Variation of AMPK Activity and Its Effects on Glycolysis in Postmortem lamb

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Abstract—AMPK activity and glycolysis indexs were observed to determine the correlation of them. The results showed that AMPK was activated in postmortem skeletal muscle, and reach the maximal value at 1hr, then begin to fall down, AMPK activity has very significantly deviation among 0-24hr postmortem($P<0.01$). Glycogen content and pH value were all decreased endlessly at 0-24hr postmortem, lactic acid content was increased at 0-24hr postmortem, and significantly difference are all very significantly at different time postmortem($P<0.01$). HK and LDH were all reached max activity at 1hr postmortem, and then step down gradually, This is correspond with the trend of AMPK activity variation. There are significantly deviation at different time postmortem for HK and LDH activity($P<0.01$). The study supports that AMPK regulates glycolysis in postmortem skeletal muscle.

***Index Terms*—AMPK, Glycolysis, Postmortem lamb**

I. INTRODUCTION

AMP-activated protein kinase(AMPK), a heterotrimeric enzyme with a, b, and g subunits(Hardie, 2004), which has the function for regulate energy metabolism. AMPK could perceived the alteration of energy metabolism state in animal organism, and through affect many links of substance metabolism to maintain energy supply and demand balance. Once activated, AMPK phosphorylates downstream substrate, the overall effect of which is to switch off ATP consuming such as glycogen and fatty acid synthesis and to switch on catabolic pathways that generate ATP such as glycolysis and fatty acid oxidation(Shen, Gerrard, & Du, 2008).

Carling(2004) and Choi, Kim, Lee, Kim, Mu, Birnbaum, Kim, and Ha(2001) showed that AMPK could switched on by an increase in the AMP/ATP ratio in muscle cells. ATP is decrease and AMP is increase in postmortem muscle cells, so AMPK was activated, in the aspect of glycometabolism it could promote glycolysis in order to reduce ATP utilization and increase ATP production. Therefore, the objective of this study was to assess AMPK activation, glycolysis and their correlation in lamb postmortem.

II. MATERIALS AND METHODS

A. Samples

Three Eighteen months old Inner Mongolia lamb were chose as subject, with three replicates after slaughter and bloodletting, biceps femoris(BF) were snap frozen in liquid nitrogen and stored at -80℃ until analyzed.

B. Measurements of AMPK activity

The extraction of AMPK was referenced by the method of Underwood, Means, and Zhu(2008). Frozen BF samples (0.1g) were powdered in liquid nitrogen and homogenized in 500ul of ice-cold homogenization buffer (0.25mol/L mannitol 0.05mol/L Tris/HCl, pH7.4 1mmol/L EDTA 1mmol/L EGTA 1mmol/L DTT 50mmol/L NaF 5mmol/L sodium pyrophosphate). Homogenates were centrifuged at 12,000g, 4℃ for 5 min. The supernatants were then used for AMPK activity determination, using a commercially available AMPK ELISA determination kit(WuHan ZhongMei technology limited company).

C. Lactic acid content measurement

With each sample, 0.3g of muscle was homogenized in 900ul of 0.9mol/L $HClO_4$. Homogenates were then centrifuged at 13,000g at 4℃ for 5 min. The supernatants were removed and neutralized with 2mol/L KOH. After centrifugation (13,000g) to precipitate potassium perchlorate, the extracts were used for lactic acid measurement(Shen, Means, Thompson, Underwood, Zhu, McCormick, Ford & Du, 2006). Lactic acid concentrations were determined by using a commercially available lactate determination kit(NanJing JianCheng technology limited company).

D. Glycogen content measurement

Using a commercially available glycogen determination kit(NanJing JianCheng technology limited company) to

measurement.

E. pH value

Muscle was homogenized in 10 times distilled water at 3000r for 1min, after 10 min standing, pH meter(Sigma) was used to determine pH value.

F. HK and LDH activity measurement

Using a commercially available glycogen determination kit, muscle sample was homogenized in 9 fold 0.9% NaCl, then centrifuged at 2000g for 10min. The supernatants were then used for HK and LDH activity determination.

G. Statistical analysis

Data were analyzed by using ANOVA, differences in the mean values within the same treatments were compared by Duncan multiple comparison. All data are expressed as mean±SE.

III. RESULTS AND DISCUSSION

A. AMPK activity variation in postmortem lamb

According to AMPK determination time extent is among 0-24hr(Shen, Underwood, Means, McCormick & Du, 2007; Shen & Du, 2005), this study decided 0hr, 1hr, 4hr, 8hr, 12hr, 24hr to determine AMPK activity variation. The results is shown in Fig1. AMPK was activated in postmortem skeletal muscle, at 1hr postmortem AMPK activity achieved the max value 107.49±2.983U/L, then begin to fall off, at 24hr postmortem the activity became 66.8±5.541U/L, decrease about 50% compared with 1hr postmortem. In addition, AMPK activity has significant deviation among 0-24hr postmortem($P<0.01$).

The muscle glycometabolism becomes glycolysis after the earlier period of animal dead, which leads to ATP decreased quickly, AMPK was activated. Shen et al(2005, 2006, 2007) showed that muscle AMPK activity has the max value often in 0.5h and 1h postmortem, then decreased rapidly. Our study has the similar result, at 1h postmortem AMPK activity reached the maximum in lamb. Because of the limited glycogen content in muscle, glycolysis stopped quickly, AMP/ATP no longer changed, So AMPK activity decreased soon after it activated.

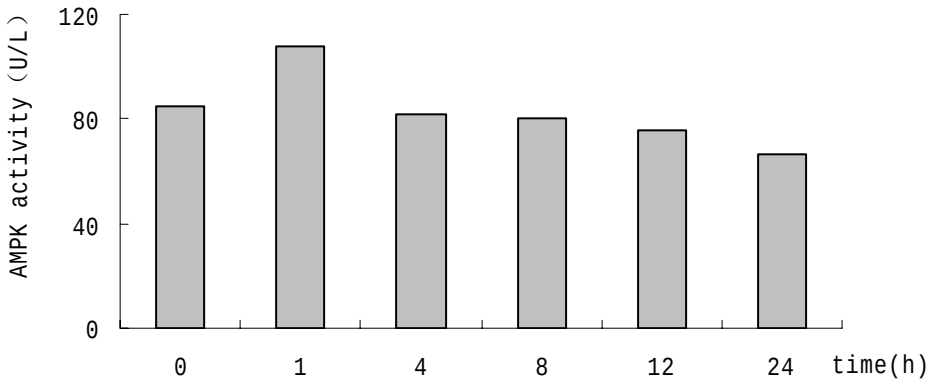

Fig1 Variation of AMPK activity(U/L) in postmortem lamb

The muscle glycometabolism becomes glycolysis after the earlier period of animal dead, which leads to ATP decreased quickly, AMPK was activated.

B. Glycolysis physical and chemical indexs variation in postmortem lamb

Glycogen content and pH value were all decreased endlessly at 0-24hr postmortem, lactic acid content was increased at 0-24hr postmortem, and their difference are all significantly at different time postmortem($P<0.01$)(Table 1).

Lactic acid is the end product of glycolysis, the degradation of glycogen leads to lactic acid accumulation, the accumulation of lactic acid leads to pH value degression.

Table1 Glycolysis physical and chemical indexs variation in postmortem lamb

Glycogen content(mg/g)	Lactic acid content(umol/g)	pH value

0h	2.28±0.004[a]	177.66±11.74[e]	6.37±0.007[a]
1h	1.83±0.037[b]	381.59±21.67[d]	6.18±0.032[b]
4h	1.25±0.035[c]	450.16±37.547[d]	6.02±0.037[c]
8h	1.08±0.068[cd]	597.32±25.583[c]	5.96±0.024[c]
12h	0.96±0.09[de]	812.37±63.373[b]	5.87±0.023[d]
24h	0.86±0.084[e]	1120.94±13.678[a]	5.60±0.03[e]

C. Glycolysis enzyme variation in postmortem lamb

Hexokinase(HK) is one of the key enzyme in glycolysis, the key enzyme Mediated irreversible reaction is the major link of glycolysis regulation, their activity influence glycolysis speed directly. Besides, LDH is a important enzyme in glycolysis that catalysis pyruvic acid dehydrogenation in order to generate lactic acid. The activity of HK and LDH could all represent glycolysis velocity. HK and LDH activity variation are showed in fig2 and fig 3.

Winder, Holmes, Rubink, Jensen, Chen, and Holloszy(2000) showed that hypodermical injection AICAR(a activator of AMPK) for rat could increase its HK($P<0.05$) and LDH($P>0.05$) activity accompanied with AMPK active step up. Our experiment results is similar to Winder, HK and LDH were all reached max activity at 1h postmortem, and then step down gradually. This is correspond with the trend of AMPK activity variation, then step down gradually. There are very significant deviation at different time postmortem for HK and LDH activity($P<0.01$).

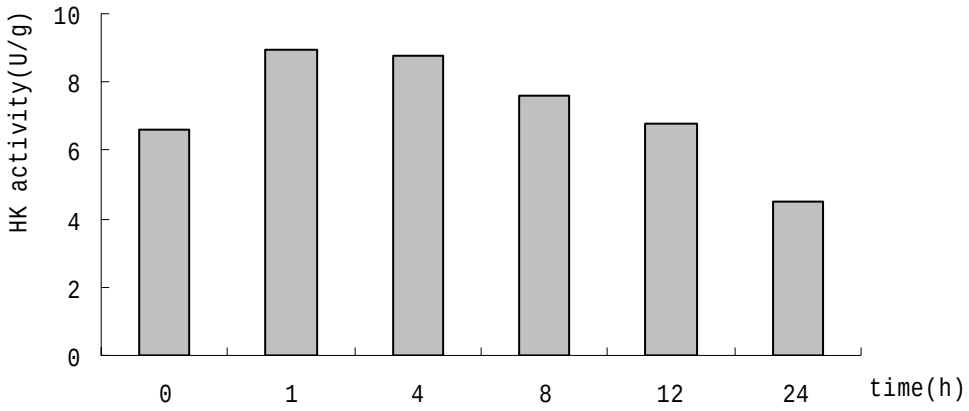

Fig2 Variation of HK activity(U/L) in postmortem lamb

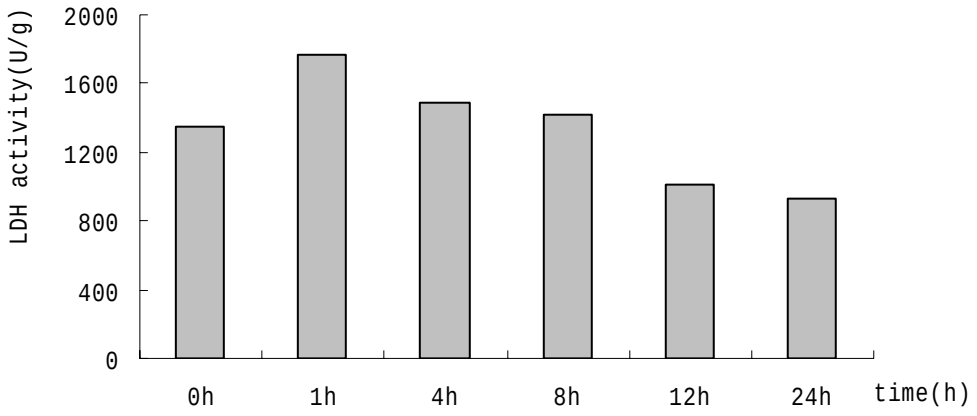

Fig3 Variation of LDH activity(U/L) in postmortem lamb

IV. CONCLUSION

Muscle glycometabolism convert to glycolysis postmortem, ATP production reduced, which leads to an increase of AMP/ATP ratio in postmortem, activated AMPK, glycogen content and pH value decreased gradually, lactic acid accumulation increased gradually.

AMPK activity has a max value at 1h postmortem, and the key enzyme HK and important enzyme LDH of glycolysis

achieved maximal value at 1hr postmortem, too. It showed that activation of AMPK could promote glycolysis. After AMPK activation, the activity of HK and LDH are step up, and glycolysis speed up.

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