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GLYCOGEN PHOSPHORYLASE DRIVES RAPID EARLY POSTMORTEM METABOLISM IN PORCINE MUSCLE

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Introduction

Development of pork quality attributes is largely governed by the rate and extent of postmortem pH decline (Briskey, 1964, Sellier and Monin, 1994). Postmortem pH decline is largely dependent on the energy status of muscle at or around slaughter.

Two major genes, halothane (HAL) and Rendement Napole (RN), alter pH decline by causing a defect in Ca^{2+} regulation and glycogen storage, respectively. Halothane positive pigs (nn) exhibit increased sarcoplasmic Ca^{2+} concentration (Cheah and Cheah, 1976, Mickelson, et al., 1989), which sustains muscle contraction, increases ATP utilization and results in accelerated postmortem glycolysis. RN mutant pigs ($RN^{-}RN^{-}$), however, possess an increased capacity for extended pH decline and low ultimate pH, which is attributed to higher muscle glycogen levels (Monin and Sellier, 1985). These genes generate inferior pork quality through distinctly different mechanisms of post mortem metabolism, yet their ability to modulate metabolism when present together is not known.

Differences in the inherent properties of rate limiting enzymes would be expected to influence the rate and extent of anaerobic metabolism. Yet, enzyme capacity does not explain abnormal glycolysis (Allison, et al., 2003, Schwagele, et al., 1996). Modulators of rate limiting enzymes alter enzyme activity based on energy charge of the cell, and this affects flux through glycolysis and contributes to aberrant postmortem metabolism. Thus, inherent variation in metabolism may control glyocolytic flux. Kastenschmidt et al. (1968) and Hammelman et al. (2003) showed disproportionate activity of glycogen phosphorylase in the first hour postmortem compared to subsequent time points in muscles undergoing normal rates of glycolysis, whereas phosphofructokinase appeared to be a more critical glycolytic control point in muscles undergoing a rapid rate of postmortem glycolysis.

Objectives

Objective: Utilize the HAL and RN genetic mutations to examine the effects of accelerated postmortem pH decline and elevated muscle glycogen, separately and in

combination, on the rate limiting reactions of glycolysis and their relationship to pork quality attributes.

Hypothesis: Different enzymes become rate limiting at different times during postmortem metabolism, and this contributes to pH decline and pork quality development.

Methodology

A heterozygous (Nn/RN$^-$rn$^+$) population was used to generate animals possessing all possible genotypes. HAL and RN genotype was determined using the polymerase chain reaction and restriction fragment length polymorphism technique outlined by O'Brien et al. (1993) and modified procedure of Meadus and MacInnis (2000), respectively. Animals homozygous for both genotypes (NN/rn$^+$ rn$^+$, n = 12; NN/RN$^-$RN$^-$, n=17, nn/ rn$^+$ rn$^+$, n = 11; nn/RN$^-$RN$^-$, n = 16) were slaughtered and exsanguination was considered time = 0.

Longissimus muscle pH values were recorded adjacent to the last rib at 0, 15, and 45 min postmortem. Muscle samples for metabolite analysis were taken from the lumbar region of the *longissimus* at 0, 30, 60, 120 min and 24 h (1440 min). At 24 h postmortem, subjective color, marbling (NPPC, 2000) and firmness (NPPC, 1991) of the *longissimus* muscle were evaluated at the 10th rib, and ultimate pH was determined on the cut muscle surface. Objective color measurements (L*, a*, b*) were determined on one 2.54 cm chop using a Hunter Colorimeter, and water holding capacity was determined on an adjacent 2.54 cm chop using the drip loss method (Rasmussen and Stouffer, 1996). Muscle glucose, glucose 6-phosphate, glycogen and lactate concentrations were determined using enzyme analytical methods (Bergmeyer, 1974) modified to a 96-well configuration (Hammelman, et al., 2003). These metabolites were used to calculate glycolytic potential (GP) using the formula outlined by Monin and Sellier (1985): GP = 2(glucose + glucose 6-phosphate + glycogen) + (lactate). ATP and phosphocreatine levels were determined by a standard sequential enzymatic assay (Passonneau and Lowry, 1993). Data was analyzed using the PROC MIXED procedure of SAS, with the REPEATED statement for variables measured at more than one time point. Significance is represented at $P < 0.05$.

Results & Discussion

The higher drip loss and lower subjective firmness scores of RN mutants compared to RN normal genotype suggests the RN$^-$ allele decreases water holding capacity. RN mutants also had lower marbling scores than the wild type. HAL mutants possessed inferior meat quality compared with the normal genotype, as evidenced by increased reflectance (L*), yellowness (b*) and drip loss values, as well as lower subjective color, firmness, and marbling scores. There were no HAL×RN genotype interactions for quality traits.

During postmortem metabolism, ATP must be generated in order to keep muscle in the relaxed state. Energy levels are first buffered by using phosphocreatine (PCr) to phosphorylate ADP to ATP. The HAL normal genotype had higher PCr concentrations

than mutant pigs at both time points. HAL normal animals maintained higher ATP concentrations than HAL mutants during the first 30 min postmortem.

The RN$^-$ allele increased GP in the *longissimus* muscle. RN mutants had higher glycogen concentrations compared to the wild type. The mutant HAL gene increased glycogen breakdown and lactate concentrations at 0, 30, 60 and 120 min postmortem (Figure 1). The rapid utilization of ATP and PCr, followed by increased rates of glycogen breakdown and lactate accumulation early postmortem, denotes the faster rate of glycolysis classically observed in HAL mutant animals. At 45 min, HAL mutants, regardless of RN genotype, possessed similar pH that was lower than HAL normal genotypes. These data imply that the higher glycogen content does not contribute to more rapid degradation of glycogen, greater lactate accumulation or lower pH at 45 min.

Glycogen debranching enzyme breaks the α-1,6 linkages of glycogen and releases free glucose, which accumulate in postmortem muscle. Glucose concentrations were increased by both mutant HAL and mutant RN genotypes (Figure 2). HAL mutants had elevated glucose concentrations at 30, 60 and 120 min and 24 h, supporting that glycogen debranching enzyme does not block rapid glycolysis and pH decline when the temperature is high. In contrast, RN mutant pigs had greater glucose concentrations at 120 min and 24 h.

Glycogen phosphorylase cleaves the outer chains of the glycogen molecules, generating glucose 1-phosphate which is isomerized to glucose 6-phosphate (G6P). HAL and RN genotype combination influenced G6P concentration over time (Figure 3). G6P concentrations in HAL mutants decreased during the first 30 min postmortem and remained at a similar level thereafter. The high rate of lactate formation in HAL mutants indicates high glycolytic flux during the first hour postmortem. Curiously, G6P concentrations in the HAL/RN mutant tended to increase in the first 30 min, and then decrease through 60 min. Despite the large difference in G6P between HAL mutant and HAL/RN mutant pigs at 30 min, the rate of lactate accumulation was similar. Thus, glycogen phosphorylase and debranching enzyme activity are capable of aggressive glycogenolysis to supply adequate G6P for the reactions of glycolysis. This suggests phosphofructokinase may be rate limiting during rapid early postmortem metabolism in HAL/RN mutants. Conversely, the high ATP levels and decreased energy demand of normal and RN mutant genotypes suggests that the decrease in G6P concentrations from 0 to 60 min is likely due to decreased glycogen phosphorylase activity.

Alternatively, during normal rates of glycolysis, greater glycogen degradation after 60 min corresponds with increased G6P accumulation. The accumulation of G6P after 60 min supports that glycogen phosphorylase activity was sufficient to meet demand, and that either phosphorylase activity was increased or phosphofructokinase activity was reduced. Acidic conditions and declining ATP concentrations after 60 min may compromise phosphofructokinase activity in muscle undergoing normal rates of glycolysis and prevent it from sustaining the glycolytic flux provided by phosphorylase.

Conclusions

Together, the HAL and RN genes maximize activation of glycogen phosphorylase by enhancing sarcoplasmic Ca^{2+} concentration and glycogen availability, and depleting ATP. The difference in G6P concentrations between HAL and HAL/RN mutants despite

high glycolytic flux indicates that phosphofructokinase may be rate limiting during aggressive early postmortem glycogenolysis. In contrast, decreased G6P concentrations in the first 60 min postmortem in muscle undergoing normal rates of glycolysis is likely due to high ATP levels and decreased energy demand, which would limit glycogen phosphorylase activity. The accumulation of glucose and glucose 6-phosphate in muscles with normal rates of glycolysis suggests phosphofructokinase is rate limiting to glycolysis after one hour postmortem. Therefore, different enzymes may be rate limiting at different times postmortem depending on the rate of metabolism. A greater understanding of glycolytic flux through phosphofructokinase is critical for minimizing adverse pork quality development.

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Tables and Figures

Figure 1. LS means of postmortem *longissimus* muscle lactate concentrations in halothane and RN genotypes.

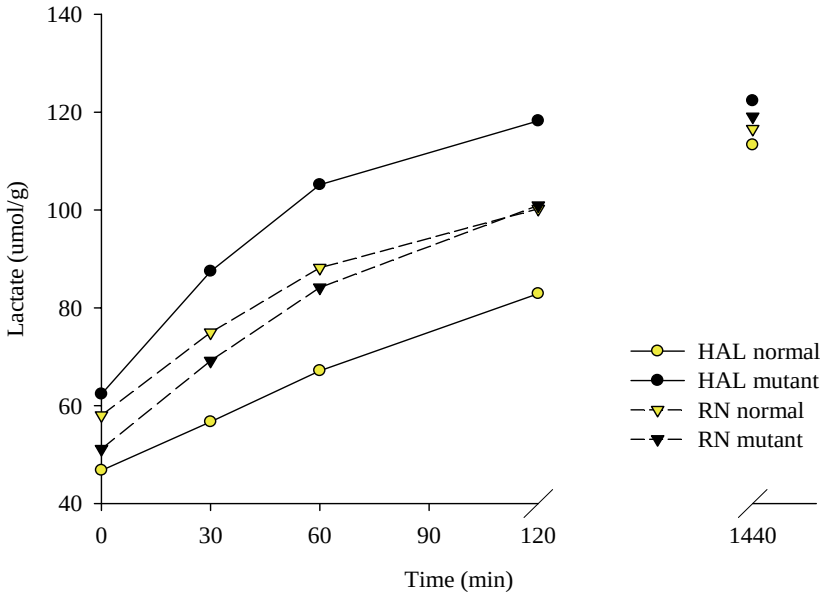

Figure 2. LS means of postmortem *longissimus* muscle glucose concentrations in halothane and RN genotypes.

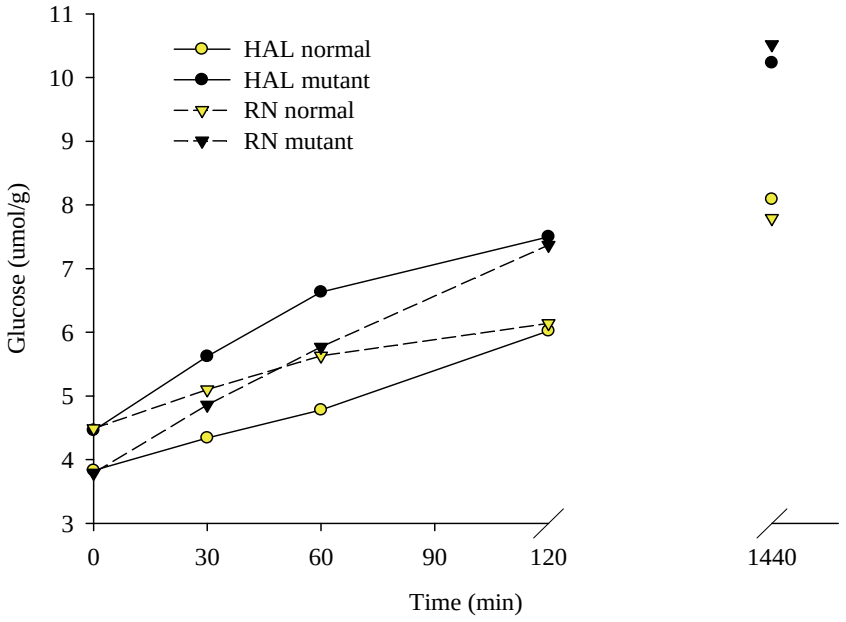

Figure 3. LS means of postmortem *longissimus* muscle glucose 6-phosphate concentrations in halothane and RN genotypes.