THE POST MORTEM ACTIVITY OF GLYCOGEN DEBRANCHING ENZYME

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Introduction

Shortly after slaughter the enzymes in meat operate in conditions quite similar to prevailing in a living muscle. To maintain the ATP level constant, energy is produced by glycogen degradation. Glycogen phosphorylase together with glycogen debranching enzyme (GDE) catalyse the breakdown of it to glucose-1-phosphate and free glucose (Brown and Illingworth-Brown, 1966). Glucose-1-phosphate is further broken down to lactate resulting in an acidification of muscles. When measuring the enzyme activities in meat, the samples are usually taken as soon as possible after slaughter. The *post mortem* pH decrease, however, continues for 24 or 48 hours in pig muscles. Thus, the glycolytic enzymes must maintain their activity a fairly long period of time. Both the rate and the extent of pH decrease differ between animals and between muscles. In Hampshire breed pigs a dominant RN^- allele in PRKAG3 gene is common (Milan *et al.*, 2000). The allele is associated with an increased glycogen content, faster pH decrease and lower ultimate pH in glycolytic muscles from RN^- carriers compared with the wild type animals (Fernandez *et al.*, 1992; Enfält *et al.*, 1997; Lindahl *et al.*, 2004). Furthermore, the ultimate pH is attained not until 48 h after slaughter in RN^- carries while in the wild type animals the pH decrease ceases 24 h *post mortem* (Lindahl *et al.*, 2004). This research focuses on the change in the *post mortem* GDE activity during the period of 0.5 h to 48 h after slaughter in *longissimus dorsi* muscle from Hampshire crossbred animals with and without presence of the RN^- allele.

Materials and Methods

Fourteen Hampshire x (Swedish Landrace x Yorkshire) crossbred pigs were stunned in CO_2 and slaughtered at a commercial slaughterhouse in Sweden. A DNA test (Milan *et al.*, 2000) was applied to identify RN^- allele carriers (RN^- carrier, n=7) and wild type animals (n=7). Samples from *M. longissimus dorsi* were obtained 0.5, 3, 5, 24 and 48 hours after bleeding, frozen in liquid nitrogen and stored at -80°C until analyzed. The pH was measured (Knick portable pH-meter equipped with a combination gel electrode, SE104, Knick Berlin, Germany) from *M. longissimus dorsi* at the last thoracic vertebra at 0.5, 3, 5, 24 and 48 hours after bleeding.

The GDE activity was measured by a method following the change in the iodine complex spectrum of phosphorylase limit dextrin described more detailed (Kylä-Puhju *et al.*, 2005). The method enables the use of crude meat extract in the GDE activity analyses. Limit dextrin is the natural substrate for GDE and it was available in our laboratory. The temperature of the reaction mixture was 39 °C and pH 6.3±0.05. The reproducibility of the GDE activity assay was determined by using an internal standard. A large muscle sample was ground in liquid nitrogen, diced, stored at -80°C and analyzed with the samples. The coefficient of variation (CV) for the internal standard was ±18.3%. The differences in the GDE activity and in pH between the genotypes were tested by using the MIXED procedure of SAS v. 8.02. The model included the fixed effects of genotype, sex, time and interaction between genotype and time and a random animal effect to account for repeated measurements performed within the same animal.

Results and Discussion

The pH 0.5 h *post mortem* was similar in the RN^- carriers (6.53±0.06) and in wild type animals (6.65±0.06). However, as reported earlier (Enfält *et al.*, 1997; Lindahl *et al.*, 2004), the rate of pH decrease was faster and the ultimate pH was lower in the RN^- carriers (5.21±0.03) than in wild type animals (5.36±0.03). The GDE activity was similar in both RN genotypes up to 5 h after slaughter (Figure 1). Estrade *et al.* (1994) found that 5 min after slaughter, both the GDE activity and the glycogen phosphorylase activity are uniform between the RN genotypes. Thus, it seems that immediately after slaughter, the glycogenolytic enzymes are not more active in RN^- carriers than in wild type animals despite the higher glycogen content in RN^- carriers. Thereby the GDE activity does not explain the faster pH decrease early *post mortem* in RN^- carriers than in wild type animals.

The GDE activity decreased slowly as a function of time *post mortem*, and the decrease was statistically significant until after 5 hours *post mortem*. At 24 h *post mortem* the GDE activity was significantly reduced in both genotypes, but the decrease was more pronounced in wild type animals. The GDE activity 24 h *post mortem* was higher in RN^- carriers compared with wild type animals, and this difference was maintained up to 48 h *post mortem*. In RN^- carries, despite the higher rate of pH decrease, the ultimate pH is attained not until 48 h after slaughter (Lindahl *et al.*, 2004). Thus, the extended pH decrease in RN^- carriers might be a result of longer maintenance of the high GDE activity in these animals. Furthermore, in some situations (like when very fast chilling is concerned), the GDE may catalyze a rate-limiting step in *post mortem* glycogenolysis and thus glycolysis (Taylor *et al.*, 1975; Kylä-Puhju *et al.*, 2005).

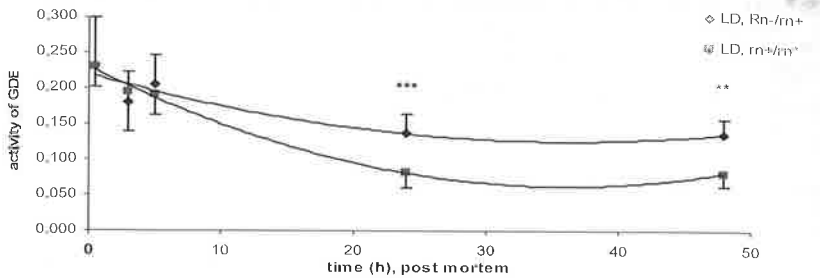

Figure 1: Activity of GDE (Δabs/Δmin) with standard deviations in RN⁻ carriers (◇) and in wild type pigs (■, rn+). Asterisks indicate significant differences between the two genotypes (*** P<0.001, ** P<0.01).

The RN⁻ mutation is located in the gene coding for adenosine monophosphate-activated protein kinase (AMPK), which is a key metabolic enzyme (Milan *et al.*, 2000). Activated AMPK inhibits the ATP-consuming pathways and stimulates ATP-generating pathways (Hardie and Carling, 1997) and is important for maintaining the activity of glycogen phosphorylase in *post mortem* muscle (Shen and Du, 2005). Thus the mutation in the AMPK gene might explain why RN⁻ carriers exhibit a prolonged high GDE activity, as found in the present study. The GDE activity was measured in laboratory, in fixed conditions. In carcass, however, the temperature decreases concurrent with pH decrease. The pH decrease has a minor effect on the GDE activity, but the effect of temperature decrease is significant (Kylä-Puhju *et al.*, 2005). Thus, carcass chilling may further rise the difference in GDE activity between RN genotypes after 5 hours *post mortem*.

Conclusions

The decrease in the GDE activity with increasing *post mortem* time is slower and less pronounced, the pH decrease is faster and the ultimate pH lower in RN⁻ carriers than in wild type animals. The long period of high GDE activity in RN⁻ carriers may enable the extended pH decrease.

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