RIGOR BONDS REDUCE LATTICE SPACING OF SKINNED PORCINE SKELETAL MUSCLE FIBRE UNDER PSE-LIKE CONDITIONS

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Abstract – We established a new model on permeabilised/skinned porcine muscle fibres to study the effects of varied pH, temperature and the presence of rigor bonds on the myofibrillar protein denaturation during PSE-like conditions. Two parameters were evaluated: 1) the isometric contraction force of the contractile machinery; 2) myofilament lattice spacing. Our data showed that rigor bond attachment for 30 min at 38°C, pH 5.5 reduced the lattice spacing by 20%, accompanied by 50% loss of active contraction force while relaxed fibres had unchanged lattice spacing and force. The present study suggests that rigor bond attachment during PSE-like conditions would diminish water-holding due to more severe myofibrillar protein denaturation affecting the filament spacing.

Key Words – lattice spacing, protein denaturation, water-holding capacity

I. INTRODUCTION

Myofibrillar protein denaturation is widely accepted as the reason for the loss of water-holding in PSE. Rapid decline of pH at high (body) temperature and the presence of rigor bonds are the three important factors affecting the extent of myofibrillar protein denaturation. In the present study, we established a new model system in skinned porcine skeletal muscle, where the intact contractile units were maintained and the environmental factors (e.g. ATP, temperature and pH) could be manipulated directly *in vitro*. This enables us to keep the muscle fibres in relaxed or rigor states under PSE-like conditions. We monitored the effects on contractile force and filament lattice. The aim was to examine the effects of rigor bonds during PSE conditions on the myofilaments, in relation to water-holding capacity.

II. MATERIALS AND METHODS

Chemically skinned muscle fibre bundles were prepared essentially as described in [Kawai and Wang] from fresh (pre rigor, < 10-15 min after eutanasia) *longissimus thoracis et lumborum* (LTL) muscles from Swedish domestic pigs. The preparations were kept relaxed in 50% of glycerol at -20 °C. A bundle of 3-6 fibres was dissected from the sample, attached to force transducer and stretched 1.3 times of the slack length to give a sarcomere length of about 2.5 µm. Relaxing, contraction and rigor solutions were prepared, with compositions calculated according to Fabiato [1]. As illustrated in Fig. 1, an initial isometric contraction was recorded in Ca^{2+}-containing solution. Then, fibres were exposed for 30 min to different conditions **B1**: relaxed (ATP, phosphocreatine/PCr containing, low [Ca^{2+}]), 22 °C, pH 7.0; B2: relaxed, 22 °C, pH 5.5; **B3**: relaxed, 38 °C pH 5.5; **B4**: relaxed, 38 °C, pH 7.0; **B5**: rigor (no ATP or PCr, low [Ca^{2+}]), 22 °C, pH 7.0; **B6**: rigor 22 °C pH 5.5; **B7**: rigor 38 °C pH 5.5; **B8**: rigor 38 °C pH 7.0. After incubation, a second contraction was initiated and recorded. The ratio between 2nd and 1st contraction force was used to evaluate the effect of the treatments on the force development. Small angle X-ray diffraction (SAXS, beamline P03, at Petra III, Hamburg) was used to determine the equatorial patterns and lateral filament spacing, before, during and after the treatments above.

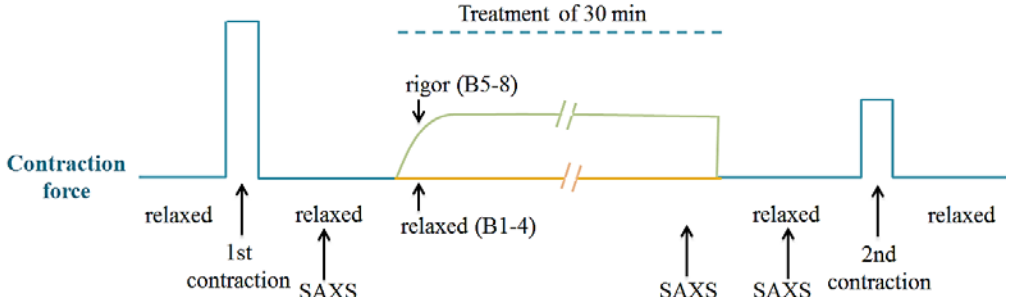

Figure 1. Schematic illustration of the experimental design.

III. RESULTS AND DISCUSSION

Fig. 2 shows a clear decline (~50%, p< 001, ANOVA) of contraction force of skinned fibres after B7 treatment. Thus rigor attachment during PSE-like conditions, i.e. pH 5.5 at 38 °C decreased the contraction force of contractile units while no changes were observed when fibres were kept relaxed at pH 5.5 at 38 °C (B3). The loss of contraction force can be an indicator of myofibrillar protein denaturation occurred close to myosin head region such as irreversible binding of actomyosin [2]. The results are consistent with previous studies showing a loss of ATPase activity in PSE muscle suggesting that the changes in the sarcomere affect both mechanical and biochemical properties of the contractile system.

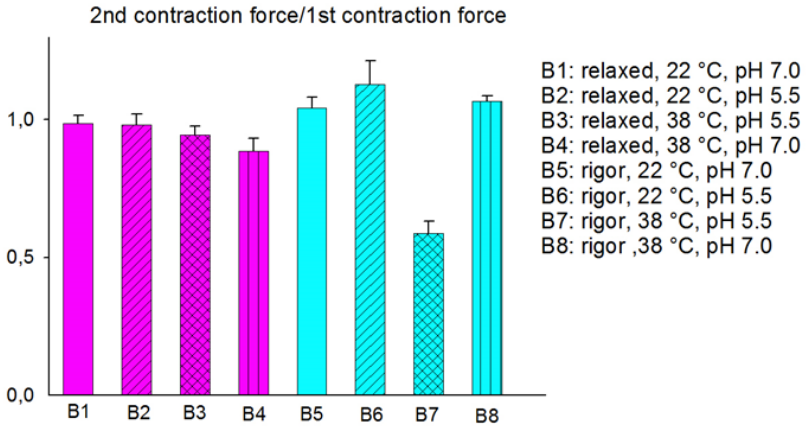

Figure 2. Relative contraction force after incubation at conditions B1-B7 (n=5-6)

We also report that that the lattice spacing (1.0 equatorial reflections) was significantly smaller after the incubation in the B7 group (from around 40 nm to 33 nm, *P*< 0.01 ANOVA), while the spacing in B3 and the other groups remained unchanged. The SAXS measurements confirmed that the muscles were in rigor in the ATP free solution and relaxed after the all treatments. This result suggests that rigor attachment between the myosin head and actin during PSE-like conditions has a negative effect on the space between thick and thin filament. Since lattice spacing determines the amount of water entrapped in the myofibrillar matrix, the more compressed lattice in B7 indicates poorer water-holding capacity.

As firstly proposed by Penny [3] and later supported by Offer [4] and [5], extreme fast glycolysis may counteract the negative effect of low pH and high temperature on myosin because of the earlier attachment of rigor bonds, which could protect myosin against further denaturation. The results from our study indicate that that rigor attachment during PSE-like conditions rather increase myofibrillar denaturation and loss of water holding.

IV. CONCLUSION

The study shows that rigor attachment between myosin and actin during PSE conditions will negatively affect myofibrillar proteins. The changes are reflected in a loss of active contraction force by the sarcomere and in a lateral shrinkage of the lattice spacing, which implies a poorer water-holding capacity.

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