THE RELATIONSHIP BETWEEN EXTREME SARCOMERE LENGTHS AND TENDERNESS IN THE M. *LONGISSIMUS DORSI* OF STEER BEEF

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Background:
It has been proposed that the degree of acto-myosin crosslinking of the sarcomere within the myofibril may contribute to post mortem (PM) toughness (Smulders *et al.*, 1990, Taylor *et al.*, 1995). Results from an Irish factory based survey, suggest that beef M. *longissimus dorsi* (LD) with sarcomere lengths (SL) above 2μm tend to have acceptable mechanical (<50N) and sensory tenderness (Maher *et al.*, unpublished data). Manipulation of carcasses to achieve more tender beef has been in practice for many years, with temperature during ageing and method of hanging playing an important role. A combination of aitch bone hanging (tenderstretch) and storage at 10°C for 48 hours was used to produce tender beef with lengthened myofibrils and long sarcomeres. Supercontracted sarcomeres were produced by hotboning and storage at 2°C for 48 hours to cold-shorten and toughen the muscle. This manipulation of the LD provided an opportunity to create a range of SL and to examine their relationship with tenderness.

Objectives:
The main objective of the experiment was to examine at the relationship between extreme SL and tenderness in the LD of steer beef PM, and to determine the accuracy of SL as a predictor of tenderness.

Method:
Steers (n=12) were slaughtered at the Meat Industrial Development Unit at the National Food Centre, Dublin. Carcasses for treatment 1 (T1) were split, the left-hand side (LHS) LD hot-boned within 1.5 hours PM and stored for 48 hours at 2°C to achieve very short SL. The control for T1 was the LHS of a carcass conventionally hung for 48 hours at 2°C (C1). Carcasses for treatment 2 (T2) were split and the right hand side (RHS) hung by the aitch bone for 48 hours at 10°C. Control for T2 was the RHS of a carcass conventionally hung for 48 hours at 10°C (C2). At 48 hours PM the LD was excised from intact carcasses. Four replicates have been completed to date. Sarcomere length was measured by laser diffraction, using a vertically orientated laser beam (Uniphase Helium Neon laser 1300) coupled with a power supply 1201-2 (Uniphase Ltd., UK) (Cross *et al.*, 1980). Samples were prepared for electron microscopy using a modified version of the Glauert (1975) method. Muscle was cut into strips (4mm wide, 40mm long and 2mm deep) and restrained at *in situ* length by tying them on sticks. Sample was fixed immediately in a primary fixative (fix 1°) containing 3% gluteraldehyde, 2.5% paraformaldehyde in 0.2M Sorensons phosphate buffer (pH 7.2), EGTA, NaCl and NaOH for a minimum of 90 minutes. The central part of the strip was then cut into pieces (1mm wide by 2mm long by 1mm deep), ensuring that the long dimension (i.e. 2mm) was parallel to the muscle fibre direction. These pieces were placed in vials containing fresh fix 1° for a further 90 minutes at 4°C. The material was rinsed in Sorensons phosphate buffer and stored at 4°C overnight and postfixed in 1% osmium tetroxide (fix 2°) on ice for 45 minutes. This was repeated with fresh fix 2°, followed by 4 washes in Sorensons phosphate buffer at 4°C and left overnight at 4°C. Samples were next dehydrated through a graded acetone series and embedded in Araldite CY212 resin (with Agar 100). Ultra-thin sections were cut (silver interference colour) with glass knives on the LKB Ultrotome III. The sections were stained with 2% uranyl acetate and lead citrate, examined and photographed in the JEOL 1200 EX TEMSCAN electron microscope at an accelerating voltage of 80kV. At 14 days PM freshly cut samples (2.5cm thick) were taken from the vac-packed muscle for Warner bratzler shear force (WBSF) analysis using the Instron model 5543 (Shackelford *et al.*, 1991). Data were subjected to ANOVA using Minitab 13.

Results and discussion:
There was a significant difference between treatments for both SL and WBSF. As was expected, T1 (hot-boned, stored at 2°C) produced cold-shortened muscle with supercontracted sarcomeres (mean±standard deviation, 1.31μm±0.151) compared to its control (C1) (1.61μm±0.099). WBSF values for T1 were (69.61N±1.20), compared 31.07N±7.54 for control (C1). Aitch bone hanging at 10°C (T2) resulted in a mean SL of 2.28μm (±0.174), which was more extended than its control (C2) (1.61μm±0.205). More tender WBSF values reflected the SL, having a T2 WBSF value of 22.14N (±10.17) compared to the control (C2) (28.48N±8.59). There was a tendency towards a relationship between SL and tenderness at 14 days PM ($r = -0.626$, $p = 0.071$), however the non-significant P-value is possibly due to the low number of replicates. In agreement with our own findings, Smulders *et al.* (1990) found a relationship between shortening and 14 day toughness in the LD, and also observed a clear positive association between long SL (greater than 1.9μm) and tenderness. They suggested that as SL decreases this relationship between SL and tenderness was less obvious, and the variation they observed within SL and WBSF could prevent SL being used as a predictor of tenderness. The electron micrographs provide a visual assessment of the contraction and relaxation of the sarcomere. Treatment 1 muscles have a supercontracted actomyosin complex, where the myosin filaments appear to be doubled on top of the actin filaments (which are attached to the Z-line) and are pushing through the opposite Z-line making it amorphous, with no trace of the I-band remaining (Fig 3A&B) (Stromer & Goll, 1967). Aitch bone hung LD show relaxed sarcomeres, with little overlap between the actin (lighter area) and the myosin (darker area) filaments (Fig.3D). Both controls had some overlap between the actin and myosin, which is evident in Fig. 3C as the I-band is darker than the I-band in Fig.3D. As observed in Fig. 3, the 48 hour aged samples show disruption of the actin filament/Z-line interaction, increased inter-myofibrillar spaces and sarcolemma widening, which was also seen by O'Halloran *et al.* (1997) at 24 hours PM.

Conclusion:
Manipulation of the LD enables investigation of the relationship between SL at 2 days PM and mechanical tenderness at 14 days PM. The data suggests that manipulating beef LD to create very long SL at day 2 PM may in turn enable SL to be used as an acceptable predictor of 14 day WBSF. This experiment is only in the initial stages and analysis is ongoing, with further factors such as pH being investigated.

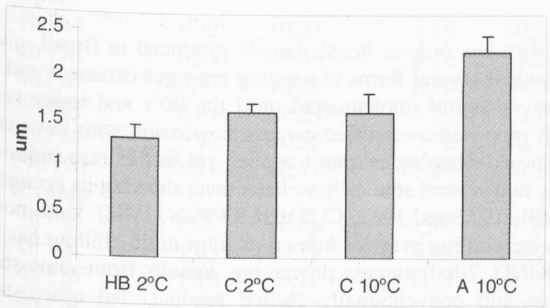

Figure 1: Sarcomere length mean and standard deviation at day 2 post-mortem of the M. *Longissimus dorsi* from 4 groups (replicates =4). Treatments were hot-boning (HB) and 2°C storage, aitch bone hanging (A) and storage at 10°C or conventionally hung controls (C) stored at 2°C and 10°C.

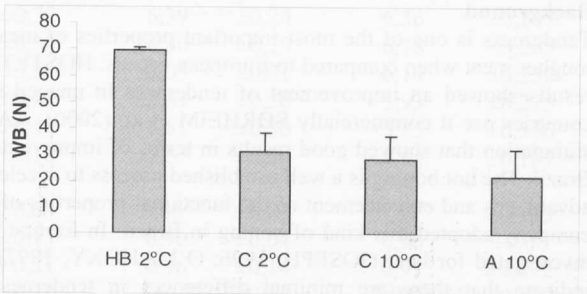

Figure 2: Warner Bratzler (WB) mean and standard deviation at 14 day post-mortem of the M. *Longissimus dorsi* from 4 groups (replicates =2-3). Treatments were hot-boning (HB) and 2°C storage, aitch bone hanging (A) and storage at 10°C or conventionally hung controls (C) stored at 2°C and 10°C.

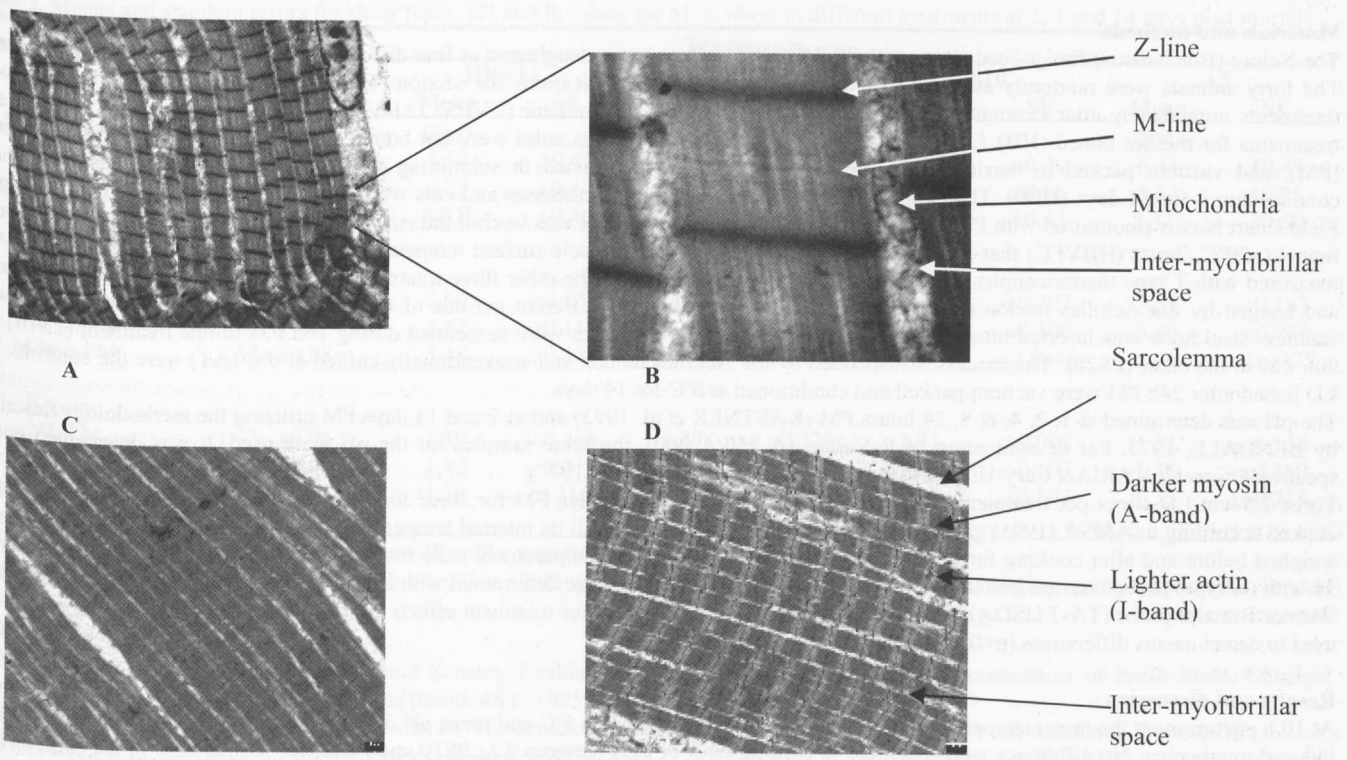

Figure 3: Electron micrographs of sections of bovine LD muscle aged for 24 hours PM. (A) Hot-boned muscle aged at 2°C to achieve very short sarcomeres (5,000X). (B) Hot-boned muscle aged at 2°C (40000X). (C) Conventionally hung controls aged at 10°C (5,000X). (D) Aitch bone muscle aged at 10°C to achieve very long sarcomeres (5,000X). Disruption of the actin filament/Z-line interaction, increased inter-myofibrillar spaces and sarcolemma widening are evident on these 48 hour aged beef samples.

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