DOES ULTRASOUND HAVE THE CAPACITY TO MODULATE THE GLYCOLYTIC METABOLISM KINETICS? AN *IN VITRO* STUDY

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I. INTRODUCTION

Post-mortem glycolysis has an influencing effect on the tenderisation of muscle based on the interaction between rate of pH and temperature decline, during the onset of rigor. Ultrasound (US) is a non-thermal novel technology that has the ability to alter the rate of glycolysis though its ability to alter enzymatic activity [1]. The application of US to early post-mortem muscle is of interest as it may present a mechanism whereby the rate of post-mortem pH decline in muscle could be manipulated with the target of improving tenderness. Results of a previous study (not published) that our group carried out on the use of US on early post-mortem muscle showed that US had a significant effect on pH decline: but enzymatic activity was not affected when examined using a glycolytic buffer system. To understand if muscle structure was providing a physical protection to the glycolytic enzymes from the effect of US, the objective of the current study was to investigate the impact of US on the glycolytic activity when US is applied directly to muscle homogenates, prepared using early post-mortem muscle.

II. MATERIALS AND METHODS

Bovine *Longissimus thoracis et lumborum* (LTL) was collected from 4 steers, 1 hr post-mortem, from a commercial slaughtering plant and transported to Teagasc Research Centre, Ashtown. The LTL was divided into 6 steaks and randomly assigned to treatments described. Each steak was cut into small pieces, snap frozen in liquid nitrogen, powdered and stored at - 80 °C. For US treatment, muscle was homogenised in water and placed in a jacketed vessel. During treatment, temperature was maintained at 30 °C, stirred constantly. The US treatments were 20 kHz: 60% amp (11.4 W/cm^2) or 100% amp (19 W/cm^2), for 15 or 30 min, using a US probe. Controls followed the same procedure, without US. Immediately after treatment, homogenates were added to an *in vitro* buffer system [2], where the solution was incubated for 1440 min at 25 °C with constant stirring. Aliquots were removed from the buffer at specific time points to determine glycogen, glucose and lactic acid content and pH. Glucose and glycogen content was determined using the DNS method. Lactic acid content was determined using a lactic acid kit (Megazyme, Ireland). Data was analysed as a repeated measures ANOVA using MIXED procedure of SAS 9.4. Treatment power and duration and their interaction were fixed effects and individual animals were considered random effects, sample time were repeated measures.

III. RESULTS AND DISCUSSION

The experimental conditions used (20 kHz US, 60/100% amp, 15/30 min) had a significant impact ($P < 0.05$) on the rate of pH decline, glucose and lactic acid content over 1440 min. US did not affect ($P > 0.05$) the rate of glycogen degradation. US treatment (100% amplitude (19 W/cm^2), 30 min) had a significant effect on pH, and glucose and lactic acid content in the glycolytic buffer, when compared to the other treatments and controls, specifically between time points 30 min and 240 min. The final readings (at 1440 min) of pH and concentration of glucose and lactic acid did not differ significantly between any treatments or control. For controls and homogenates where US treatment (60% amp for 15 and 30 min, 100% amp for 15 min) had no effect, pH declined from 7.4 to 5.4 over 1440 min, with

the majority of the decline taking place within the first 90 min. pH decline mirrored lactic acid production (Fig 1A). In these samples, glycogen content decreased rapidly in the first 90 min and then more slowly up to 1440 min. Glucose concentration increased rapidly in the first 240 min and at a slower rate thereafter (Fig 1B). However, following the use of 100% amp US for 30 min, pH decreased at a significantly slower rate with the pH of the glycolytic buffer system not reaching pH 6 until after 240 min, compared to the control, where pH 6 was attained between 30 and 90 min. This corresponded with a delay in lactic acid production (Fig 1A). The accumulation of glucose accelerated, where the concentration of glucose was significantly greater ($P < 0.05$) then the controls between 30 and 240 min of the reaction. However, at 1440 min, there was no significant differences ($P > 0.05$) observed (Fig 1B). The results indicate that the 100% amp US for 30 min slowed down the consumption of glucose in the buffer system, and impacted reactions further down-stream in the glycolytic pathway, delaying the production of lactic acid, and subsequent pH reduction. Enzymes that may have been affected by the US treatment are hexokinase or phosphofructokinase. Since similar ultimate pH and metabolite concentrations were achieved after 1440 min when compared to controls, the results suggest that the effect of US may involve the significant reduction in enzyme activity.

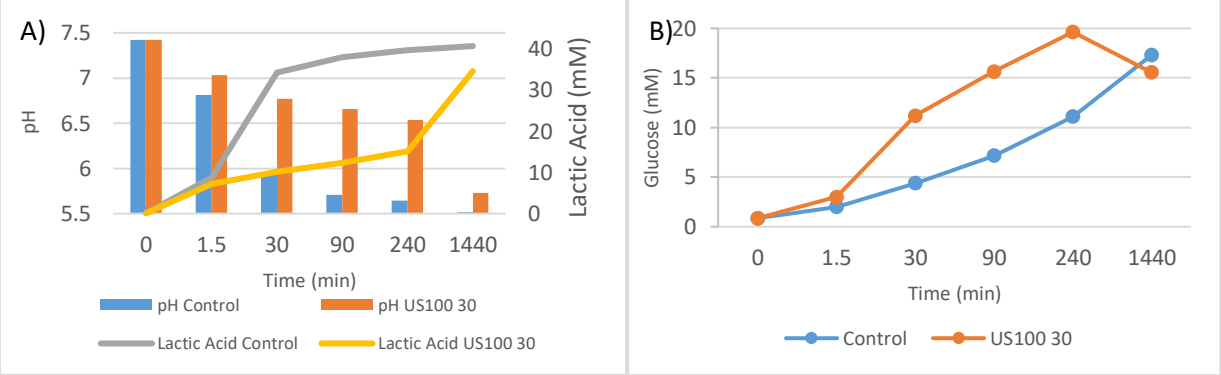

Figure 1. *In vitro* pH, lactic Acid (A) and glucose content (B) following 100% amp, 30 min US and control.

IV. CONCLUSION

Under the experimental conditions, US power and duration (100% amp (19 W/cm2) US for 30 min) had a significant interactive effect on the rate of pH decline, and glucose and lactic acid accumulation, when enzymatic activity of muscle homogenates was assessed *in vitro* using a glycolytic buffer system. This US treatment decreased the rate of pH decline and reduced lactic acid production in the first 240 minutes, while glucose was seen to accumulate. However, no significant differences were observed at 1440 min, indicating that US has the capacity of slow down the rate of glycolysis but not completely arrest it. Further research is required to identify the specific enzymes affected by US treatment.

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