PRE-SLAUGHTER TRANSPORT STRESS ACCELERATED GLYCOLYSIS OF POSTMORTEM PORK: THE INITIAL REGULATORY ROLE OF S-NITROSYLATION ON INTRACELLULAR CALCIUM

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I. INTRODUCTION

Nitric oxide and its mediated protein S-nitrosylation have been reported to regulate the activity, localization, stability, and interaction of proteins and play an important role in determining meat quality [1]. In our previous study, pre-slaughter transport stress induced NO overproduction by promoting NO synthase activity, which thereby notably increased overall protein S-nitrosylation levels in postmortem pork [2]. Additionally, abnormal calcium release and uptake in the sarcoplasmic reticulum could induce intense muscle contraction, increased glycogenolysis, and faster pH drop. This work aimed to further explore the effect of pre-slaughter transport stress on intracellular calcium levels from the view of the regulatory role of S-nitrosylation on calcium transporters including ryanodine receptor 1 (RyR1) and sarcoplasmic reticulum calcium ATPase 1 (SERCA1) as well as thus the change in muscle glycolysis.

II. MATERIALS AND METHODS

2.1 Sample collection

A total of sixteen castrated crossed (Duroc × Landrace × Yorkshire) with an average weight of 110 kg were randomly selected and assigned to two treatments (n = 8): three-hour transport without resting (transport-induced stress group, TS group) and three-hour transport followed by three-hour resting (low-stress control group, CON group). The transportation and slaughter conditions have been stated in detail in our previous report [2]. At 30 min postmortem, the *longissimus thoracis* muscles between the 8th and 10th thoracic vertebrae were collected and stored at -80 °C for further analysis.

2.2 Glycogen content and lactic acid concentration

Glycogen content and lactic acid concentration of postmortem muscle were detected according to the methods of Ma et al [2].

2.3 Sarcoplasmic calcium concentration

The calcium concentration in the sarcoplasm was measured by employing inductively coupled plasma optical emission spectrometry referring to the procedures of Liao et al [3].

2.4 SERCA1 and RyR1 S-nitrosylation levels

The S-nitrosylated protein was enriched and purified using a pierce™ S-nitrosylation western blot kit (90105, Thermo Scientific, USA). The S-nitrosylation levels of the target protein were calculated by the ratio of enriched S-nitrosylated protein to total protein.

2.5 Statistical analysis

All data were presented as the mean ± standard deviation. The T-test was applied for significance analysis (Chicago, IL, USA). $P > 0.05$ means no statistical difference while $p < 0.5$ means significant differences.

III. RESULTS AND DISCUSSION

As Figure 1 (A and B) shows, compared with CON group, the glycogen content in TS group decreased by 28.9%, while the lactic acid concentration increased by 25.2% ($p < 0.05$), indicating an accelerated glycolysis metabolism in TS group muscle samples. The sarcoplasmic calcium concentration in the TS group was remarkably higher with an increase of 18.1% relative to CON group (Figure 1C, $p <$

0.05). An increased level of sarcoplasmic calcium in the early postmortem period was the primary contributor to the accelerated glycolysis in postmortem muscle [4]. Importantly, higher S-nitrosylation levels of RyR1 and SERCA1 in TS group were also found relative to CON group (Figure 1D-F, $p < 0.05$). The elevated S-nitrosylation levels of RyR1 and SERCA1 resulted from pre-slaughter transport stress could be responsible for the calcium overload in the cytoplasm through activation of RyR1 and inhibition of SERCA1.

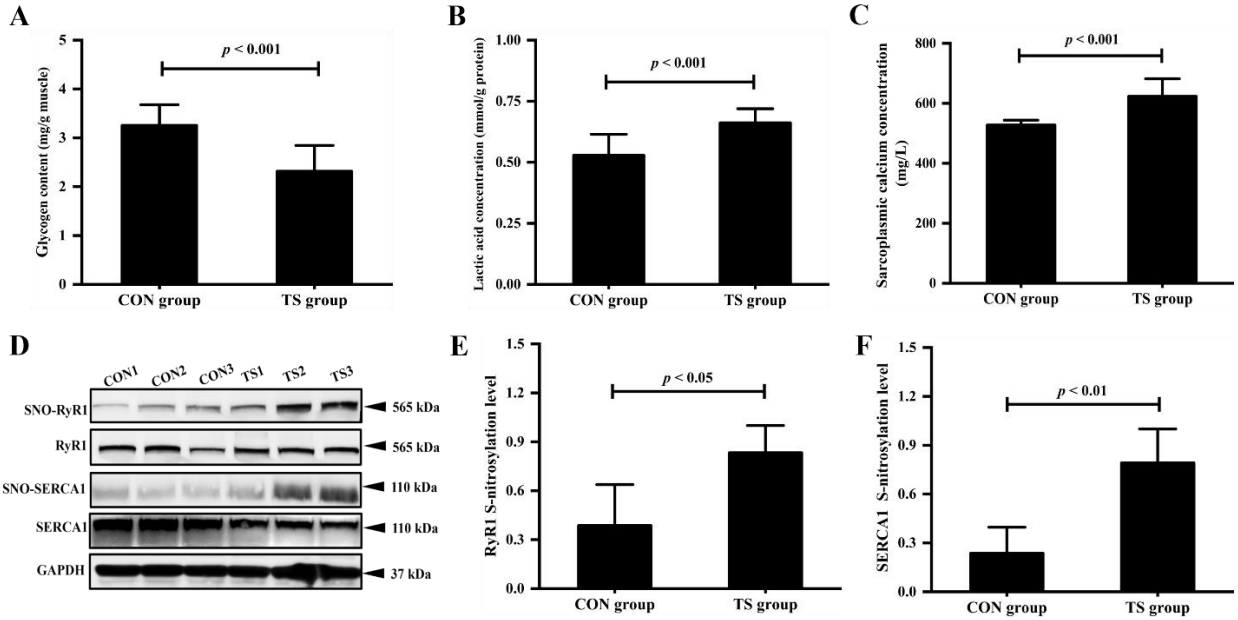

Figure 1. Differences in glycolytic metabolism (A and B), sarcoplasmic calcium levels (C), and calcium transporter S-nitrosylation levels (D-F) of postmortem *longissimus thoracis* muscle from pigs with different pre-slaughter stress levels. CON: control group; TS: transport-induced stress group.

IV. CONCLUSION

In summary, this study highlights that the increased S-nitrosylation levels of RyR1 and SERCA1 induced by pre-slaughter transport stress caused an increase in sarcoplasmic calcium, which further led to the acceleration of muscle glycolysis metabolism and thus the alteration of pork quality.

ACKNOWLEDGEMENTS

This work was supported by the earmarked fund for China Agriculture Research System (CARS-35).

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