Exercise, fasting, and epinephrine administration - effects on muscle glycogen degradation in vivo in pig *M. longissimus dorsi*

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Introduction

Many meat quality characteristics are dependent on the rate or the extent of the pH decrease post mortem. The pH decrease is the result of anaerobic degradation of glycogen to lactate. The concentration of muscle glycogen at the time of slaughter will thus be a very important factor for the course of the pH decrease. Transportation and preslaughter handling of the animals are known to effect the pH development post mortem, most likely by reducing the amount of glycogen available for the post mortem energy metabolism. Previously, conditions for transportation, like time/distance, density of animals, temperature and humidity have been used to standardize preslaughter treatment. However, very little information is available regarding the extent and how the variation of these conditions actually influences the physiological prerequisites, and how much the individual energy sources actually needs to be altered in order to obtain a significant effect on pH development. An alternative way to clarify the effects of preslaughter treatments on pH development would be to standardize directly on physiological variables of which the amount of glycogen is considered the most important. The series of preliminary experiments presented here were aimed to identify methods by which the energy levels at the time of slaughter could be manipulated and standardized. We have mainly focused on the effects on muscle glycogen, however, as the amount of creatine phosphate may also be of significance, results regarding effects on creatine phosphate will be presented in the belonging poster.

Material and methods

42 animals at a live weight of 100 kg, Danish Landrace x Large White cross breeds (halothane free), both females and castrated males were used in the six preliminary experiments. For each experiment the animals were balanced by litter and sex. Muscle sampling was performed in the *longissimus dorsi* muscle by the needle biopsy technique. The biopsies were immediately frozen in liquid nitrogen and stored until analysis. Glycogen (glucose units) was measured spectrophotometrically after boiling in HCl for two hours. These techniques were applied in all experiments. In the last experiment the animals were slaughtered and the pH was recorded during the first 24 hours.
In experiment (1) the effect of administration of 0.3 mg epinephrine (injected subcutaneously in the neck region) pr kg live weight were investigated in 6 animals. Samples were taken before and 6, 17 and 24 hours after treatment. In experiment (2) we investigated the effect of injection of 0.2 mg epinephrine/kg live weight (n=10). In order to reduce physical and emotional stress due to repeated sampling (involving catching, fixation and sampling) which may effect the results, the ten animals were divided into two groups of five pigs. One group from which samples were taken before and after 5, 10, 15, 20 and 25 hours of injection another group from which samples were taken before and after 15, 20, 25, 38 and 50 hours.
Experiment (3) using 0.2 mg epinephrine/kg live weight was conducted to elucidate the initial decrease in glycogen in combination with a short burst of physical exercise (2 minutes with a speed of 4.5 km/hour). Samples were taken in pairs of animals after 1, 3 and 6 hours after injection, one from each of the pair immediately after a 2 minutes sprint on the treadmill (n=6). A similar experiment without application of epinephrine was also conducted (experiment 4). Samples were taken before (at rest) and after the exercise (n=4). The 5th experiment involved fasting for 24 hours, followed by exercise for 30 min (speed 3.8 km/hour) and ten minutes of rest. Samples were taken immediately before fasting and after the ten minutes of rest (n=6). Finally to verify the effect on pH development post mortem the 6th experiment was performed. One group of pigs (n=5) was subjected to 10 minutes of physical exercise on a treadmill immediately before slaughter and the other group (n=5) was given a dose of 0.2 mg epinephrine/kg 15 hours prior to slaughter. pH was measured 1, 10, 30, and 45 minutes post mortem and further after 1, 3, 6, and 24 hours.

Results

Using the two different concentrations of epinephrine we showed that injection of 0.3 mg/kg reduced the glycogen concentration approximately 73% of resting levels after 6 hours and remained constant throughout the experimental period, while administration of 0.2 mg/kg resulted in maximal effect (62%) after 10 hours with subsequent decreasing effect, and even 50 hours after the administration the glycogen was still reduced by 25% compared to resting levels, as seen in Figure 1. Experiment 3 showed a gradual decrease in glycogen during the initial 6 hours, corresponding to 24, 49 and 61% of initial glycogen concentration after 1, 3 and 6 hours, respectively. Moreover, the results indicated that a short burst of physical exercise may only reduce the glycogen content insignificantly. Physical exercise alone (Exp. 4) caused a reduction of 12%. Fasting for 24 hours followed by 30 minutes of exercise on a treadmill and subsequent resting of the animals to for 10 minutes before sampling implied a reduction in the glycogen content of only 19% compared to initial glycogen content. Administration of 0.2 mg/kg epinephrine 15 hours prior to slaughter compared to exercise for 10 minutes before slaughter resulted in two distinctly different courses of pH decrease, as seen in Figure 2. In both groups was observed a temporary increase in pH at 45 minutes after slaughter.

Discussion

Epinephrine is known to enhance the glycogenolytic activity by activation of phosphorylase and phosphofructokinase in both cases via cyclic AMP (Sutherland and Cori, 1951, Sutherland and Robison, 1966). The capacity of the hormone to reduce glycogen levels have been demonstrated in several animal species including man and is known to affect the pH-development post mortem in meat from various farm animals. It has been shown that the effect of the hormone was related to dosage as well as time of administration prior to slaughter.

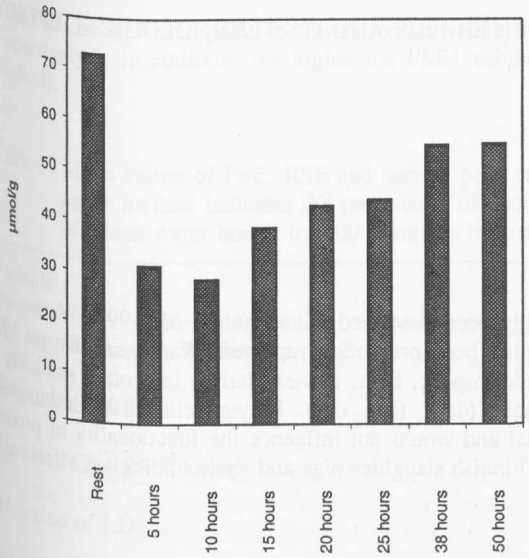

Fig 1. Mean values of glycogen after administration of 0.2 mg/kg epinephrine.

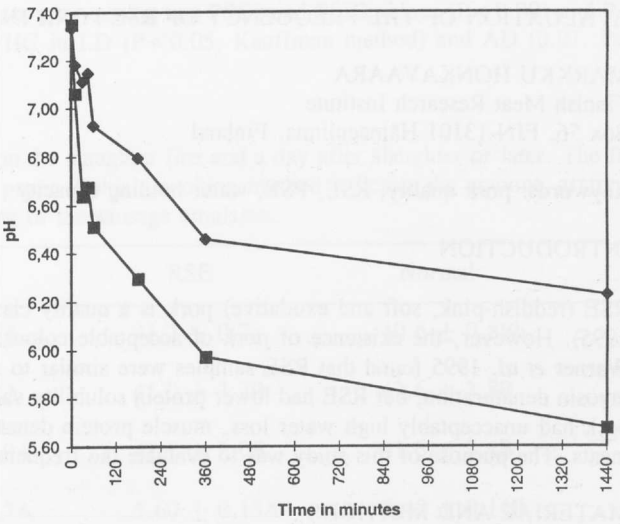

Fig. 2. pH development after epinephrine administration (diamonds) and after exercise (squares).

Furthermore, the response to administration has been found to be dependent on fiber type characteristics of the muscle (Jensen et al., 1989). The effect of exercise on glycogen depletion has likewise often been reported. The effect being dependent on intensity and duration of the exercise. Fasting has been used to a lesser extent. However, considerably, more time than we have used in the present experiment, is needed, approximately 70 hours, before any significant effect on pH development can be observed in white muscles. The presented results confirm these earlier observations. The fact that administration of 0.2 mg epinephrine/kg still exerts a strong effect even 50 hours after administration may appear surprising, however, it may not actually be caused by a continuous action of epinephrine, as there are evidence to suggest that complete recovery from glycogen depletion may take at least 50 hours. Furthermore, it cannot be ruled out, that the repeated sampling of the animals may have influenced the muscle glycogen concentrations at that time as well. Exercise in combination with a proceeding fasting period, might be expected to result in a pronounced effect on the muscle glycogen, if we consider, that the lower liver glycogen would have reduced the availability of blood-glucose to the muscle. However, this was not the case as the total reduction in glycogen only amounted 19%. Fernandez et al. (1992) stated that both the extent as well as the rate of pH decrease can be affected independently, by manipulating the time between epinephrine administration and slaughter. Earlier investigations by Bendall (1951) have focused on the importance of creatine phosphate, which when present, was able to provide the initial necessary energy requirements post mortem and only when creatine phosphate levels are below 10% of resting levels a further reduction in glycogen could be registered (the delay phase). This may explain the dual effect of epinephrine injections. The increase in pH in experiment (6) may indicate, that creatine phosphate still was present at sufficient concentrations 45 minutes after slaughter in both experiments. Consequently, the intensity of the work bout was not sufficiently high to significantly reduce the creatine phosphate concentrations at slaughter. We also have indications, that this increase in pH 45 minutes after slaughter may be caused by a local conversion of lactate to glycogen. The reason for investigating both the effect of short term high intensity exercise and long term exercise with lower intensity before slaughter is the expected difference in effect on the creatine phosphate levels. High intensity exercise is expected to result in a low concentration of creatine phosphate and thus a faster rate of pH decrease, whereas low intensity work for longer periods of time exclusively will influence ultimate pH. Analysis of the phosphorous compounds are ongoing and will be presented on the poster. Only by standardizing on physiological variables prior to slaughter, (primarily metabolite levels) will it be possible to obtain more detailed information on the importance of the energy metabolism post mortem for the pH development and its effect on other technological and organoleptic meat quality characteristics. Epinephrine administration and exercise as well as combinations of these treatments are powerful tools for this purpose.

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