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COULD THE RELATIVELY LOW ACTIVITY OF GLYCOGEN DEBRANCHING ENZYME ATTENUATE A SUDDEN PH FALL?

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Introduction

The rate and extent of pH decrease in muscles *post mortem* are the most important factors determining meat quality (1, 2). The pH decrease is a result of efforts to maintain the ATP level constant by the anaerobic breakdown of glycogen to lactate. Normally the ATP turnover rate and thus *post mortem* pH decrease are faster in porcine than in bovine muscles and in fast twitch and glycolytic (FG) than in slow twitch and oxidative (SO) (3). Furthermore, the time of onset of anaerobic glycolysis depends on the amounts of myoglobin-bound oxygen in muscles (4), which is generally higher in bovine than in porcine muscles (3).

After slaughter, pH decreases to the ultimate values of around 5.4-6.3, depending on the metabolic and contractile type of a muscle (1, 5), if the glycogen content in muscles before slaughter is not a limiting factor (6). However, it has been showed that some glycogen always remains in bovine muscles after the ultimate pH had been attained (7). Several factors, including glycogen debranching enzyme (GDE), have been suggested to stop *post mortem* glycolysis.

GDE together with glycogen phosphorylase (PHOS) are responsible of the complete degradation of glycogen (9). Glycogen molecules consist of branched glucose chains (10). The GDE breaks down the branching points of glycogen (so-called limit dextrin state), enabling the further action of PHOS (9). The activity of PHOS has been studied quite extensively in meat production animals. Less attention, however, has been paid to GDE despite suggestions of its role as a rate limiting factor in *post mortem* glycogenolysis (8, 11). Hence, the GDE may have an influence on formation of PSE meat, on tenderness and on shelf-life of meat.

Objectives

The aim was to study the activity of GDE and the relationship between the activities of PHOS and GDE in porcine and in bovine muscles, which differ in rate of contraction (slow and fast) as well as in aerobic capacity (oxidative and glycolytic).

Methodology

Muscle samples (fast twitch and glycolytic (FG): *longissimus dorsi*; slow twitch and oxidative (SO): *masseter*) from 27 pigs and from 19 bovines were obtained from a commercial abattoir about 35 min after stunning. The samples were cut into small pieces and frozen and stored in liquid nitrogen.

The activity of GDE was determined using the method of Nelson et al. (12) with minor modifications (8). The method is based on monitoring the shift in the absorbance due to conversion of limit dextrin to glycogen by GDE. The GDE activity measurements were made in triplicate and the activity was calculated from the slope of the linear portion of the absorbance curve. The reaction times used were 1, 1.5, 2.5 min for porcine *longissimus dorsi* muscle, 1, 2.5, 4 min for porcine *masseter* muscle and 1, 3, 6 min for bovine muscles. In addition, the absorption spectra between 375 nm and 800 nm of the method blank and the reaction mixtures were obtained and compared to each other to ensure the conversion of limit dextrin to glycogen (results not shown).

Lactate content, glycogen content as total glucose content, and the activity of phosphorylase were measured as described Kylä-Puhju et al. (8). Glycolytic potential was calculated according to Monin et al. (17): glycolytic potential (mmol LA equiv./kg) = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate].

The pH values were measured from meat extracts (1 g muscle + 10 ml 5 mM Na-Iodoacetic acid solution) using a Knick Portamess 752 pH-meter equipped with a Mettler-Toledo Inlab 427 electrode. Extracts were made from muscles which were frozen immediately after sampling (pH_{35}), and from samples which were frozen 24 h (porcine muscles) or 48 h (bovine muscles) *post mortem*. Two latter ones were designated the ultimate pH (pH_u). To perform the measurement, the frozen samples were homogenised in icy Na-Iodoacetic acid and all pH values were measured at room temperature.

Data analysis was conducted using SPSS 10.0 for Windows (13). The GLM procedure was applied when calculating the estimated marginal means for variables of porcine muscles. When the error variances were unequal, Dunnet's T3 test was used, otherwise Tukey's HSD test. Tukey's test was applied also when comparing porcine and bovine muscles. The differences between bovine muscles were tested using independent samples t-test. Pearson correlations were calculated between parameters of a given muscle.

Results & Discussion

The results of the present study indicated that porcine muscles have a higher capacity to degrade glycogen than bovine muscles. In particular the activity of GDE was higher in porcine muscles than in bovine muscles (Table 1). The activities of GDE and PHOS were higher in porcine than in bovine *longissimus dorsi* muscle. In porcine *masseter* muscle the activity of GDE was higher and there was also a tendency ($p=0.0588$) of higher activity of PHOS than in bovine *masseter* muscle. The activity of glycolytic enzymes together with the aerobic capacity and the rate of ATP turnover have an effect on the rate of *post mortem* glycolysis, which is frequently higher in porcine than in bovine muscles (3). In the present study, the lower rate of anaerobic glycolysis in the bovine *longissimus*

dorsi muscle shortly after slaughter was seen as a lower lactate content and higher pH at the sampling compared to the corresponding porcine muscle.

The activities of GDE and PHOS increased with the fast twitch and glycolytic character of a muscle of a given animal (Table 1). Tsutou et al. (14) obtained similar results with rabbit slow and fast muscles. Both in pigs and in cattle, the activity of GDE was about two times higher in FG *longissimus dorsi* muscles than in SO *masseter* muscles. However, there was even greater increase in the activity of PHOS, and thus the PHOS/GDE ratio was higher in FG muscles than in SO muscles. Ylä-Ajos et al. (15) obtained similar results with chicken FG breast muscle and SO leg muscle, however, in chicken the ratios were much higher than that found in the present study.

The lower PHOS/GDE ratios in SO muscles compared to FG muscles may indicate that glycogen degrading enzymes were more in balance in the SO muscles. Consequently, in SO muscles the degradation of glycogen may proceed without a delay caused by the low activity of GDE. Alternatively, the proportionally low activity of GDE in relation to the activity of PHOS in FG muscles may be a protective mechanism against a sudden pH decrease, as suggested for chicken muscles (15). FG muscles are capable of short-term strenuous contractile activity, but they fatigue quite easily (19). Thus, in strenuous physical stress, the high PHOS/GDE ratio in FG muscles enables a short burst of glycolysis, which leads to fast increase in H^+ content. The high buffering capacity of FG muscles (18, 20) protects these muscles against a sudden pH decrease, but the proportionally low activity of GDE compared to the activity of PHOS may be needed to further restrain the glycogenolysis. Since GDE and PHOS activities were not measured in same units the ratio does not quantify the real difference between the activities of these enzymes within a muscle. The ratios between muscles, however, are comparable.

The fast glycolysis in FG muscles was enabled by the high glycolytic potential (Table 1) in association with the high activity of glycogen degrading enzymes, allowing a rapid conversion of glycogen into lactate. However, rapid glycolysis is not essential for SO muscles. In these muscles aerobic energy production is preferred, because of better blood supply, higher myoglobin content, more mitochondria and a more active pathway from glycogen through the Krebbs cycle to CO_2 and H_2O than in FG (3, 17, 18). Several papers have reported that enzymes in the pathway from glycogen to lactate are more active in FG than in SO muscles (17, 18, 19), however, until now the activity of GDE in the muscles of meat production animals has not been extensively studied.

The activity of GDE may play a role in controlling the rate of *post mortem* pH decrease. Although the activity of GDE did not correlate with the pH_{35} or with the pH_u for either porcine or bovine muscles, a negative relationship between the activity of GDE and pH_u was seen when the results for individual muscles of a given species were combined (Figure 1 a, b). The ultimate pH remained high in SO muscles where also the activity of GDE was low. However, one should bear in mind that there were differences also in glycogen content and in the activity of PHOS between individual muscles of a given animal and it is well known that particularly glycogen content has an influence on muscle pH_u. Although the activity of GDE may not have a critical role in normal *post mortem* reaction sequence, its role may be significant e.g. in a situation where muscle glycogen is low at slaughter and the carcass is chilled rapidly. When the glycogen content is low, muscles soon end up in a situation where glycogen limit dextrin has to be broken down by GDE to maintain the ATP level. At that moment the rate and continuation of the

pH decrease is determined by the activity of GDE rather than phosphorylase. However, the decrease in the activity of GDE is substantial when muscle temperature is lowered by rapid chilling (8). Furthermore, low activity of GDE may reduce the incidence of PSE meat by restraining fast glycolysis in porcine muscles, thus giving time to temperature decrease before reaching the critical pH.

Conclusions

In porcine muscles the activities of glycogen degrading enzymes, GDE and PHOS, are higher than in bovine muscles, thus providing a chance for rapid pH decrease in porcine muscles. The activities of both GDE and PHOS increase with the increasing fast twitch and glycolytic character of a muscle of a given animal, however, the increase in the activity of PHOS is more considerable. The relatively low activity of the GDE may restrict the rate of glycolysis in fast twitch muscles, and thus act as a protective mechanism towards a sudden pH decrease in living muscle.

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Tables and Figures

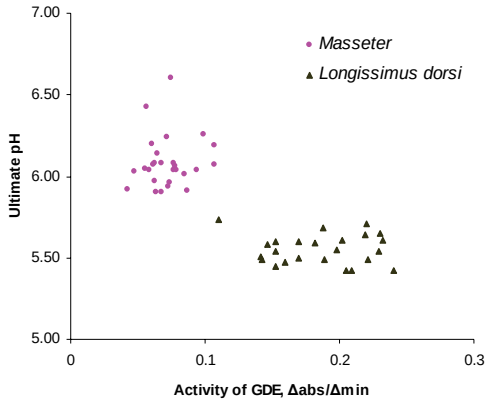

Figure 1a. The ultimate pH in relation to the activity of GDE in porcine muscles

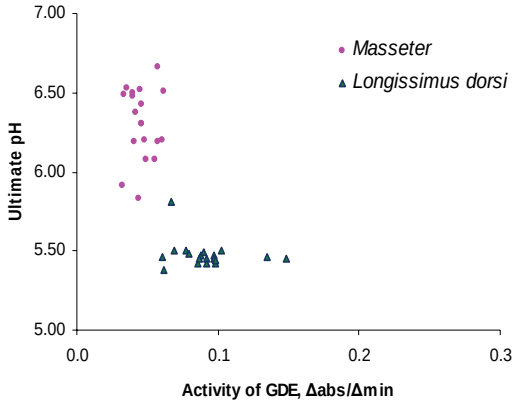

Figure 1b. The ultimate pH in relation to the activity of GDE in bovine muscles

Table 1. The activity of GDE (Δabs/Δmin), the activity of PHOS (U/g muscle), the ratio between PHOS and GDE activities, pH_{35} and pH_u values in porcine and bovine muscles[4]

	Porcine muscles				Bovine muscles			
	longissimus dorsi (n=27)	*masseter* (n=27)	S.E.[1]	p-val.[2]	*longissimus dorsi* (n=19)	*masseter* (n=19)	S.E.[1]	p-val.[2]
GDE	a0.187	c0.073	0.006	<0.001	b0.091	d0.046	0.004	<0.001
PHOS[3]	a12.6	3.2	0.3	<0.001	b8.1	1.3	0.3	<0.001
PHOS/GDE[3]	a70	46	4	<0.001	b89	29	6	<0.001
Glycogen	a66.7	c51.4	3.2	0.022	b105.3	d31.5	3.3	<0.001
Lactate	a49.5	19.3	2.0	<0.001	b28.4	18.5	1.3	<0.001
GP	a182.9	c122.1	5.7	<0.001	b239.0	d81.5	6.3	<0.001
pH_{35}	a6.71	6.81	0.03	0.073	b6.97	6.79	0.03	<0.001
pH_u	5.56	c6.08	0.03	<0.001	5.47	d6.30	0.04	<0.001

[1]S.E. = standard error of the mean, [2]p-value for a difference between muscles of a given animal, [3]for porcine muscles n=27, for bovine muscles n=12, [4]Different letters within rows indicate significant differences (P<0.05) between porcine and bovine *longissimus dorsi* muscles (a, b) or between *masseter* muscles (c, d)