The Significance of the Activity of Glycogen Debranching Enzyme in Glycolysis in Porcine and Bovine Muscles

Maria Ylä-Ajos*, Marita Ruusunen, Eero Puolanne
Department of Food Technology, PO Box 66, 00014 University of Helsinki, Finland

Correspondence to: Maria Ylä-Ajos

Department of Food Technology

PO Box 66, (Agnes Sjöbergin katu 2)

00014 University of Helsinki

FINLAND

Tel: +358 9 19157948

Fax: +358 9 19158460

E-mail: maria.yla-ajos@helsinki.fi

* Corresponding author

Abstract

The purpose of the study was to examine the activity of glycogen debranching enzyme, GDE, in porcine and bovine muscles differing in rate of contraction and in oxidative capacity. The activity of GDE, the activity of phosphorylase, total glucose content, lactate content and pH were measured from meat samples taken 35 min *post-mortem* and ultimate pH 24 or 48 h *post-mortem*.

Both GDE and phosphorylase are needed for the complete degradation of glycogen. In porcine muscles the activities of these glycogen degrading enzymes were higher than in bovine muscles. The activities were increasing with the increasing fast twitch and glycolytic character of a muscle of a given species. However, the increase in the activity of phosphorylase was greater than the increase in the activity of GDE. It was concluded that the GDE may restrict the rate of glycolysis in fast twitch muscles.

Keywords: Glycogen debranching enzyme; Phosphorylase; Oxidative and glycolytic muscles; Pig; Cattle

1 Introduction

The rate and extent of pH decrease in muscles *post-mortem* are important factors determining meat quality (Bendall & Swatland, 1988; Briskey, 1964; Monin & Ouali, 1991). The pH decrease is a result of efforts to maintain the ATP level constant by the anaerobic breakdown of glycogen to lactate. Normally higher rate of ATP turnover (Pearson & Young, 1989; Pösö & Puolanne, 2004) and shorter time to onset of anaerobic glycolysis due to lower oxygen stores (Hamm & El-Badawi, 1991; Livingston & Brown, 1981; Pearson & Young, 1989) is observed in porcine than in bovine muscles, thus the rate of *post-mortem* pH decrease being faster in the former.

Muscles can be classified into three groups according to the contractile and metabolic characteristics of their main fibre type (fast twitch and glycolytic (FG), slow twitch and oxidative (SO), fast twitch and oxidative (FOG)) (Pearson & Young, 1989). In living muscles, SO fibres tend to maintain their energy-rich phosphate levels aerobically, while FG and FOG muscles have a greater ability to maintain their ATP supply anaerobically (Pearson & Young, 1989), thus the *post-mortem* rate and extend of pH decrease are higher in FG than in SO muscles.

After slaughter, the pH decreases to the ultimate values of around 5.4-6.3, depending on the type of a muscle (Bendall & Swatland, 1988; Fernandez & Tornberg, 1991; Rao & Gault, 1989) if the glycogen content in muscles before slaughter is not a limiting factor (Henckel, Karlsson, Jensen, Oksbjerg, & Petersen, 2002; Przybylski, Vernin & Monin, 1994). However, Immonen and Puolanne (2000) showed that some glycogen always remains in bovine muscles after the *post-mortem* reaction sequence, and in muscles with pH_u lower than 5.75 the residual glycogen content varied considerably, from 10 to 85 mmol/kg meat. Several factors have been suggested to stop *post-mortem* glycolysis, including adenosine monophosphate deficiency (Scopes, 1971), a shortage of ADP or glucose (van Laack, Kauffman & Greaser, 2001) and inhibition of glycolytic enzymes by pH decrease (Pearson & Young, 1989). Kylä-Puhju, Ruusunen and Puolanne (2005) suggested that in some cases *post-mortem* glycolysis may be delayed by the inhibition of glycogen debranching enzyme due to muscle temperature decrease.

The degradation of glycogen is achieved by cooperation of two enzymes: glycogen phosphorylase (PHOS) and glycogen debranching enzyme (GDE) (Brown & Brown, 1966). Glycogen molecules consist of branched glucose chains attached to protein core (Goldsmith, Sprang & Fletterick, 1982; Gunja-Smith, Marshall, Mercier, Smith & Whelan, 1970;

Meléndez-Hevia, Waddell & Shelton, 1993). In vivo most of the enzymes needed in glycogen metabolism are bound to these glycogen-protein particles (Lees, Chen & Williams, 2004; Nelson, White & Watts, 1972; Taylor, Cox, Kernohan & Cohen, 1975). The GDE breaks down the branching points of glycogen (so-called limit dextrin state), enabling the further action of PHOS (Brown & Brown 1966; Nelson, Kolb & Larner 1969).

The activity of PHOS has been studied quite extensively in meat production animals, particularly in pigs (e.g. Dalrymple, Kastenschmidt & Cassens, 1973; Estrade, Ayoub, Talmant & Monin, 1994; Fernandez, Neyraud, Astruc & Sante, 2002; Monin, Mejenes-Quijano, Talmant & Sellier, 1987; Schwägele, Lopez Buesa & Honikel, 1996; Suuronen, 1995) and also in cattle (Talmant, Monin, Briand, Dadet & Briand, 1986). However, less attention has been paid to GDE despite suggestions of its role in slowing down the rate of *post-mortem* glycogenolysis and thus glycolysis, particularly when the glycogen limit dextrin state is concerned (Kylä-Puhju et al., 2005; Taylor et al., 1975). Hence, the GDE may have an influence on formation of PSE meat in pig, on tenderness and on formation of heat ring in cattle and on the shelf-life of meat from both species.

The aim of the present work was to study the activity of GDE and the relationship between the activities of PHOS and GDE in porcine and in bovine muscles. The purpose was to examine muscles with differences in rate of contraction (slow and fast) as well as in aerobic capacity (oxidative and glycolytic).

2 Materials and methods

2.1 Animals and sampling

Muscle samples from 27 pigs (fast twitch and glycolytic (FG): *M. longissimus dorsi, M. semimembranosus*; slow twitch and oxidative (SO): *M. infraspinatus, M. masseter*) and from 19 bovines (FG: *M. longissimus dorsi* and SO: *M. masseter*) were obtained from a commercial abattoir about 35 min after the stunning. The samples were cut into small pieces, frozen and stored in liquid nitrogen. Freezing took place within 1 min of sampling. The GDE analyses were made within one week of sampling.

2.2 Biochemical analyses

The activity of GDE was determined using the method of Nelson, Palmer & Larner (1970) with minor modifications (Kylä-Puhju et al., 2005). The method was based on monitoring the change in absorbance after certain reaction times, due to conversion of limit dextrin to glycogen by GDE. The activity of GDE was calculated from the slope of the linear portion of the absorbance curve. The reaction times used were 1, 2.5, 4 min for porcine *masseter* and *infraspinatus* muscles, 1, 1.5, 2.5 min for porcine *longissimus dorsi* muscle, 1, 2, 3 min for porcine *semimembranosus* muscle and 1, 3, 6 min for bovine muscles. The reaction times were determined in preliminary experiments for each muscle. In addition, the absorption spectra between 375 nm and 800 nm of the method blank and the reaction mixtures were obtained to ensure the conversion of limit dextrin to glycogen (results not shown). GDE is a monomeric protein containing two independent catalytic activities: a glycan transferase (EC 2.4.1.25) and amylo-1,6-glucosidase (EC 3.2.1.33) (Nelson et al., 1970; Gordon, Brown & Brown, 1972; White & Nelson, 1974; Taylor et al., 1975). The method used in the present study measures the combined action of GDE.

Lactate and glycogen determinations were made concomitantly. Muscle samples were homogenised in ice-cold 0.1 M phosphate buffer (pH 7.0) with a Polytron homogeniser. Lactate content was determined spectrophotometrically (365 nm) using a commercial kit (Boeringer-Mannheim no. 139 084). Glycogen content was determined as total glucose content, i.e. the resulting value includes also free glucose and glucose phosphates present in the muscle. Muscle homogenate was hydrolysed in 1 M HCl at 100 °C for 2 h, after which pH was adjusted to 6.5-7.5 (Lowry & Passoneau, 1973) and glucose was determined with Roche diagnostic kit no. 1447521.

Glycolytic potential was calculated according to Monin et al. (1987): glycolytic potential (mmol LA equiv./kg) = 2([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate]. In the present study, the formula was used in form: glycolytic potential = 2[total glucose content] + [lactate].

The activity of phosphorylase was measured spectrophotometrically (absorbance 340 nm, Perkin Elmer Lambda 2 spectrometer, Űberlingen, Germany) according to Bass et al. (1969) following the release of glucose-1-phosphate from glycogen with L-cystein, NaDP (N8129, Sigma-Aldrich, St. Luis, USA), glucose-1,6-diphosphate, AMP, phosphoglucomutase (rabbit muscle, 79440, Fluga Chemie GmbH, Buchs, Switzerland) and glucose-6-phosphate-dehydrogenase (127671, Roche, Indiapolis, USA).

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*, ultimate pH (pH_u). The frozen samples were homogenised in ice cold Na-Iodoacetic acid and the pH values were measured at room temperature.

The reproducibility of the assays was determined as follows: a large muscle sample was ground in liquid nitrogen, divided into small pieces and stored at –80 °C. This sample was analysed every time the samples were analysed. The coefficients of variation (CV) were ±8.2% for lactate content, ±13.9% for glycogen content, ±11.8% for activity of PHOS and ±21.3% for activity of GDE. The GDE activity measurements were made in triplicate, glycogen content, activity of PHOS and pH measurements in duplicate.

2.3 Statistical analysis

The General Linear Model was applied when calculating the estimated marginal means and standard errors of activity of GDE, activity of PHOS, PHOS/GDE ratio, glycogen content, lactate content, glycolytic potential, pH_{35} and pH_u for porcine muscles. The model included a fixed effect of muscle and random effect of slaughter date to compensate for environmental effects. When the error variances were unequal, Dunnet's T3 test was used (for porcine PHOS, GDE, pH_{35}, glycogen, lactate, GP), otherwise Tukey's HSD test (for porcine PHOS/GDE ratio, pH_u). When testing the differences in bovine muscles, independent samples t-test was applied. Estimated marginal means were considered to be significantly different when $P<0.05$. Pearson correlations were calculated between parameters of a given muscle. Data analysis was conducted using SPSS 10.0 for Windows (SPSS Inc., 1994).

3 Results

3.1 Porcine muscles

The activities of glycogen degrading enzymes were the highest in FG *longissimus dorsi* muscle and the lowest in SO *masseter* muscle (Table 1). The activity of GDE was more than

two times higher and the activity of PHOS more than three times higher in the FG muscles than in the SO ones. Thus, the ratio between the two glycogen degrading enzymes PHOS/GDE was higher in the FG muscles than in the SO muscles, but was similar in both SO muscles and in both FG muscles, respectively. Since GDE and PHOS activities were not measured in the 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.

In FG muscles the glycolytic potential was higher than in the SO muscles (Table 1). Furthermore, in the FG muscles the *post mortem* pH decrease was faster and the ultimate pH was lower than in SO muscles. The glycolytic potential and pH at 35 min *post-mortem* were the lowest and the pH_u was the highest in the *infraspinatus* muscle.

The activity of PHOS correlated positively with the ultimate pH in FG *longissimus dorsi* muscle, r=0.588 p=0.001, and *semimembranosus* muscle, r=0.421 p=0.029. Furthermore, in the same muscles the correlation between the activity of PHOS and glycolytic potential was negative, r=-0.439 p=0.022 and r=-0.416 p=0.031, respectively. However, no correlation between the activity of GDE and the measured parameters was found.

3.2 Bovine muscles

In bovine muscles the activities of glycogen degrading enzymes were also higher in FG *longissimus dorsi* muscle than in SO *masseter* muscle (Table 2). The activity of GDE was almost two-fold higher in *longissimus dorsi* muscle compared to *masseter* muscle. However, the activities were lower than in porcine muscles. Again, the difference was even greater in the activity of PHOS, and the PHOS/GDE ratio was almost three times higher in *longissimus dorsi* muscle than in *masseter* muscle.

As expected, glycolytic potential and pH_{35} were higher and pH_u was lower in *longissimus dorsi* muscle than in *masseter* muscle (Table 2). No significant Pearson correlations either between GDE and measured parameters or between PHOS and measured parameters were found (results not shown).

The activity of GDE did not correlate with pH_u for either porcine or bovine muscles. However, when the results for the porcine muscles (Fig. 1a) or bovine muscles (Fig. 1b) were combined, a trend could be seen. The ultimate pH remained high in SO muscles where also the activity of GDE was low. However, it must be kept in mind that there were differences also in the activity of PHOS and the glycogen content between individual muscles of a given animal and it is well known that the glycogen content has an influence on muscle pH_u.

4 Discussion

4.1 Porcine versus bovine muscles

The rate of the hydrolysis of ATP determines the rate of *post mortem* glycolysis (Bendall, 1973), which is higher in porcine than in bovine muscles (Pearson & Young, 1989). The physiological purpose of glycolysis is to maintain the ATP level constant in the muscle (Hamm, 1977), leading to anaerobic degradation of glycogen after slaughter. The results of the present study indicated that porcine muscles have a higher capacity to degrade glycogen than bovine muscles. The GDE activity was about two times higher in porcine muscles than in corresponding bovine muscles. Also the activity of PHOS was somewhat higher in the porcine muscles.

The rate of glycolysis is affected by the rate of ATP turnover, aerobic capacity and the activity of glycolytic enzymes. Firstly, the myofibrillar ATPase activity (Laborde, Talmant & Monin, 1985; Talmant et al., 1986) and the ATP turnover rate are higher in porcine muscles

compared to bovine muscles. The rate of ATP turnover in porcine *longissimus dorsi* muscle can be as high as 4 μmol ATP/(min*g) as has been reported for of stress susceptible Pietrain pigs (Bendall, 1973). Even though this does not represent a normal case, it is much higher than the ATP turnover rate of 0.15 μmol ATP/(min*g) for bovine *longissimus dorsi* muscle during the first three hours *post-mortem* (Hamm, 1977). Secondly, porcine muscles are less aerobic and produce ATP by oxidative phosphorylation for a shorter period after slaughter than bovine muscles. Thus, the delay in the time of onset of anaerobic glycolysis in bovine muscles is a result of high amounts of oxygen binding myoglobin, abundant mitochondria and high activity of oxidative enzymes (Lawrie, 1985, Pösö & Puolanne, 2004). Thirdly, the activities of glycolytic enzymes, such as phoshorylase (Talmant et al. 1986), glyceraldehydes-3-phosphate dehydrogenase (pig, Laborde, Talmant & Monin, 1985; bovine, Talmant et al., 1986) and lactate dehydrogenase (Hamm & El-Badawi, 1991) depending, however, on the muscle concerned, are higher in porcine muscles than in bovine muscles. The present study showed that besides the PHOS activity, the activity of GDE is also higher in porcine muscles than in bovine muscles. Together these factors explain the faster pH decrease after slaughter in porcine muscles compared with bovine muscles.

In the present study, the slower rate of anaerobic glycolysis in the bovine *longissimus dorsi* muscle shortly after slaughter was seen as a higher pH at the sampling compared to the corresponding porcine muscle. Although this difference was not observed between the *masseter* muscles, porcine muscles can be considered on average a more fast twitch and glycolytic type than bovine muscles, as stated by Talmant et al. (1986). The GP's for porcine *infraspinatus*, *longissimus dorsi* and *semimembranosus* muscles were similar to those reported by Fischer and Dobrowolski (2002) for the same muscles. However, the GP of bovine FG *longissimus dorsi* muscle was higher than in the corresponding porcine muscle, which partly originates from the sensitivity of pigs to pre slaughter stress. Porcine muscles are

very glycolytic and glycogen reservoirs begin to degrade even under light stress whereas in more oxidative bovine muscles the break down of glycogen begins as a consequence of long-term stress (Lawrie, 1985). The GP and pH_{35} were very low particularly in porcine *infraspinatus* muscle.

4.2 Porcine and bovine fast twitch and glycolytic versus slow twitch and oxidative muscles

The activities of GDE and PHOS increased with the fast twitch and glycolytic character of a muscle of a given animal. Tsutou et al. (1985) obtained similar results with rabbit slow and fast muscles. Both in pigs and in cattle, the activity of GDE was twice as high in FG muscles as in SO ones. However, there was even greater increase in the activity of PHOS. Several other papers have reported that enzymes in the pathway from glycogen to lactate are more active in FG muscles than in SO ones (Beecher, Cassens, Hoekstra & Briskey, 1965b; Hamm & El-Badawi, 1991; Laborde et al., 1985; Monin et al., 1987; Talmant et al. 1986). Until now, the role of the activity of GDE on glycogen degradation in the muscles of meat production animals has not been extensively studied.

Rapid glycolysis is not essential for SO muscles. In these muscles aerobic energy production is preferred, because of better blood supply, higher amounts of myoglobin, more mitochondria and more active pathway from glycogen through the Krebs cycle to CO_2 and H_2O than in FG ones (Beecher, Briskey & Hoekstra, 1965a; Beecher et al. 1965b; Bendall, 1975; Laborde et al., 1985; Pearson & Young, 1989; Talmant et al., 1986; Pösö & Puolanne, 2004). Also the glycogen content is lower in SO than in FG muscles (Karlsson, Klont & Fernandez, 1999; Lefaucheur et al., 1989; Monin et al., 1987) and similar results was obtained in the present study. The present study showed that besides lower PHOS activity, the activity of GDE was also lower in the SO muscles than in the FG muscles. Altogether, the fast glycolysis in FG muscles is caused by the high glycolytic potential in association with the high activity of

glycogen degrading enzymes, allowing rapid conversion of glycogen into lactate. Furthermore, the ultimate pH was lower in FG muscles than in SO ones as has been reported earlier (Beecher et al., 1965b; Bendall & Swatland, 1988; Fernandez & Tornberg, 1991; Hunt & Hedrick, 1977; Laborde et al., 1985; Monin et al., 1987; Rao & Gault, 1989; Talmant et al., 1986).

In porcine FG *semimembranosus* and *longissimus dorsi* muscles, the activity of PHOS correlated negatively with GP, different to the report of Estrade, Ayoub, Talmant & Monin (1994) for porcine *longissimus dorsi* muscle. Furthermore, the activity of PHOS correlated positively with the ultimate pH in FG porcine muscles. The observed correlations could be due to faster *pre mortem* glycolysis in muscles that showed high activity of phosphorylase. Such a correlation was not found in SO muscles or in bovine muscles.

4.3 The activity of GDE in relation to the activity of PHOS

The PHOS/GDE ratio was higher in FG muscles than in SO ones, in both species. The activity of PHOS increased more than the activity of GDE with the increasing fast twitch and glycolytic character of a given animal, thus increasing the PHOS/GDE ratio. The ratio was lowest in bovine SO *masseter* muscle, but highest in bovine *longissimus dorsi* muscle. The authors have no explanation to the latter observation. The PHOS/GDE ratio is also higher in FG chicken breast muscle than in SO leg muscle, however, the values of the ratios are much higher than found in the present study (Ylä-Ajos, Ruusunen, Puolanne, unpublished data). In porcine muscles the ratio was relatively constant within a muscle type.

The lower PHOS/GDE ratios in all SO muscles compared to FG muscles indicated 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. FG muscles are capable for a short-term strenuous contractile activity, but they fatigue quite easily (Lawrie, 1985). Also the potential for amplification of the glycogenolytic cascade is much higher in FG muscles than in SO muscles (Cohen, 1978). Thus, in strenuous physical stress, a high PHOS/GDE ratio in FG muscles enables a short burst of glycolysis, which leads to a rapid increase in H^+ content. The high buffering capacity of FG muscles (Davey, 1960; Kylä-Puhju et al., 2004; Puolanne & Kivikari, 2000; Rao & Gault, 1989; Talmant et al. 1986) 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 glycogenolysis.

4.4 The potential role of GDE in muscle metabolism post-mortem

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). However, one should bear in mind that there were differences also in glycogen content and activity of PHOS between individual muscles of a given animal. In the present study, the activity of GDE did not correlate with glycolytic potential within any muscle in either of the given species. However, Lees et al. (2004) demonstrated with rat skeletal muscle sarcoplasmic reticulum that an activity which leads to a reduction in muscle glycogen content, also reduces GDE content. Furthermore, Daly, Richards, Gibson, Gardner & Thompson (2002) reported that the bovine *longissimus dorsi* muscles with higher glycogen content reached pH 6 at higher temperatures during chilling than the muscles that contained less glycogen. Thus, *post-mortem* glycolysis was faster in animals having more glycogen indicating that substrate availability is one of the factors determining glycolytic rate.

The present study was carried out with carcasses randomly selected from the slaughter line and thus representing a normal commercial situation. The activity of GDE may neither slow down nor stop the glycogenolysis in normal porcine or bovine muscles during normal *post-mortem* reaction sequence. However, the possibility that the activity of GDE may have a critical role in glycogenolysis can not be excluded. This kind of a situation could arise when muscle glycogen is low at slaughter and the carcass is chilled rapidly. When the glycogen content is low, muscles end up in a situation where glycogen limit dextrin has to be broken down to keep on glycolysis. At this time GDE determines the rate and continuation of the pH decrease rather than phosphorylase. Furthermore, low activity of GDE may reduce the incidence of PSE meat by restraining fast glycolysis in porcine muscles, thus giving time for the temperature to decrease before reaching the critical pH. GDE may also play a role in the formation of dark, coarse band (heat ring) as a result of rapid chilling in non-electrically stimulated bovine muscles. Orcutt, Dutson, Cornforth and Smith (1984) showed that the pH value of *longissimus dorsi* muscle was higher just beneath the subcutaneous fat cover than in the central regions at12 and 24 h *post mortem*, but similar at 48 h. They proposed that the slower glycolytic rate in the outer parts of the muscle was due to the higher chilling rate. Furthermore, Kylä-Puhju et al. (2005) showed that decrease in muscle temperature radically decreases the activity of GDE. Hence, the relationship of the activity of GDE both to the rate of pH fall and to the ultimate pH in muscles where glycogen content prior slaughter is low or high warrants further study.

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. The activities of 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 greater than the increase in the activity of GDE and thus the latter enzyme may restrict the rate of glycolysis in fast twitch muscles.

Acknowledgements

The present study was supported by the Finnish Graduate School Program 'Applied Bioscience - Bioengineering, Food & Nutrition, Environment' (ABS) and the Finnish Association of Academic Agronomists. The authors thank the slaughterhouses Koiviston Teurastamo Oy and Paimion Teurastamo Oy for providing the muscle samples and for help in sampling. Laboratory Technician Irja Korhonen is acknowledged for her technical assistance in biochemical analyses.

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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

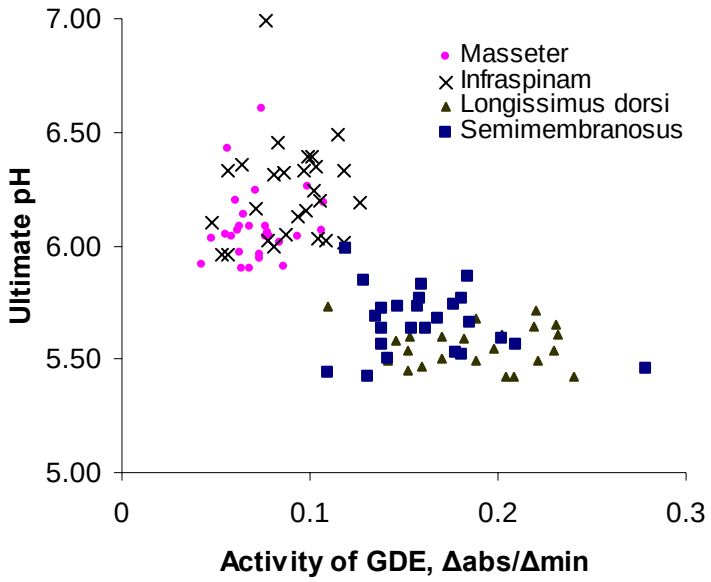

Figure 1a.

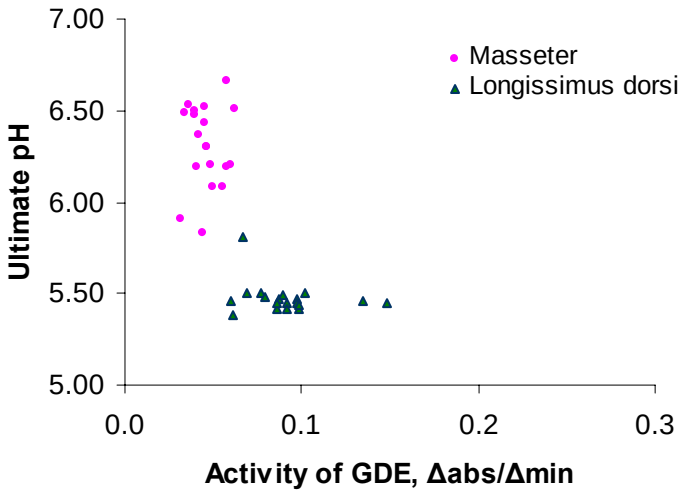

Figure 1b.

Table 1. The activity of GDE (Δabs/Δmin), the activity of PHOS (U/g muscle), the ratio between PHOS and GDE activities, glycolytic potential (mmol LA equiv./kg) and pH_{35} and pH_u values in porcine muscles. N = 27.

	Slow Twitch and Oxidative muscles		Fast Twitch and Glycolytic muscles		
	M. infraspinatus	*M. masseter*	*M. longissimus dorsi*	*M. semimembranosus*	S.E.[1]
GDE	0.089a	0.073b	0.187c	0.160c	0.006
PHOS	4.0a	3.2a	12.6b	12.1b	0.3
PHOS/GDE	46a	46a	70b	79b	4
GP	96.4a	122.1b	182.9c	160.5c	5.7
pH_{35}	6.64a	6.81bc	6.71ab	6.87c	0.03
pH_u	6.22a	6.08b	5.56c	5.69d	0.03

[1]S.E. = standard error of the mean. Different letter within a row means a significant difference ($P<0.05$) between the values.

Table 2. The activity of GDE (Δabs/Δmin), the activity of PHOS (U/g muscle), the ratio between PHOS and GDE activities, glycolytic potential (mmol LA equiv./kg) and pH_{35} and pH_u values in bovine muscles.

	Slow Twitch and Oxidative muscle *M. masseter*	Fast Twitch and Glycolytic muscle *M. longissimus dorsi*	S.E.[1]
GDE	0.047a	0.091b	0.004
PHOS	1.3a	8.0b	0.3
PHOS/GDE	30a	86b	6
GP	82.3a	239.5b	6.3
pH_{35}	6.80a	6.98b	0.02
pH_u	6.30a	5.48b	0.04

n=19 except for phosphorylase and ratio PHOS/GDE when n=12.
[1]S.E. = standard error of the mean. Different letter within a row means a significant difference (P<0.001) between the values.