Investigating the effect of ultrasound on glycolysis in early post-mortem bovine muscle

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Objectives: Ultrasound (US) is a technology that has the ability to modify enzyme activity, and thus holds potential to manipulate/ optimise glycolytic kinetics in early post-mortem muscle. This is of interest as it will allow a better understanding of how these metabolic processes can be manipulated to optimise final meat quality. While numerous studies have used US to modify proteolytic enzyme activity in post-rigor muscle, few have focused on the effect on pre-rigor glycolysis. Therefore, the objective of the study is to investigate the effect of US on the glycolytic related enzymes in early post-mortem muscle using an *in vitro* buffer system.

Materials and Methods: Bovine *Longissimus thoracis et lumborum* (LTL) was collected 1 hour postmortem from 4 heifers (18-26 months) from a commercial slaughtering plant (Kepak Group, Kilbeggan, Ireland) and transported to Teagasc Research Centre, Ashtown. The LTL was cut into 12 steaks (1.5 cm thickness), for controls (6) and treatments (6). The steaks were vacuum packed prior to US: 25 or 45 kHz for 15, 30 or 45 minutes, in a water bath <10 °C. Controls underwent the same processing, with no US applied. After treatment, one half of the steak was used to monitor pH decline in the intact muscle for 24 hr (1440min); and the other half was snap frozen and processed for use in an *in vitro* glycolytic buffer according to England, Matarneh (1). Aliquots were removed at specific times to determine glycogen, glucose, lactic acid and pH values over time (0 to 1440mins). Glycogen and glucose were determined using DNS method. A commercial kit (Megazyme, Ireland) was used for lactic acid. pH decline was modelled to the equation: $K= a-b \times e^{(-c \times t)}$ (2) to determine the kinetics of pH decline (*c*) using the mosaic package in RStudio (3). Data was analysed by ANOVA using MIXED procedure of SAS, considering intensity and duration of treatments and its interaction as a fixed effect, and individuals as random effect. pH and metabolites were evaluated as time-repeated measures using Tukey's test. When analysing kinetics of pH decline, Friedmans test was carried out in Genstat with Samples (Animals) as blocks, comparing 9 treatments.

Results: The *in vitro* buffer system was an effective tool to determine the effect of US on the inherent activity of enzymes within bovine muscle. Under the conditions of this experiment, US (25/45 kHz, 15/30/45 min) did not have a significant effect (p-val- ue>0.05) on the rate of change for pH, glycogen, glucose or lactic acid when analysed over 1440 min in the glycolytic buffer. A similar outcome was observed in the pH profile of intact muscle. Interestingly, while glucose concentrations initially increased, in general a shortlived decrease was observed around the 30 or 90 min time points. Glucose levels were highly correlated with pH over the reaction period (R=-0.75156, P<0.0001), where glucose content increased exponentially. The inflexion point of the expo- nential curve was when pH declined below pH 6, indicating a change in the glucose production/consumption balance. This could be a result of a change in the enzymatic activity of phosphofructokinase or hexokinase, as the pH declines over time, where there appears to be a faster rate of glucose production compared to its consumption, allowing it to accumulate towards the later stages of the reaction.

Conclusion: Under our experimental conditions, US appeared to have no effect on the rate of glycolysis, as observed in the *in vitro* glycolytic buffer and intact muscle. However, a shift in the process of glycolysis was observed in the buffer system for all treat- ments as the pH of the buffer made the transition to below pH 6, where the degradation and accumulation of glycogen and lactic acid, respectively, slowed and glucose accumulated in the system

References:

1. England EM, Matarneh SK, Oliver EM, Apaoblaza A, Scheffler TL, Shi H, et al. Excess glycogen does not resolve high ultimate
   pH of oxidative muscle. Meat science. 2016;114:95-102.
2. Gagaoua M, Troy D, Mullen AM. The Extent and Rate of the Appearance of the Major 110 and 30 kDa Proteolytic Fragments dur- ing Post-Mortem Aging of Beef Depend on the Glycolysing Rate of the Muscle and Aging Time: An LC MS/MS Approach to De- cipher Their Proteome and Associated Pathways. Journal of Agricultural and Food Chemistry. 2021;69(1):602-14.
3. Pruim R, Kaplan DT, Horton NJ. The mosaic Package: Helping Students to 'Think with Data' Using R. The R Journal. 2017;9(1):77-102.

Key words: Glycolysis, Ultrasound, pH, Post-mortem metabolism