	28.10	34.82	29.43	4.42	0.55
	20	52.24	48.42	46.60	3.68	0.56
	45	72.56	60.04	52.02	5.83	0.08
	1440	102.28	97.56	101.48	4.64	0.76
pH	0	6.49	6.50	6.50	0.04	0.95
	45	5.99	6.15	6.31	0.07	0.03

Muscle from β-GPA treated animals must have additional means to offset the loss of buffering capacity by PCr. During contraction in fast-twitch white muscle of β-GPA fed animals, deamination of AMP is initiated sooner, resulting in increased

inosine monophosphate (IMP) formation (Tullson, Rundell, Sabina & Terjung, 1996). Whereas 'normal' muscle has PCr to buffer adenine nucleotide levels, β-GPA muscle must compensate by increasing AMP deaminase activity in order to blunt the increase in AMP and ADP that result from greater ATP hydrolysis. Because both AMP and ADP are allosteric stimulators of glycolytic enzymes, including glycogen phosphorylase, delaying their accumulation would slow glycolytic metabolism postmortem. Moreover, in muscle of control and β-GPA fed rats, 5 Hz stimulation resulted in two-fold greater acid accumulation in control muscle; this was associated with a nearly two-fold increase in lactate (Meyer, Brown, Krilowicz & Kushmerick, 1986). Additionally, β-GPA muscle degraded ~33% less glycogen during stimulation and contained lower levels of inorganic phosphate (P_i). The dramatic increase in glycolytic metabolism in muscle of control rats was attributed largely to P_i generated from hydrolysis of PCr, which potently stimulates glycogenolysis. Thus, reducing available PCr decreases glycolytic flux and slows postmortem metabolism.

IV. Conclusion

Dietary supplementation with 2% β-GPA reduced PCr levels in pig *longissimus* muscle. β-GPA treatment and reduced muscle PCr contributed to reduced glycolytic flux, as indicated by decreased glucose, G6P, and lactate, as well as increased muscle pH at 45 min postmortem. This slower rate of metabolism corresponded with meaningful improvements in pork quality, including decreased reflectance, more desirable reddish-pink color, and lower drip loss. Although it was not determined in this study, P_i from greater PCr hydrolysis is likely the main reason for enhanced glycogenolysis. In summary, PCr level and its rate of hydrolysis are critical factors influencing the rate of postmortem muscle metabolism and meat quality development.

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References

Bergmeyer, H. U. (1974). *Methods of enzymatic analysis*. Academic Press, New York.

Briskey, E. J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. *Advances in Food Research,* 13, 89-178.

Chevli, R. & Fitch, C. D. (1979). β-guanidinopropionic acid and phosphorylated β-guanidinopropionic acid are substrates for creatine kinase. *Bioechemical Medicine,* 21, 162-167.

Copenhafer, T. L., Richert, B. T., Schinckel, A. P., Grant, A. L. & Gerrard, D. E. (2006). Augmented postmortem glycolysis does not occur early postmortem in AMPKγ3-mutated porcine muscle of halothane positive pigs. *Meat Science,* 73, 590-599.

Essen-Gustavsson, B., Karlstrom, K. & Lundstrom, K. (1992). Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. *Meat Science,* 31, 1-11.

Meyer, R. A., Brown, T. R., Krilowicz, B. L. & Kushmerick, M. J. (1986). Phosphagen and intracellular pH changes during contraction of creatine-depleted rat muscle. *American Journal of Physiology,* 250, C264-274.

Monin, G. & Sellier, P. (1985). Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period: The case of the Hampshire breed. *Meat Science,* 13, 49-63.

NPPC. (1991). Procedures for evaluating market hogs. Des Moines, IA: National Pork Producers Council.

NPPC. (2000). Pork composition and quality assessment procedures. Des Moines, IA: National Pork Producers Council.

Passonneau, J. V. & Lowry, O. H. (1993). *Enzymatic analysis: A practical guide*. Humana Press, Totowa, NJ.

Rasmussen, A. & Andersson, M. (1996). New method for determination of drip loss in pork muscles. In: *Proc. 42nd Int. Congr. Meat Sci. Technol.*, (pp 286-287), Lillehammer, Norway.

Tullson, P. C., Rundell, K. W., Sabina, R. L. & Terjung, R. L. (1996). Creatine analogue β-guanidinopropionic acid alters skeletal muscle AMP deaminase activity. *American Journal of Physiology,* 270, C76-85.