EFFECT OF MUSCLE STRETCHING ON TITIN AND CALPAIN 3: IMPLICATIONS FOR TENDERNESS

Stevenson B.J., Morton J.D., Ilian, M.A., Bickerstaffe R.
Molecular Biotechnology Group, Animal and Food Sciences Division, Lincoln University, Canterbury, New Zealand

Background

The tenderness of aged meat results from the breakdown of specific muscle proteins in the post-mortem period. It is widely accepted that the calpain proteases have an important role in this process[1,2] but it is unclear which of the calpain isozymes are important. Recent studies have shown an association between the levels of the muscle specific calpain, p94 or calpain 3, and meat tenderisation[3,4]. Unfortunately, researchers have been unable to demonstrate activity of this enzyme *in vitro*, presumably because of autolysis during extraction[5]. One of the objectives of this study was to determine whether enhancing the rate of tenderisation will cause an increase in the autolytic activation of calpain 3. Autolytic activation of calpain 3 can be measured by Western blotting[4].

There is also considerable interest in the substrates of proteolysis. The ageing of meat does not involve the wholesale breakdown of muscle proteins. It appears that tenderisation is due to the proteolysis of a few key proteins[6]. A prime candidate is titin. This giant protein, with a size of about 3000 kDa, is the largest single polypeptide chain known and makes up about 10% of the muscle protein mass[7]. It stretches across half a sarcomere and has an important role in maintaining muscle structure. During ageing it is broken down to smaller fragments, principally T2 (2100 kDa) and T3 (1200 kDa)[8]. Calpain 3 has been observed bound to titin although it is unclear whether titin is a calpain 3 substrate[9].

The technique used in this study is to enhance the rate of tenderization in lamb by stretching the *longissimus* muscle (LTL). Previous studies have shown that stretching muscles will lead to more rapid ageing and more tender meat[10,11]. In this experiment extension of the LTLwas achieved by hanging the carcasses in different postures.

Objectives

To determine whether increasing the sarcomere length of post-mortem *longissimus* muscle would alter the rate of degradation of titin and autolytic activation of calpain 3

Methods

Twelve Coopworth lambs were slaughtered by captive bolt and exsanguination at Lincoln University. Six of the dressed carcasses were hung by the Achilles tendon ("conventional ') while the other six were hung over a bar at the pelvic region with all four legs tied together (bar-hanging). Samples (10mm width) were removed from the central region of the LTL at 1,2,3 and 7 days after slaughter for analysis. An additional 100mm sample was taken for tenderness testing at 7 days.

Myofibrils were isolated from each sample[12] and the sarcomere length determined by phase contrast microscopy (Leica DM IRB). Images were captured with a Spot digital camera and analysed using Image-Pro Plus software.

Protein samples were prepared by adapting an earlier protocol[13] to allow simultaneous preparation for titin and calpain analysis. The six samples from each treatment at each time were pooled to determine the changes in the titin and calpain 3 with time to minimise the effect of carcass variation. The samples for titin were separated by SDS-PAGE on a 4% acrylamide gel[14] and stained with Coomassie brilliant blue. The stained gels were dried, scanned and analysed using the Biorad program Quantity One. Calpain 3 was separated by SDS-PAGE on an 8% acrylamide gel [15] and visualised by Western blotting using an antidody to the IS2 region[4].

Statistical data was analysed using the Genstat 5 package.

Results and discussion

Measurement of the sarcomere lengths at one-day post-mortem confirmed that bar-hanging had extended the muscles. The mean sarcomere length for the LTL of bar-hung carcasses was 2.4µm (S.D. ± 0.39µm), which was significantly greater than the 1.55µm mean (S.D. ± 0.3µm 9) for conventionally hung carcasses. Over the next six days of aging both sets of muscles stretched further with the sarcomere lengths at seven days post-mortem being 2.5 (S.D. ± 0.13µm) and 1.8µm (S.D. ± 0.34µm) respectively. This increase in sarcomere length in conventionally hung carcasses over time has been reported previously[16].

It is established in the literature that mechanical stretching of muscle causes improvement in LTL tenderness especially in animals with inherently tougher meat.[10,11] The fundamental concept is that stretching muscles pre-rigor and allowing them to complete rigor in the stretched state will result in a significant reduction in the overlap region between the thick and thin filaments of the sarcomere and in the number of permanent cross bridges between actin and myosin. In this paper we examined whether improvement in tenderness associated with stretching skeletal muscles is only due to alteration in the lengthening state of the sarcomere or whether it also involves change in the rate of degradation of specific myofibrillar proteins by the calpain proteolytic system.

The breakdown of titin over the seven days of ageing is shown in Figure 1. The decline in the intensity of the T1 band corresponds with an increase in the largest titin fragment, T2. Nebulin, the second largest protein in muscle, also disappears but there is no evidence in a change in the density of the myosin heavy chain. Stretching has clearly enhanced the rate of titin breakdown (Figure 1). Because we used pooled samples it is not possible to determine whether these differences are significant. Since the level of titin degradation was higher in stretched LTL compared to non-stretched one at all time points studied we believe the differences are real.

The calpain 3 Western blot shows total loss of the p94 band by 3 days post-slaughter (Figure 2). This was accompanied by an increase in the p52.5 fragment. In other calpains this pattern is typical of activation and subsequent autolysis. Furthermore, the level of autolysis of calpain 3 was higher in stretched LTL compared to non-stretched LTL at 1d and 2d post-mortem.

A possible reason for the enhanced breakdown of titin is that the myofibril matrix opens during stretching, allowing -access by proteases such as calpain 3 to the cleavage sites of titin. Earlier, our studies on the role of calpain 3 in meat tenderization revealed, for the first time, a strong correlation between the variations in meat tenderness and the expressions of calpain 3 at the mRNA[3] and protein[4] levels. The finding in this study indicate that the improvement in tenderness by stretching is not only governed by a change in the sarcomere density but may also be related to changes in the localization pattern of calpain 3 at the sarcomere and the degradation rate of titin. These results provide additional experimental evidences that calpain 3 is an important enzyme in tenderization of meat.

Conclusions

Bar-hanging results in a significant extension of the sarcomeres in the LTL. This leads to more rapid degradation of the structural protein, titin, and the protease, calpain 3.

Pertinent literature

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Figure 1. Post-mortem degradation of titin and nebulin in lamb longissimus which has been hung conventionally (C) and by bar-hanging (S).

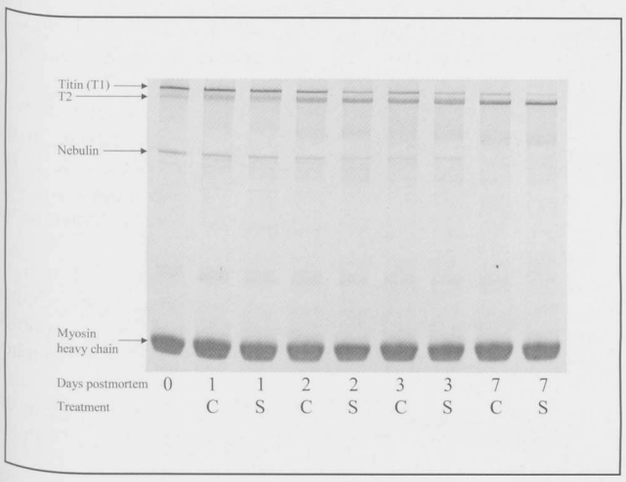

Figure 2. Post-mortem degradation of calpain 3 in lamb longissimus which has been hung conventionally (C) and by bar-hanging (S).