Application of ^{31}p-NMR to Measure the Biochemical Characteristics of the Postmortem Muscle of PSS Pigs

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SUMMARY: The purpose of this study was to apply ^{31}PNMR to investigate the changes of phosphorus related compounds of muscle, and coordinated with the biochemical tests including pH value, lactic acid, ATP, CPK, CrP and ATPase activity to assure the changes of biochemical characteristics of muscles of the procine stress susceptible (PSS) and normal pigs.

The results were as follows:From the spectra of muscle, creatine phosphate (CrP) and ATP resonance of PSS pigs almost disappeared within 30 minutes postmortem, but the inorganic phosphate level of the muscle increased with the postmortem time increased. However, the CrP and ATP resonance of the normal pig muscle remained constant within 30 minutes postmortem. The CrP and ATP depleted completely after 60 minutes postmortem. Sugar Phosphate decreased with the postmortem time increased. The pH value of PSS pig muscle was significantly lower ($p<0.05$) than the normal pigs during 6 hours postmortem, and lactic acid content of PSS pig muscle was significantly lower ($p<0.05$) than the normal pigs. The temperature of PSS pig muscle was significantly higher ($p<0.05$) than the normal pigs, but gradually descented. The ATPase and LDH activities of the muscle had no significant difference between the PSS and normal pigs, while CPK activity of the PSS pig muscle was significantly higher ($p<0.05$) than the normal pigs during 3 hours postmortem.

INTRODUCTION: Our lab has used 31P-NMR to study phosphorus related compounds of blood of stress sensitive pigs (Yang et al., 1991). From the NMR-spectra of blood it could be observed that the level of ATP was very low, and the inorganic phosphate resonance increased with the period of electrical stress. Because the stress sensitive pigs had more rapid metabolism rate, it produced more lactate causing the pH value of blood dropped. As a result of changing the chemical shift of the inorganic phosphate resonance moved to higher field. The content of ATP and Pi of blood had significant difference between the normal and PSS pigs. Creatine phosphate kinase and lactate dehydrogenase activities of blood for PSS pigs were significantly higher than the normal pigs observed during periods of ischaemia, exercise and recovery, and some researchers are interested in the applications of NMR to investigate the biochemical processes resulting from electrical stimulation of carcasses of isolated muscles soon after slaughter of animals. It is not easy to evaluate the role of NMR in such studies, partly because to the author's knowledge, so far these have been very few publications in this general area of research. Our lab has used 31P-NMR to study phosphorus related compounds of blood of stress sensitive pigs (Yang et al., 1991). From the NMR-spectra of blood it could be observed that the level of ATP was very low, and the inorganic phosphate resonance increased with the period of electrical stress. Because the stress sensitive pigs had more rapid metabolism rate, it produced more lactate causing the pH value of blood dropped. As a result of changing the chemical shift of the inorganic phosphate resonance moved to higher field. The content of ATP and Pi of blood had significant difference between the normal and PSS pigs. Creatine phosphate kinase and lactate dehydrogenase activities of blood for PSS pigs were significantly higher than the normal pigs.

The purpose of this experiment was to study the changes of metabolites of phosphates in the postmortem muscles from the normal and PSS pigs by NRM-spectrometry. At the same time, the biochemical processes of the muscle metabolism of PSS pigs were also determined.

MATERIALS and METHODS:

1. Testing animals

Halothane test was used to screen the normal and PSS pigs. 50-90 Kgs of body weight of pigs were tested.

2. Muscle samples

The animals were sacrificed after fixed and anaethesized, M. Longissimus dorsi at 6th rib was excised and incubated at 25 ℃ for 0.5, 1, 2, 3, 6, and 12 hours postmortem and frozen in liquid nitrogen until prepared the muscle extract for running the ^{31}P-NMR spectrometry. The muscle extract was prepared by the procedures described by Vogel et al. (1985).

3. Phosphorus- ^{31}NMR

NMR-spectra were measured by NMR-spectrometer 7.05 Tesla, Varian Instrument Ltd, USA.

4. Biochemical properties analysis

pH value was determined by LTP-SP 35 digital pH/MV meter, Suntex Instrument Co.

Myofibrillar proteins of ATP activity were prepared by the procedure described by Honikel and Cheon(1987) and the ATP activity was determined by the method described by Briskey and Fukazawa(1971).

Creatine Phosphokinase activity(CPK) was determined by the method of Tanger and Gilvary(1959).

Lactate Dehydrogenase activity was measured by the method of Caband and Wroblewsk(1958).

ATP content was determined by the method of Dieter and Juttu(1981) and creatine phosphate(CrP) content was determined by the method of Heinz and Herwig(1981).

RESULTS and DISCUSSION

Fig.1 and 2 showed the ^{31}P-NMR spectra of the PSE muscle resulted from the PSS pigs at 0.5, 1, 2, 3, 6, and 12 hr after slaughter. When the PSS pigs expose to the stressors such as transportation, driving and crowding, the animals can't adapt to these conditions, which cause sympathetic nervous system release higher level of catecholamine, resulting in causing glycongen breakdown rapidly, simultaneously cause the peripheral blood vessel contraction.

Consequence of stress, and metabolic adjustments associated with it, can be noted in the muscles. These adjustments usually place an increase demand on the muscle for contraction, so there is a need for an increase rate of blood flow in the muscles, but this circulating adjustment is still unable to provide the quantity of the blood needed to supply adequate oxygen to the muscle.

Fig. 1 indicated that CrP and ATP disappeared from the NMR-spectra of the PSE muscle in 30 min. after slaughter, but only sugar phosphate and inorganic phosphate presented in the spectra. This was due to that the temperature of PSE muscle was higher than that of the normal muscle, and caused higher metabolic rate. And sugar phosphate was absent until 2 hours postmortem, only inorganic phosphate presented in the spectra (Fig.2) .

The reasons for ^{31}P-NMR only can be used for qualitative analysis but not for quantative. were NMR technique only can measure high speed movement compounds, but can not detect the comnpounds combining with other materials becoming immobile. It is very difficult to measure absolute concentration because relaxation time of each compound after impulse thus the signal intensity are also different. However, the biochemical properties are used to aid the changes of CrP and ATP in muscle. The changes of ATP levels in the normal and PSE muscles 6 hours postmortem were shown in Table 1.

The level of ATP in PSE muscle was less half than the normal muscle one hour postmortem, and significantly lower ($p<0.05$) in ATP concentration of the PSE muscle than the normal muscle 2 hours postmortem. The ATP in PSE muscle depleted completely at 2 hours postmortem and then tended to be stable. However, ATP level in the normal muscle degraded, muscle contraction and metabolism lowered gradually as storage time extended, but change rate was lower than the PSE muscle.

Table 2 showed that CrP change of the normal and PSE muscles during 6 hours postmortem. CrP level of the PSE muscle was significantly lower than that of the normal muscle one hour postmortem($p<0.05$), and tended to stable. CrP level of the normal muscle decreased with the storage time increased.

The level of CrP in the normal muscle 6 hour postmortem was the same as the level of the PSE muscle at one hour postmortem.

The change of carcass temperature of the normal and PSE muscles were shown in Fig. 3. The temperature of PSE muscle rose up to 42.8 ℃ at 0.5 hour postmortem and still reminded at 41℃ one hour postmortem. The carcass temperature change is usually used as one of the methods for identify the muscle whether the normal or PSE muscle or not (Honikel and Cheou, 1987; Dobroeno, 1989). In this experiment, the L. dorsi muscle samples were removed from the carcass within 20 min. after slaughter and place in insulated box in order to delay temperature drop. However, inspite of the normal or PSE muscle it was able to find the temperature of the muscle dropped rapidly, but the dropping rate of the PSE was slower than the normal muscle.

Fig.4 and 5 showed the changes of pH and lactate of the normal and PSE muscles after slaughter. The PSE muscle had higher temperature and caused glycogen brokendown rapidly. The pH of the PSE muscle was dropped below 5.9 at 0.5 hour, and below 5.8 one hour postmortem. The lactate contents of the PSE muscle were 74.4 m mole/Kg at 0.5 hour postmonten, and 83.03m mole/Kg one hour postmortem, respectively. The lactate was accumulated to 94.58m moles/Kg. The change of pH of the normal muscle was 6.3 0.5 hour, and lactate content was 32.89 m mole/Kg, at 0.5 hour postmortem but pH value reached to 5.8 and tended to be stable, the lactate content was 80.53 m mole/Kg at 3 hour postmortem. The results could be observed that the lactate content of the normal muscle was significantly ($p<0.05$) lower than that of the PSE muscle.

The changes of myofibrillar ATPase activity for the normal and PSE muscles were shown in Table 3. There was no significant difference for ATPase activity between the normal and PSE muscles. However, as muscle pH of PSS pig dropped below 5.30, the ATPase activity was significantly lower than the PSE muscle of pH 5.4. The ATPase activity for pH 5.3 and pH 5.4 muscles were 0.0068 and 0.011 m mole/mg, respectively. These results could be found that the pH of the muscle had not dropped below 5.3 the ATPase was not denaturated and the activity of ATPase for the PSE muscle was not different from the normal muscle, even, it was pale in appearance. However, as pH dropped below 5.3, the ATPase was denaturated and its activity depleted remarkably.

CPK activity of the normal and PSE muscles were shown in Table 4. CPK activity of the PSE muscle was significantly($p<0.05$) higher than the normal muscle 3 hour postmortem. Generally, CPK activity decrease with postmortem time extend, however, the reason for the results of the experiment needed furthe study.
No difference for LDH activity between the normal and PSE muscles was detected from 3 hour postmortem (Table 5).

REFERENCES:

1. BRISKEY, E. J., and T. FUKAZAWA, (1971). Myofibirllar proteins of skeletal muscle. Adv. Food res. 18; 279.
2. CABAND, P. G., and F. WROBLEWSKI (1958): Colorimetric measurement of lactic dehydrogenase activity in body fluids. Am. J. Clin. Pathol. 30;234.
3. DIETER, J. and W. JUTTA (1981): Method of enzymatic analysis. 3th edition Bergmeyer publishing comp.7; 340.
4. DOBRENOV, B. (1989): Studies on DFD and PSE pig meat in Australia state of Queensland. Fleischwirtsh 69; 869.
5. GUPTA, R. K., L. BENORIC, and E. B. ROSE. (1978): The determination of free magnesium level in the humen red blood cell by 31P-NMR. J. Bio. Chem. 253, 6172.
6. GUPTA, R. K. and R. D. MOORS. (1980): 31P-NMR studies intracellular free Mg in intact frog skeletal muscle. J. Bio. Chem. 255, 6172.
7. HEINZ, F. and W. HERWIG (1981): Method of enzymatic analysis. 3th edition Bergmeyer publishing comp. 8; 507.
8. HONIKEL, K. O., and J. K. CHEON (1987): Causes of the development of PSE pork. Fleischwirtsch international 1; 7.
9. SUNG, S. K., T. ITO, and T. FUKAZAWA (1976): Relationship between contractility and some biochemical properties of myofibrills prepared from normal and PSE porcine muscle. Food Sci. 41; 102.
10. TANZER, M. J., and C., GILVARG (1959): Creatine kinase measurement. J. Biol. Chem. 234; 3201.
11. VOGEL, H. J. and P. LUNDBERG (1985): Postmortem energy metabolismt in bovinemuscles studied by non-invasive phosphorus-^{31} nuclear magnetic resonance. Meat Sci. 13; 1.
12. YANG, W. D., M. T. CHEN and D. C. LIU. (1991). Application of ^{31}P-NMR to measurethe biochemical characteristics of blood of pss pigs. J. of the chinese society of Anim. Sci. (in press).

Table 1. Adenosine triphosphate (ATP) concentration of muscle from stress-susceptible and normal pigs during 6 hours postmortem

	mmole / Kg			
Time post-mortem (hr.)	1	2	3	6
Stress-susceptible	0.32	0.0696*	0.0567*	0.053*
Normal	0.676	0.4829	0.3976	0.194

* There is significant difference between two treatments ($P < 0.05$)

Table 2. Creatine phosphate (CrP) concentration of the muscle from stress-susceptible and normal pigs during 6 hours postmortem

	mmole / Kg			
Time post-mortem (hr.)	1	2	3	6
Stress-susceptible	0.34*	0.40	0.27*	0.35
Normal	0.92	0.73	0.71	0.31

* There is significant difference between two treatments ($P < 0.05$)

Table 3. Myofibrillar ATPase activity of the muscle from stress-susceptible and normal pigs

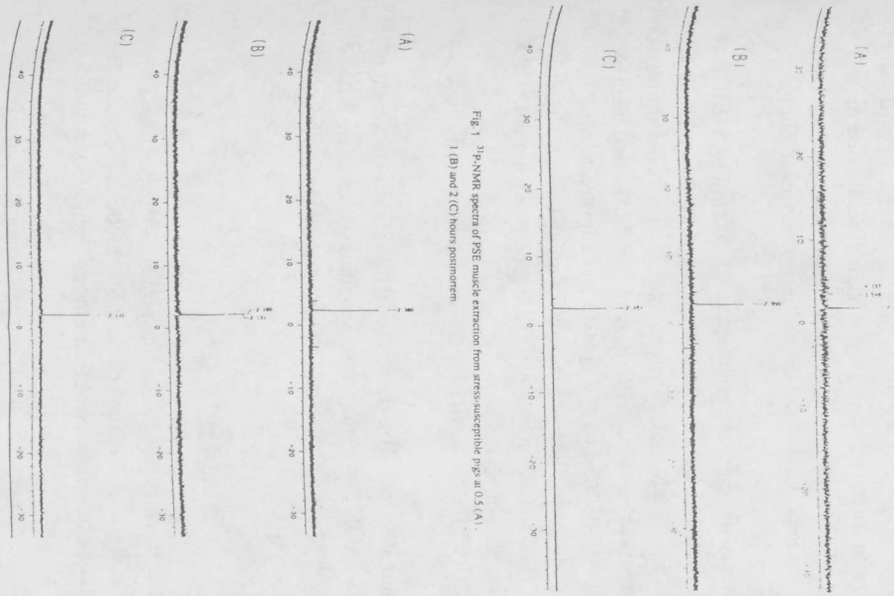

Fig. 1 ^{31}P-NMR spectra of PSE muscle extraction from stress-susceptible pigs at 0.5 (A), 1 (B) and 2 (C) hours postmortem.

Fig. 2 ^{31}P-NMR spectra of PSE muscle extraction from stress-susceptible pigs at 3 (A), 6(B) and 12(B) hours postmortem

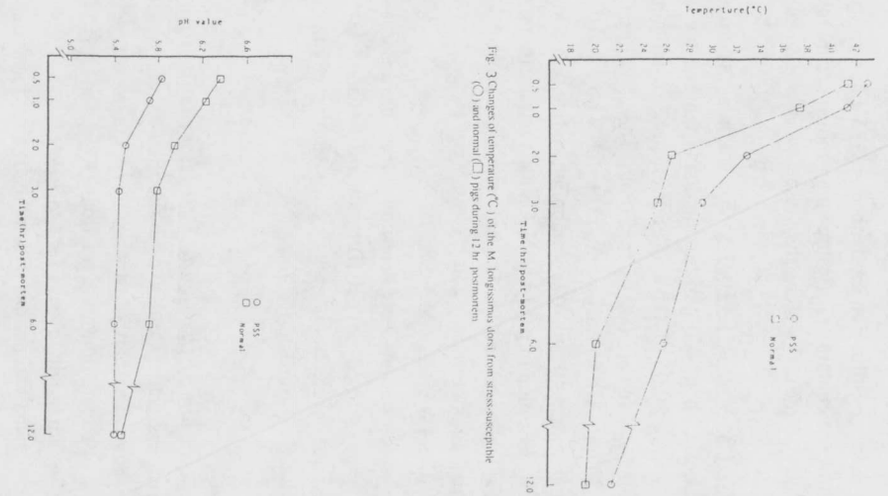

Fig. 3 Changes of temperature (℃) of the M. longissimus dorsi from stress-susceptible (○) and normal (□) pigs during 12 hr postmortem

Fig. 4 Changes of pH Value of the M. longissimus dorsi from stresssusceptible (○) and normal (□) pigs during 12 hr postmortem

Table 3. Myofibrillar ATPase activity of the muscle from stress-susceptible and normal pigs during 6 hours postmortem

	mmole / mim / mg protein*			
Time post-mortem (hr.)	1	2	3	6
Stress-susceptible	0.0125 ± .0014	0.0130 ± .0012	0.0121 ± .0009	0.0120 ± .0021
Normal	0.0120 ± .0009	0.0118 ± .0009	0.0124 ± .0008	0.0157 ± .0015

* Values are mean ± SD.

Table 4. Creatine phosphate Kinase (CPK) activity of the muscle from stress-susceptible and normal pigs during 3 hours postmortem

	IU / 1		
Time post-mortem (hr.)	1	2	3
Stress-susceptible	328.61* ± 61.7	243.27* ± 70.6	240.12* ± 72.8
Normal	204.87 ± 35.9	182.51 ± 50.9	155.79 ± 66.3

* There is significant difference between two treatments ($P < 0.05$)

Table 5. Lactate dehydrogenase (LDH) activity of the muscle from stress-susceptible and normal pigs during 3 hours postmortem

	berger-broida unit / ml*		
Time post-mortem (hr.)	1	2	3
Stress-susceptible	2032.40 ± 19.92	2037.16 ± 15.89	1962.60 ± 25.48
Normal	2007.70 ± 46.64	1993.28 ± 66.55	2048.12 ± 38.35

* Values are mean ± SD