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ATP regulates protein phosphorylation and degradation at -1.5°C in postmortem ovine muscle (#374)

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Introduction

It has been widely reported that ATP is related to rigor mortis of postmortem. The reversible protein phosphorylation played an important role in meat (ovine, beef, pork and et al) quality and the phosphorylation levels of myofibrillar proteins were significantly different between tender and tough muscle. However, the relationship between ATP, phosphorylation level and protein degradation was of less concern. The aim of this study was to demonstrate the effect of ATP on protein phosphorylation and degradation.

Methods

2.1 Sample preparation

The *Longissimus thoracis lumborum* (LTL) muscles from both sides of six sheep were collected. After removing visible fat and connective tissue, the LTL muscles were grounded. Every 50 g of ground muscles was added with 3.5 mL of 0.1 M ATP solution (dissolved in 10 mM $MgCl_2$) or the same volume of 10 mM $MgCl_2$ solution as the ATP treated/control group. Muscle samples were stored at -1.5°C for 21 days.

2.2 ATP content analysis

ATP content was measured by a commercial kit (MAK190, Sigma-Aldrich, St Louis, MO, USA) with colorimetry.

2.3 Phosphorylation levels and degradation of myofibrillar proteins

The samples at 0 h were loaded onto each gel as a reference for the densitometric analysis (Quantity One 4.6.2 software).

The degradation of -calpain, desmin and troponin T were measured with western blot and the blot were imaged by ChemiDoc™MP Imaging System of Image Lab 5.1 software.

2.4 Statistical analysis

SPSS Statistic 21.0 software (SPSS Inc., Chicago, IL, USA), Duncan's multiple range tests and Independent-samples T-test (5% significance level) were performed.

Results

3.1 ATP contents

ATP contents were decreased with prolonged storage time both in ATP treated and control groups (Fig. 1). The ATP contents of ATP treated group were significantly higher than that of the control group ($P < 0.05$). In postmortem muscle, ATP synthesis was reduced sharply due to glycolysis and ATP consumption was ongoing for biochemical reaction. The existence of ATP at 21 day indicated that ATP consumption was inhibited for the lower temperature.

3.2 Phosphorylation level

The phosphorylation levels of myofibrillar proteins were shown in Fig. 2. No matter in ATP treated or control group, the phosphorylation levels of myofibrillar proteins increased first and then decreased slightly. The phosphorylation levels of myofibrillar proteins were higher in ATP treated group than that of the control group after 12 h($P < 0.05$), which indicated that high ATP contents promoted phosphorylation levels.

3.3 The degradation of -calpain, desmin and troponin T

Compared with the control group, the degradation of -calpain, desmin and troponin T were inhibited in ATP treated group which revealed that ATP played a negative role in protein degradation (Fig. 3). In ATP treated group, -calpain degraded from 80 to 76 kDa at 12 h while at 0 h in control group. Fast ATP depletion leaded to the eariler autolysisi of -calpain through the release of Ca^{2+}, further promoting the degradation of troponin T and desmin. On the other hand, the higher phosphorylation level of myofibrillar proteins in ATP treated group might restrain protein degradation.

Conclusion

Higher ATP contents could significantly promote the phosphorylation levels of myofibrillar proteins. The ATP contents inhibited protein degradation through phosphorylation and the autolysisi of -calpain.

Notes

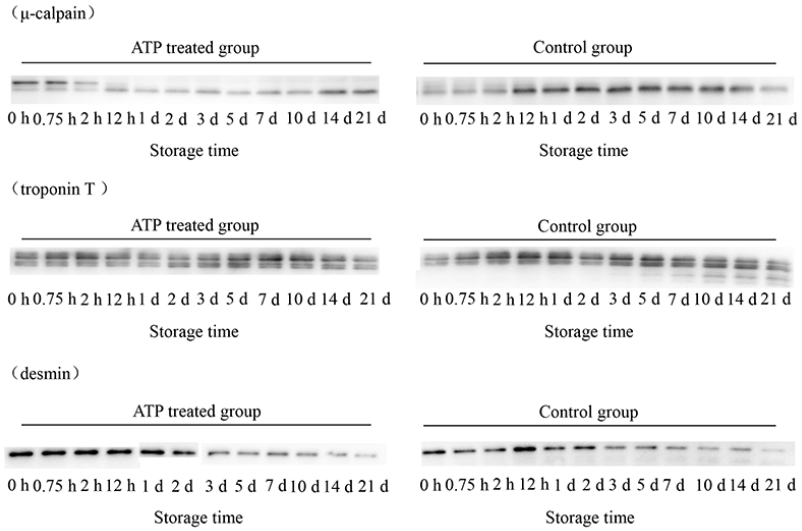

Fig. 3
The degradation of μ-calpain, desmin and troponin T in ground ovine muscle.

Notes

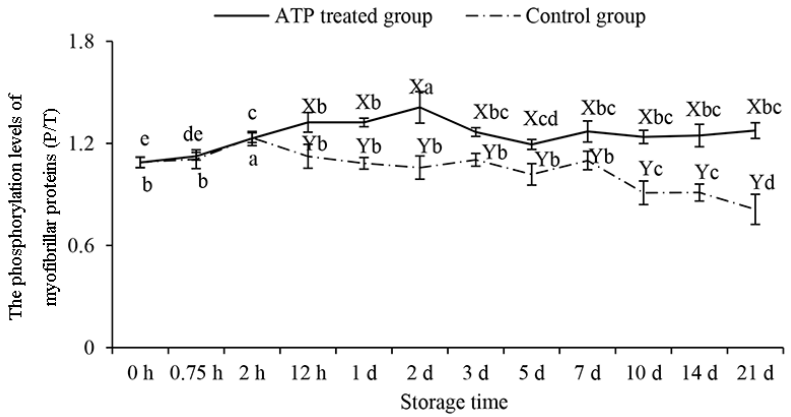

Fig. 2 The phosphorylation levels of myofibrillar proteins in ground ovine muscle.

Different small letters (a~e) represent significant difference within the same group ($P < 0.05$). Different capital letters (XY) represent significant difference between ATP treated and control groups at the same storage time ($P < 0.05$).

Notes

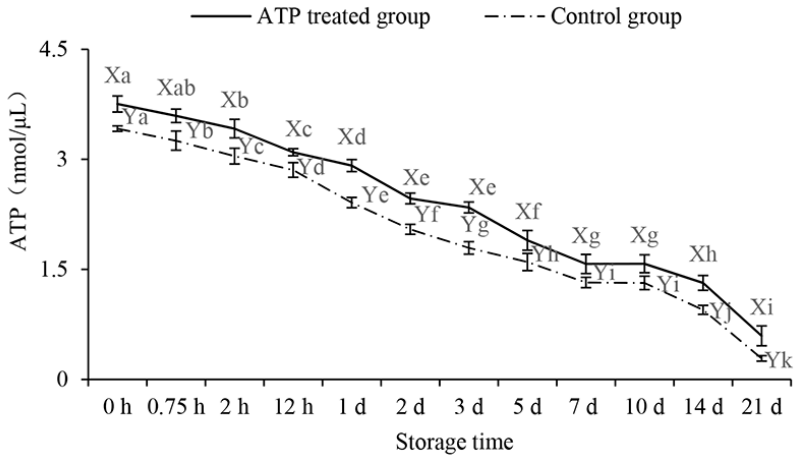

Fig. 1 The changes of ATP contents in ground ovine muscle.
Different small letters (a~k) represent significant difference within the same group ($P < 0.05$). Different capital letters (XY)represent significant difference between ATP treated and control groups at the same storage time ($P < 0.05$).

Notes