^{31}P NMR Study of Postmortem Metabolism in Porcine and Bovine Muscles

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Abstract. ^{31}P NMR spectroscopy was used to evaluate interspecies differences in muscle fibre types and related postmortem metabolism. M. longissimus thoracis (MLT) and m. pectoralis superficialis (MPS) of bulls and MLT of pigs were investigated. In perchloric acid extracts NMR resonances for sugar phosphates (SP), inorganic phosphate (P_i), glycerophosphorylcholine (GPC), phosphocreatine (PCr), adenosine triposphate (ATP), adenosine diphosphate (ADP) as well as for NAD^+/NADH could be distinguished. Also, glycogen and lactate contents and pH were determined. The relative contents of phosphorus compounds in bovine muscles of similar participation of muscle fibre are similar. Bovine muscles contain a relatively large proportion of PCr (48 % of all phosphates 15 minutes post-mortem in MPS) whereas porcine MLT show lower PCr content (11 % 15 minutes post-mortem). On the other hand, the ATP content is relatively higher in porcine MLT when compared with bovine muscles in the early phases of the postmortem processes. No NMR-detectable levels of GPC were measured in porcine MLT in contrast to bovine muscles. This suggests that the GPC content does not depend solely on the fibre participation but is also animal species determined.

The 24 hour postmortem metabolism patterns of bovine and porcine muscles have many common traits. CP disappeared first followed by ATP. Simultaneously, the P_i concentrations increased. However, the content of SP remained relatively constant in porcine, but not in bovine muscles where it increased only gradually. The significantly higher concentrations of SP and lactate as well as the lower values of glycogen and pH measured for porcine as compared with bovine muscles suggest an enhanced glycolysis during the early phases of postmortem processes in porcine muscles.

Key words: Muscle fiber types — Skeletal muscle — ^{31}P NMR spectroscopy — Phosphates — Porcine and bovine muscles

Introduction

The quality of meat depends on biological changes that occur during the first hours post-mortem.

At the death of an animal, the blood flow to and from the muscle ceases and, as a result, there are no new sources of energy for muscle function, and the supply of oxygen is cut off. In addition, the products of metabolism can no longer be removed and thus they accumulate in the tissue.

ATP is split into ADP and P_i by the ATPase. The concentration of PCr decreases gradually, and after PCr disappears, glycolysis remains the only source of ATP. No new glucose is transported to the muscle cell post-mortem; therefore, the main energy source for the muscle is glycogen stored in it. This results in decreasing glycogen content and increasing concentrations with lactate decreasing pH (for a review see Greaser 1986).

In the present study changes of phosphorus compounds and those of glycogen, lactate and pH post-mortem were investigated in muscles of bulls and pigs. The aim was to establish the interspecies differences in the participation of muscle fibre types and the related differences in postmortem metabolism in muscles. ^{31}P NMR spectroscopy was used to monitor phosphorus compounds. This method has been succesfully employed in different studies of tissue metabolism (for a review see Roth 1984; Avison et al. 1986; Fernandez and Clark 1987; Sapega et al. 1987; MacKenzie and Gooley 1988).

Materials and Methods

Animals: Seven halothane resistant F_2 generation Landrace × Duroc breed pigs were used. The animals were slaughtered at the age of 180 days, when they reached an average weight of 100—110 kg. Four bulls of Slovakian Pied breed slaughtered at the age of 450—500 days (live weight 450 kg) were used. Samples were taken from m. longissimus thoracis (MLT) of the pigs, from MLT and m. pectoralis superficialis (MPS) of the bulls. The animals were slaughtered avoiding any stress.

Samples: About 200 g of each were taken immediately after the exsanguination. In pigs, MLT samples were taken at the level of vertebrae XII to XIV, in bulls at the level of vertebrae XI—XIII from MLT and from the middle of the pars sternocostalis from MPS.

Samples for histochemical analysis were obtained from the centre of the cranial portion (approx. 1 cm^3). They were immediately frozen in liquid nitrogen and used for subsequent analyses.

The samples for biochemical analyses were placed into a thermostat and kept at 20 °C for 15; 35; 55; 75; 105 min; 6 and 24 hours. At the respective intervals, 5 g of the inner part of the muscle were cut and immediately frozen in liquid nitrogen for subsequent analyses.

Fibre types characterization: Based on the succinate dehydrogenase (E.C.1.3.99.1., SDH) reaction, muscle fibres were classified into βR (slow-twitch oxidative, red), αW (fast-twitch glycogenolytic, white), and αR (fast-twitch oxidative glycogenolytic, intermediate) types (Ashmore and Doerr

Table 1. Muscle fiber types in bovine M. Pectoralis superficialis (MPS), and M. Longissimus thoracis (MLT), and in porcine M. Longissimus thoracis (MLT^+)

	Myofiber Type, %			Cross Section Area, %		
	βR	αR	αW	βR	αR	αW
a) Bovine MPS ($n = 4$)	26.2 ± 4.5	28.5 ± 3.4	45.3 ± 4.9	24.8 ± 3.9	27.7 ± 3.5	47.5 ± 3.3
b) Bovine MLT ($n = 4$)	24.0 ± 5.2	27.0 ± 5.5	49.0 ± 6.6	20.5 ± 5.3	25.0 ± 5.3	54.5 ± 7.0
c) Porcine MLT^+ ($n = 7$)	17.0 ± 6.4	10.6 ± 3.4	72.4 ± 7.8	13.5 ± 6.3	9.5 ± 4.0	77.0 ± 8.4

a, $b - p > 0.05$; *a*, $c - p < 0.05$; *b*, $c - p < 0.05$

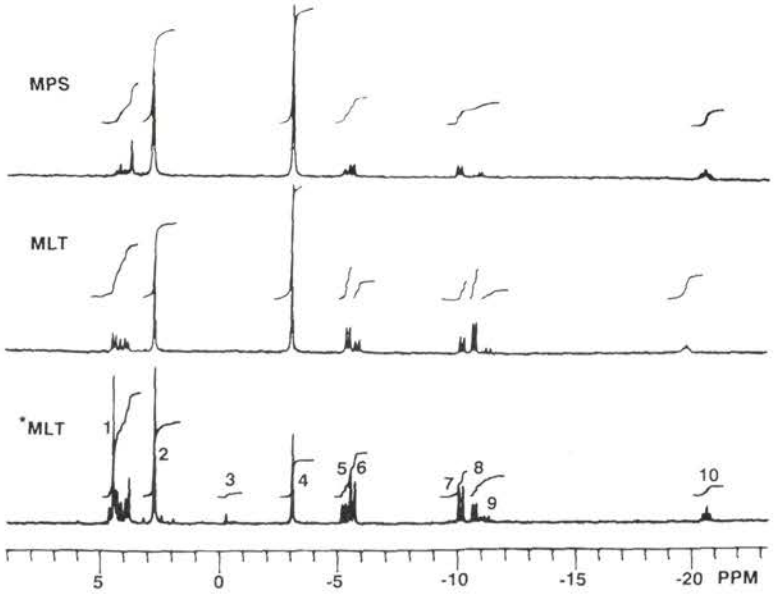

Fig. 1. ^{31}P NMR spectra of bovine m. pectoralis superficialis (MPS), and m. longissimus thoracis (MLT) and of porcine m. longissimus thoracis (MLT). The resonances have been marked based on their chemical shift values: 1 — sugar phosphate (SP; intermediate products of glycolysis-mainly glucose-6-phosphate, fructose-1, 6-diphosphate and 3-phosphoglyceric acid, in this region are also included resonances of adenosine monophosphate-AMP and inosine monophosphate-IMP; 2 — inorganic phosphorus (Pi); 3 — glycerophosphorylcholine (GPC); 4 — phosphocreatine (PCr); 5 — γ-phosphate of ATP; 6 — β-phosphate of ADP; 7 — α-phosphate of ADP; 8 — γ-phosphate of ATP; 9 — phosphate moiety of NAD^+/NADH; 10 — β-phosphate of ATP. The integral for ADP was calculated as the difference of integrals of resonances (5 + 6) and 10, that for NAD^+/NADH was calculated as the difference of integrals of resonances (7 + 8 + 9) and (5 + 6).

1971). Samples for histology were cut on a cryostate perpendicular to the longitudinal fiber axis and 10—12 μm thick sections were prepared, stained for SDH activity and photographed. The photographs were used to count fibre types and to measure their areas. Three photographs from each sample were evaluated by the square grid method (Uhrin and Kulišek 1980). Percentages of βR, αR and αW fibres, and those of the respective areas occupied by βR, αR, and αW fibres were calculated. These values were used to calculate mean fibre diameters.

NMR analyses: Extracts from samples were prepared according to the method of Renou et al. (1986) by treating the samples with 0.6 mol.l^{-1} perchloric acid at 0 °C. After centrifugation the supernatant was neutralized to pH 7—8 with 3.0 mol.l^{-1} potassium carbonate. Potassium perchlorate was removed by centrifugation and the clear supernatant was lyophilised and redissolved in 1 ml H_2O/D_2O (1:9) containing 25 nmol.l^{-1} EDTA (Renou et al. 1986).

All ^{31}P NMR spectra were recorded on a Varian VXR-300 spectrometer operating at 121.415

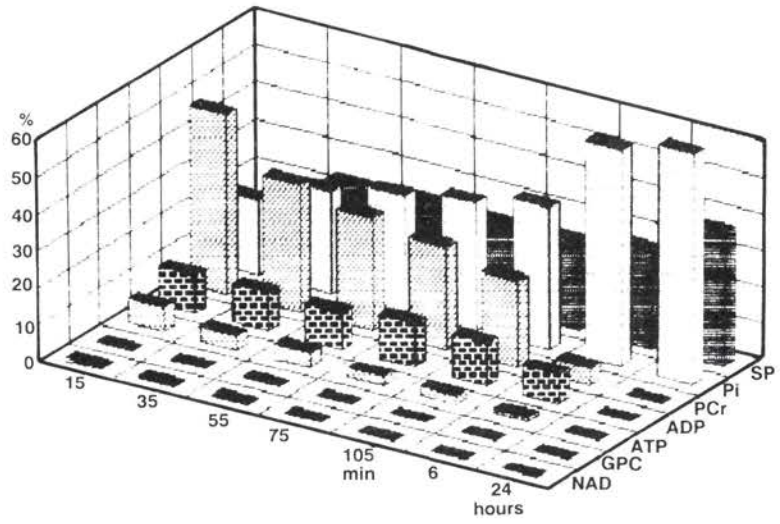

Fig. 2. The kinetics of changes in the contents of the phosporus compounds in bovine MPS. SP — sugar phosphate, Pi — inorganic phosphorus, PCr — phosphocreatine, ADP — adenosine diphosphate, ATP — adenosine triphosphate, GPC — glycerophosphorylcholine, NAD — nicotineamide adeninedinucleotide, oxidized and reduced form.

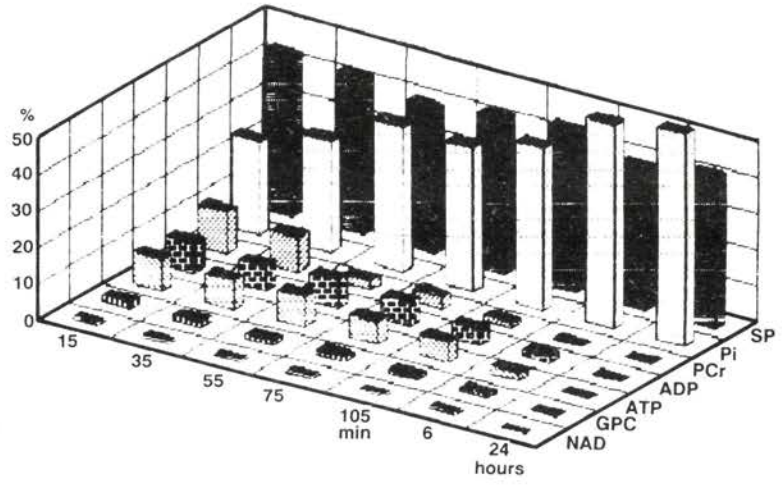

Fig. 3. The kinetics of change in the contents of the phosphorus compounds in bovine MLT. For symbols see Legend to Fig. 2.

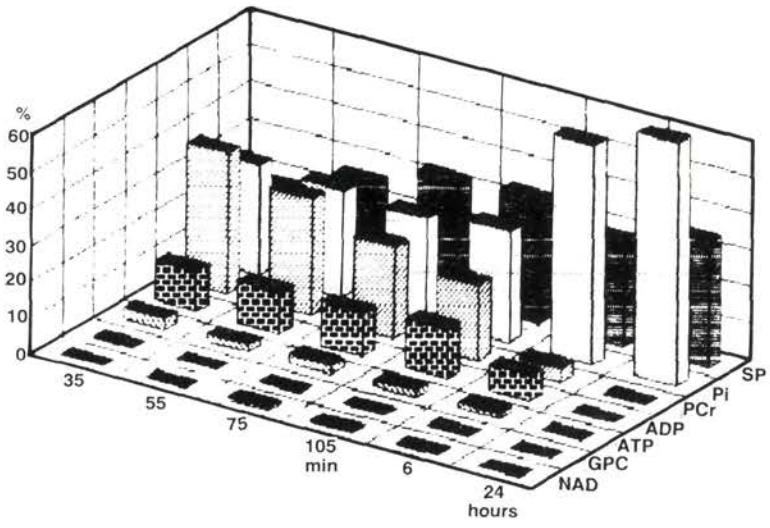

Fig. 4. The kinetics of changes in the contents of phosphorus compounds in porcine MLT. For symbols see Legend to Fig. 2.

MHz frequency. During NMR measurements, the spectrometer was locked on deuterium resonance of the solvent (HDO) and the temperature of the samples was kept at 22 °C. The acquisition parameters were set to: spectral width 6277.5 Hz, number of data points 8192, acquisition time 0.652 s, delay after acquisition 20 s, pulse width 20 μs (90° pulse angle), number of transients 32; no decoupling was applied. Prior to the Fourier transformation, the spectra in the time-domain were weighted with the appropriate exponential function (line broadening 2 Hz). Chemical shifts were related to the phosphocreatine resonance (-3.075 ppm). Under the acquisition conditions used the relative integrated intensities of spectral lines retained their quantitative significance.

Glycogen was determined according to the method of Korec (1967). Lactate was determined by the fully enzymatic test (Boehringer).

pH measurement: A small cut was made into muscle sample into a depth of approx. 1.5 cm. An electrode was slipped into this cut so as to establish a perfect contact with the tissue. A pH meter Radelkis OP 211/1 with combined silverchloride/glass contact electrode 404 T (Ingold) was used.
Statistic evaluation: Values in tables are given as mean $\pm$SD. The paired t-test was used to analyse the differences between bull MLT and MPS, and Student's t-test was used to analyse those between bull and pig.

Results

The results of the histochemical analyses of pig and bull MLT and MPS are given in Table 1. All the analysed muscles are mixed, composed of three types of muscle fibres. Both bull muscles investigated show similar proportions of muscle fibre types. Pig MLT contains more αW fibres.

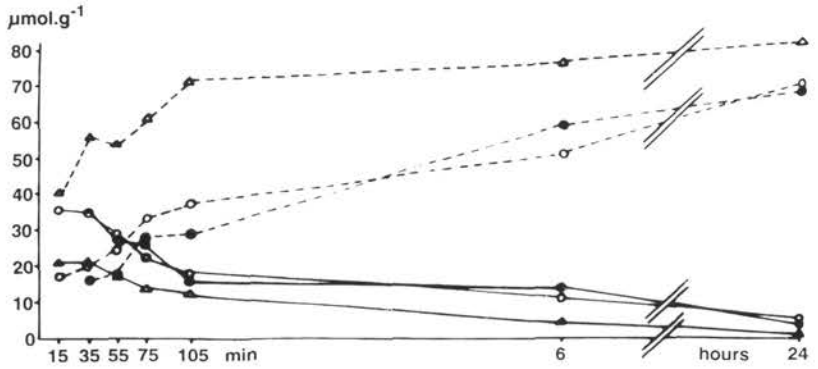

Fig. 5. Postmortem changes of glycogen and lactate in porcine and bovine MLT and bovine MPS. △———△ glycogen, △- - -△ lactate, porcine MLT; ●———● glycogen, ●- - -● lactate, bovine MLT; ○———○ glycogen, ○- - -○ lactate, bovine MPS.

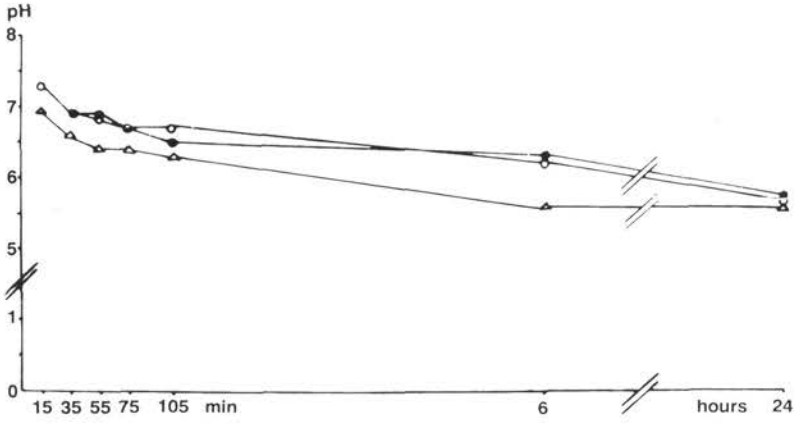

Fig. 6. Post mortem changes of pH in porcine and bovine MLT and bovine MPS. △———△ MLT, pigs, ●———● MLT, bulls, ○———○ MPS, bulls.

Figure 1 shows the ^{31}P NMR spectra of bull MPS and bull and pig MLT 15 minutes post-mortem. The kinetics of the postmortem changes concerning the relative contents of phosphorus compounds in bull and pig muscles are shown in Figs. 2, 3 and 4. The relative concentrations of phosphorus compounds in bovine muscles of similar participation of muscle fibre types showed no significant differences. Bovine muscles contain larger amounts of PCr (48 % of all phosphates 15 minutes post-mortem in MPS, 32—35 % in both muscles 35 mi-

nutes post-mortem). On the other hand, the ATP values are relatively low in both muscles. The concentration of SP increased during the period of observation in both bovine muscle types. During the last interval of observation, also ADP started decaying. The relative molar concentrations of P_i increased gradually throughout the period of observation, and reached the value of 63—66 % after 24 hours.

Fifteen minutes post-mortem, pig MLT contains 4 times less PCr than does bull MLT. On the other hand, the values of ATP and SP for pig MLT are higher than those for bovine muscles. Pig MLT contained approx. 1 % PGC throughout the period of observation, whereas no GPC was detected in bull MLT or MPS. The concentrations of P_i increased, and reached 51 % 24 hours post-mortem.

The contents of glycogen and lactate in pig and bull muscles are given in Fig. 5. No significant differences in glycogen were observed between bull MLT and MPS in any of the intervals studied. Thirty-five minutes post-mortem, the values reached 34—35 μmol . g^{-1} with a decrease to less than 50 % 6 hours post-mortem, and to 10—20 % of the initial values 24 hours post-mortem. The concentration of glycogen in pig MLT during the early phases of the postmortem processes is significantly lower in comparison with the bull muscles.

The concentration of lactate in bovine muscles is only 50 % 35 minutes post-mortem of those measured for pig MLT in the same interval.

pH values are shown in Fig. 6. Significantly lower values were measured for pig MLT in the early phases of the post-mortem processes than for bovine muscles. This suggests a more intense glycolysis in pig MLT than in bovine muscles in the early phases of the postmortem processes.

Discussion

The results show that there are no significant differences in the kinetics of postmortem changes of glycogen, lactate, pH and phosphorus compounds between bull MLT and MPS which have similar participation of muscle fibre types.

The kinetics of postmortem changes of phosphorus compounds in bovine and porcine muscles have several common traits. CP disappeared first, followed by ATP, and P_i concentrations simultaneously increased.

In muscles studied post-mortem, the content of P_i rises as a result of the ATP splitting into ADP and P_i (1).

$$\mathrm{ATP} \xrightarrow{\mathrm{ATPase}} \mathrm{ADP} + P_i \qquad (1)$$

ATP is then rephosphorylated by glycolysis or by reaction with PCr (2). However, PCr is gradually depleted and glycolysis cannot keep up with ATP resynthesis.

$$PCr + ADP \xrightarrow{\text{creatinkinase}} ATP + \text{creatine} \quad (2)$$

As a consequence, splitting of two molecules ADP into one molecule ATP and one molecule AMP is activated (3).

$$2\,ADP \xrightleftharpoons{\text{myokinase}} ATP + AMP \quad (3)$$

AMP is then deaminated to inosine monophosphate (IMP) (4).

$$AMP \xrightarrow{\text{AMP deaminase}} IMP + NH_2 \quad (4)$$

IMP is further degraded to hypoxanthine and ribose-1-phosphate or hypoxanthine and ribose (Lee and Newbold 1963). As a result of (3) and (4), the concentration of ADP which formed by reaction (1) gradually decreases. Twenty-four hours post-mortem both pig and bull muscles contain only P_i and SP at NMR detectable level.

The kinetics of postmortem changes in pig MLT is more rapid as compared with bovine muscles. In pig MLT, PCr disappears within 2 hours post-mortem, whereas it is still present approx. 6 hours post-mortem in bovine muscles. Similarly, the significantly higher concentrations of SP and lactate, and the lower concentrations of glycogen and a lower value of pH in porcine MLT as compared with bovine muscles suggest a more intense glycolysis in the early phases of the post-mortem processes.

Our observation correspond with data reported by Lundberg and Vogel (1987) who found higher rates of postmortem phosphorus metabolism in porcine muscles than in bovine muscles, and with data of Kastenschmidt (1970) who observed a drop by 30 to 50 % of glycogen concentrations in porcine muscles ten minutes post-mortem. An intense activation of glycolysis is also suggested by the high SP concentrations in porcine muscles in the early stages of the postmortem processes.

Interesting is the fact that in porcine MLT the levels of PCr are lower in the early stages of the postmortem processes in comparison with bovine muscles, whereas ATP levels are higher. This may indicate that porcine MLT is much less adapted to deficiency induced by a depression of aerobic ATP production than bovine muscles. A constantly high rate of glycolysis in porcine muscles may partly explain these differences, since in the presence of an already enhanced glycolysis the reserve allowing further acceleration is smaller.

PCr content depends on the type of muscle fibres. Meyer et al. (1985) found differences in PCr contents between fast-twitch and slow-twitch muscles. M. biceps femoris with a more than 75 % proportion of fast-twitch muscle fibres had higher PCr/P_i ratio than did m. soleus with 92 % slow-twitch muscle fibres. In correspodence with this, Jørgensen and Grasdalen (1986) found higher values of PCr (14 mmol . l^{-1}) in the red muscle of the cod (9 mmol . l^{-1}), whereas other phosphorus metabolites were had similar values in both muscles. However, in our experiments lower PCr values were measured for porcine MLT than for bovine MLT and MPS, although porcine MLT contains more fast-twitch white fibres than does bovine muscle. The reason may be a high rate of glycolysis in porcine MLT due to which a great portion of PCr is decayed 15 minutes post-mortem.

GPC was identified after addition of this compound into the muscle extract. According to Renou et al. (1986) the occurrence of this compound seems to be associated with slow contractile type fibres. However, the occurrence of this compound seems to depend also on the animal species. In our experiments GPC was present in relatively high concentrations in porcine MLT, but bovine muscles with a higher proportion of slow contractile red muscles were free of this metabolite. This compound and related metabolites arise from phospholipids and might be involved in preserving the membranes from phospholipids breakdown (Satrústegui et al. 1988).

In summary there are no significant differences in the relative occurrence of phosphorus compounds and the kinetics of their postmortem changes in bovine MLT and MPS with similar participation of muscle fibre types. During the early stages of the postmortem processes these muscles have higher glycogen and PCr contents and lower lactate, ATP and SP contents and higher pH values as compared with porcine MLT. GPC was not present in bovine muscles at NMR detectable level in contrast to porcine MLT. The patterns of postmortem metabolism in bovine and porcine muscles are similar. However, the rate of postmortem metabolism is higher in porcine muscles.

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Final version accepted September 11, 1990