INTERMUSCULAR VARIATION IN DEGRADATION OF HIGH MOLECULAR WEIGHT PROTEINS IN BOVINE MUSCLES DURING POSTMORTEM AGING

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Background

Beef tenderisation during postmortem aging differs among muscles in an individual carcass (Olson et al., 1976; Koohmaraie et al., 1988; Ilian et al., 2001). Although the sequence of postmortem metabolic events that indicate final meat tenderness remains unexplained, research has implicated the involvement of protein degradation. High molecular weight proteins, such as titin (□3,000 kDa) and nebulin (□800 kDa), are unique in size and play important roles in maintaining muscle structure, thus there is potential for degradation of these proteins to impact on meat tenderisation (Taylor et al., 1995; Huff-Lonergan et al., 1996).

The activities of Ca^{2+}-dependent proteases (calpains) and the inhibitor (calpastatin) are suggested to play an important role in protein degradation (Koohmaraie et al., 1988; Whipple and Koohmaraie, 1992; Ilian et al., 2001). Recently, the novel calpain 3 (94 kDa) isoform has been of particular interest as not only it is expressed almost exclusively in skeletal muscle, at least two binding sites for this protease are located on the titin molecule (Sorimachi et al., 1995). The expression of calpains 1, 2, and 3, and calpastatin have been shown to vary between muscles of different fibre type (Ilian et al., 2001; Jones et al., 1999). Furthermore, muscle fibre type has been suggested to have an effect on the extent and rate of postmortem degradation. Skeletal muscles can be characterized into fast and slow muscles according to the distribution of four muscle fibre types, which contract at different rates according to the expression pattern of myofibrillar protein isoforms, such as myosin heavy chains (Schiaffino and Reggiani, 1996). The degradation of myofibril proteins has been reported to occur faster in bovine white (faster) muscles, such as longissimus thoracis (LT) and semitendinosus (ST), than in red (slower) muscles, such as psoas major (PM) (Olson et al., 1976). There appears to be a complex relationship between protein degradation, muscle fibre type, and the calpain system, and their ultimate effect on meat tenderness.

Objectives

The objective of this study was to determine the effect of degradation of high molecular weight proteins on the rate of tenderisation among four bovine muscles during postmortem aging.

Materials and methods

The diaphragm (DP), psoas major (PM), longissimus thoracis (LT), and semitendinosus (ST) muscles for 0 d postmortem samples were excised from a Japanese Black steer within 1 h after slaughter. After overnight hanging of the carcass at 2˚C, the muscle blocks were excised from the carcass, bagged and aged at 2˚C. At 1, 3, 7, and 14 d postmortem, muscle samples were prepared for immediate Warner Bratzler shear force measurement (kg/cm^2), along with electrophoresis samples that were frozen at -40˚C until analysis.

For SDS-PAGE analysis, crude myofibrils were extracted from the muscles by 0.1 M potassium phosphate buffer (pH 6.8) with 0.6 M KCl and protease inhibitor cocktail (SIGMA, Saint Louis, Missouri), and analysed on the same day to avoid degradation. Myofibrils were applied to SDS-PAGE using a 3% gel without stacking gel (ATTO, Tokyo, Japan), and run at room temperature for 1 h at a constant current of 10 mA, followed by 1 h at a constant current of 20 mA. Protein bands were detected by fluorescent stain using SYPRO Ruby gel stain (Bio-rad, Hercules, CA) for 16 h overnight.

Results and discussion

There was intermuscular variation in the rate of tenderisation, as shown in Figure 1. Shear force measurement showed a faster rate of tenderisation in LT compared to ST, with PM and DP being intermediate. Although published data could not be found on the tenderisation of the diaphragm, the trend in

the rate of tenderisation of LT being higher than ST and PM is consistent with previous research (Koohmaraie et al., 1988; Ilian et al., 2001).

Figure 2 shows the degradation pattern of titin and nebulin among the four muscles. The 1,200 kDa degradation product of titin is evident in Figure 2, however T1 and T2 could not be clearly distinguished, therefore the intermuscular variation in degradation of titin could not be observed in this experiment. There was a difference in degradation of nebulin among the muscles, as intact nebulin was absent by 3 d of postmortem aging in LT compared to by 7 d in ST. The degradation of nebulin in PM and DP was observed after 1 d postmortem.

Degradation of two unidentified high molecular weight proteins was evident soon after death in this study, as can be seen in Figure 3 (band *x* and band *y*). Band *x* was present at 0 d in LT and ST muscle, and was absent by 3 d in LT and by 7 d in ST, which is a similar degradation pattern to that of nebulin in the LT and ST muscles. Band *x* was not detected in PM or DP muscles. The detection of the degradation of band *x* was due to the highly sensitive fluorescent staining, as compared to Coomassie brilliant blue which has been conventionally used in previous studies (Fritz et al., 1993; Taylor et al., 1995; Huff-Lonergan et al., 1996). Band *y* appears to be filamin, which was shown to degrade to a 240 kDa fragment (Huff-Lonergan et al., 1996). In this experiment, band *y* degradation was observed to occur from 3 d in LT, and from 7 d in ST and PM, however band *y* was not degraded in DP. In addition, a degradation fragment migrating slower than band *x* was observed from 7 d of postmortem aging in PM, with trace amounts evident also from 7 d in LT, but was not detected in ST or DP, as evident in Figure 3. This fragment may be a product of titin degradation (Taylor et al., 1995).

The degradation of myofibril proteins has been reported to occur faster in bovine white (faster) muscles, such as LT and ST, than in red (slower) muscles, such as PM (Olson et al., 1976). However, in this study, the protein degradation patterns and meat tenderisation of LT and ST were different. LT exhibited a higher postmortem tenderisation rate, and in addition, the degradation of nebulin, band *x*, and band *y* was faster in the LT muscle compared to ST. The lower tenderisation rate in ST may have been the result of relatively slower degradation of nebulin, band *x*, and band *y*. These differences may be due to intermuscular variation in the expression of calpains and calpastatin. According to Ilian et al (2001), the higher relative tenderisation rate in LT compared to ST (in ovine) and in LT compared to PM (in bovine) was due to a higher level of expression of calpain 3 in LT. These results suggest that, other than muscle fibre type, the relative proportion of calpains and calpastatin in different muscles may also influence meat tenderisation.

The diaphragm is classified as a slower type muscle (Muroya et al., 2002), and has been shown to express slow type isoforms which have different degradation patterns to fast type isoforms (unpublished data). Nebulin degradation was observed in the diaphragm, however band *x* was not present and there was no degradation of band *y*. The data suggests that the degradation of myofibrillar proteins differs among the isoform types, due to the different susceptibility to proteolysis. Thus, the difference in tenderisation rate between the diaphragm and LT may indicate that muscles with different isoform composition are tenderised at different rates.

Conclusions

The results revealed that postmortem degradation of high molecular weight proteins varies among bovine muscles of the same carcass. There appears to be a relationship between the degradation of high molecular weight proteins and meat tenderisation, with the possibility of other influencial factors such as different expression levels of calpains and calpastatin among the muscles.

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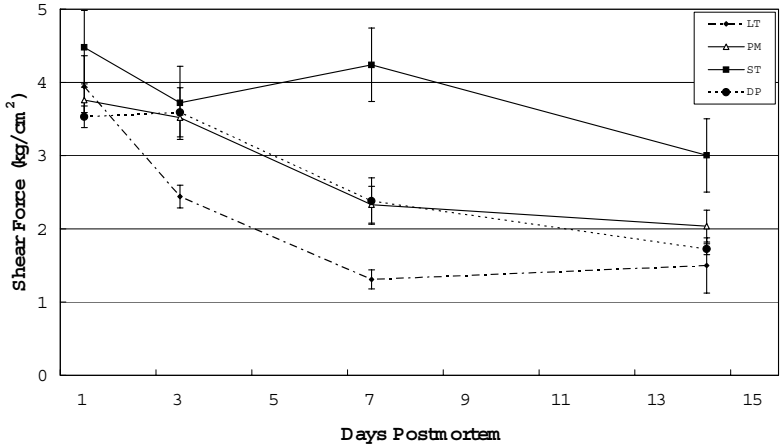

Figure 1. Shear force (kg/cm^2) of bovine longissimus (LT), semitendinosus (ST), psoas major (PM) and diaphragm (DP) at 1, 3, 7 and 14 days postmortem.

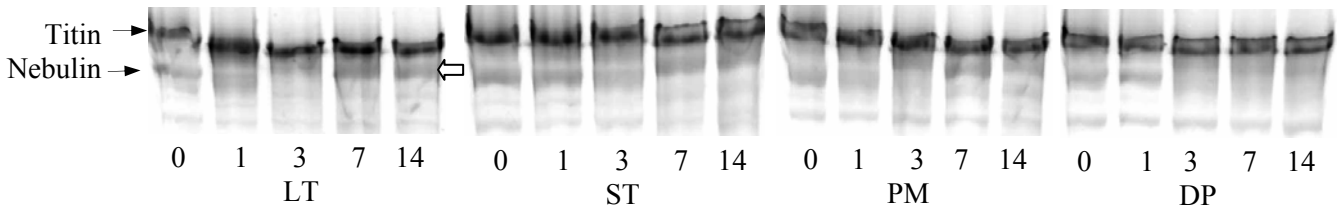

Figure 2. Postmortem degradation pattern of titin and nebulin. Numbers indicate days postmortem. LT: longissimus thoracis, ST: semitendinosus, PM: psoas major, DP: diaphragm. Open arrow designates the position of the titin 1,200 kDa fragment.

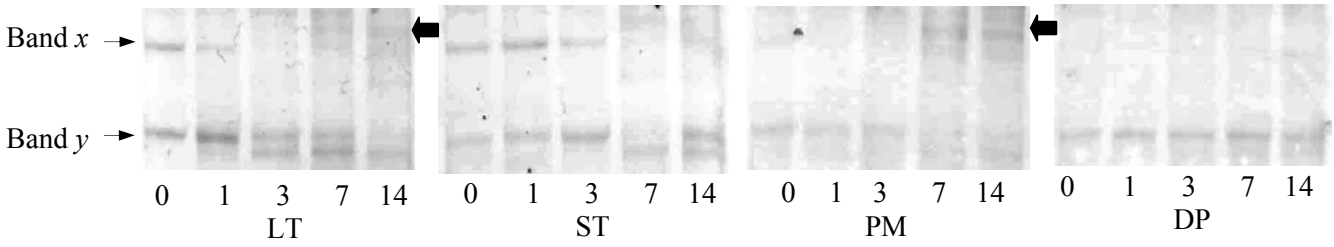

Figure 3. Postmortem degradation pattern of band *x* and band *y*. Numbers indicate days postmortem. LT: longissimus thoracis, ST: semitendinosus, PM: psoas major, DP: diaphragm. Closed arrow indicates degradation fragment, migrating slower than band *x*.